LBL-32188
UC-000

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

To be published as a chapter in *Advances in Internal Medicine*, Vol. 38, G.H. Stollerman, Ed., Mosby-Year Book, Inc., Chicago, IL, September 1992

HDL and Coronary Artery Disease

D.L. Tribble and R.M. Krauss

April 1992

U. C. Lawrence Berkeley Laboratory
Library, Berkeley

FOR REFERENCE

Not to be taken from this room

Dor

Biology & Medicine Division

LBL-32188
Copy 1
Bldg. 50 Library.

Prepared for the U.S. Department of Energy under Contract Number DE-AC03-76SF00098

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

LBL-32188
UC-000

HDL AND CORONARY ARTERY DISEASE

Diane L. Tribble, Ph.D. and Ronald M. Krauss, M.D.

Donner Laboratory
University of California

and
Department of Molecular and Nuclear Medicine
Life Sciences Division
Lawrence Berkeley Laboratory
University of California
Berkeley, CA 94720

Address reprint requests to:
Dr. Diane L. Tribble
Lawrence Berkeley Laboratory
University of California
Donner Laboratory, Room 465
1 Cyclotron Road
Berkeley, CA 94720

This work was supported in part by NIH Grants HL 18574 and HL 33577 from the National Heart, Lung, and Blood Institute of the National Institutes of Health, a grant from the National Dairy Promotion and Research Board, and was conducted at the Lawrence Berkeley Laboratory (Department of Energy contract DE-AC03-76SF00098 to the University of California).

TABLE OF CONTENTS

1. Introduction

2. Definition and Characterization of HDL

3. Clinical Measurement of HDL

4. HDL Metabolism

   A. Metabolic Origins of HDL

   B. Intravascular Remodeling of HDL

      1. Interactions of HDL with Other Lipoproteins and Cells

      2. Interconversions Among HDL Subclasses

   C. Plasma Clearance and Catabolism of HDL and HDL Constituents

5. Regulation of Plasma HDL and HDL Subclass Levels

   A. Metabolic Considerations

   B. Factors Influencing Plasma HDL Levels

      1. Intrinsic factors

      2. Extrinsic factors

6. Epidemiological Evidence of a Relationship between HDL and Coronary Artery Disease

7. Direct Evidence of a Relationship between HDL and Coronary Artery Disease

   A. Animal Studies

   B. Human Studies

8. Proposed Mechanisms Underlying the Antiatherogenic Properties of HDL

9. Concluding Remarks

1. Introduction

Plasma levels of high-density lipoproteins (HDL) and distributions of HDL subclasses, most notably among HDL_2 and HDL_3, are recognized increasingly as important determinants of atherosclerosis susceptibility. Numerous epidemiologic studies have shown an inverse relationship between plasma HDL cholesterol concentrations and the occurrence of coronary artery disease (CAD). HDL and HDL subclass levels vary in relation to constituitive and environmental factors and may represent a final pathway by which many of these factors influence CAD risk. Although properties underlying the apparent antiatherogenicity of HDL are as yet unknown, participation of this lipoprotein in the removal of excess cholesterol from cells and other circulating lipoproteins for return to the liver, a process known as 'reverse cholesterol transport', has been cited as a possible mechanism. Alternately, HDL levels may provide a surrogate measure of other compounds and/or metabolic events which influence the disease process. The purpose of this review is to examine the relationship between HDL and atherosclerosis by considering the physicochemical and metabolic properties of HDL and surveying evidence of the involvement of HDL in CAD in human populations.

2. Definition and Characterization of HDL

Plasma lipoproteins, of which HDL represent a major class, are spherical lipid-protein complexes consisting of a core of esterified cholesterol and variable amounts of triglycerides surrounded by a phospholipid monolayer with interdigitated free cholesterol and apolipoproteins. HDL are the smallest and most dense of the lipoprotein classes. Particles range in diameter from 75-120 Å, molecular mass from 200-400 (x 10^3) daltons, and density from 1.063-1.21 g/ml (Krauss, 1982). The increased density of HDL relative to other lipoproteins is attributed to the greater protein content, which ranges from 40 to 60% of HDL mass, with reciprocal variations in the percent contribution of lipids including phospholipids (PL), cholesterol esters (CE), unesterified cholesterol (UC), and triglycerides (TG). Apolipoproteins (apo) A-I and A-II constitute the major protein constituents and are found almost exclusively in the HDL fraction in normal fasting plasma. Apo E and apo C (C-I, C-II, C-III) are minor protein constituents of HDL, and also are

associated with other lipoproteins. The enzyme lecithin:cholesterol acyl transferase (LCAT) and lipid transfer proteins are important protein constituents, and are predominantly responsible for the intense metabolic activity associated with HDL.

HDL include a heterogenous collection of particles, the bulk of which can be separated into discrete molecular entities of characteristic size, density, and composition (Krauss, 1982; Musliner and Krauss, 1988). By analytical ultracentrifugation, HDL appears as a spectrum of particles with flotation (F) rates ranging from 0 to 9 which is often bimodal in distribution with particles clustering at F rates between 0 and 3.5 and 3.5 and 9. This bimodality distinquishes the two major HDL subclasses, HDL_3 and HDL_2 respectively, which also can be separated by preparative ultracentrifugation at d=1.125 g/ml (Krauss, 1982; Musliner and Krauss, 1988). The larger, more bouyant HDL_2 particles are characterized by a relative enrichment of cholesteryl esters (~50% more than HDL_3) and the presence of an additional molecule of apo A-I (Eisenberg, 1985). Distinctions between HDL_2 and HDL_3 are important not only from a physicochemical standpoint but also with regard to CAD risk as indicated by several studies which show that HDL_2 levels are somewhat more predictive of risk than HDL_3 (Miller, 1987A). Subspecies within these two subclasses have recently been identified by density gradient ultracentrifugation and gradient polyacrylamide gel electrophoresis, including at least two HDL_2 (Anderson et al, 1978) and three HDL_3 subspecies (Blanche et al, 1981). HDL_3 heterogeneity also has been demonstrated by zonal ultracentrifugation (Patsch et al, 1980). A third relatively minor subclass, designated HDL_1, has been separated on the basis of density (Albers et al, 1972). This subclass floats at d<1.063 overlapping with the low density lipoprotein distribution.

Further sources of HDL heterogeneity are indicated by other techniques, including column chromatography (Kostner and Holasek, 1977) and isoelectric focusing (Mackenzie et al, 1973). Subfractionation of HDL particles on the basis of apoprotein composition has recently been accomplished. HDL subspecies containing apo A-I without apo A-II (designated Lp A-I) are separated from HDL containing apo A-I and apo A-II (Lp A-I:A-II) by immunoaffinity chromatography (Cheung and Albers, 1984; Cheung et al, 1988). These two HDL species exhibit distinct

metabolic characteristics (Barbaras et al, 1987; Radar et al, 1991), and appear to be differentially predictive of CAD risk (Brunzell et al, 1984; Koren et al, 1987; Maciejko et al, 1983; Miller, 1987A). An apo E-containing HDL subspecies (HDL_c) within the HDL_2 distribution has been isolated from cholesterol-fed animals, and has been shown to exhibit distinct metabolic properties (Mahley et al, 1975; 1978). However, in general, the biological roles and cardiovascular significance of HDL subspecies remains unclear.

3. Clinical Measurement of HDL

Clinical quantification of HDL is typically accomplished by measurement of one or more of its chemical constituents following separation from the other lipoprotein classes. Although numerous approaches are utilized to separate lipoproteins for research purposes, most are too cumbersome for routine clinical use (see Warnick and Dominiczak, 1990). Sequential density centrifugation, which separates the three major lipoprotein classes in fasting plasma (VLDL, LDL, and HDL) on the basis of hydrated density, has been a cornerstone in research laboratories. However, centrifugal separations are laborious and technically demanding, and require expensive instrumentation not available in many clinical laboratories. It has now become commonplace to use chemical precipitation, either in conjunction with centrifugation or alone, as a means of separating lipoproteins for quantification. Lipoproteins can be precipitated directly from plasma or serum by addition of a polyanion (heparin, dextran sulfate, sodium phosphotungstate) and a divalent cation (Mn^{2+}, Mg^{2+}). Using incremental additions and/or various combinations of these agents, procedures have been developed to sequentially precipitate apo B-containing lipoproteins (VLDL, IDL, LDL, and Lp(a)) and HDL_2, leaving total HDL and HDL_3, respectively, in the supernatant for quantification (Bachorik, 1989; Gidez et al, 1982; Sjoblom et al, 1989; Warnick and Dominiczak, 1990). Estimates of total HDL and HDL subclass concentrations obtained by this approach are highly correlated with those obtained using centrifugal (Gidez et al, 1982; Patsch et al, 1989) or other techniques (Sjoblom et al, 1989).

Once separated, lipoproteins are usually quantified by measuring associated cholesterol. Enzymatic cholesterol determinations are convenient, and are reasonably accurate and precise when

appropriate quality control measures are performed (Bachorik, 1989; Warnick et al, 1990). However, use of cholesterol as a means of evaluating HDL (and other lipoproteins) has several inherent problems. Error due to incomplete separation can be potentiated by reliance on cholesterol as the means of quantification. Whereas cholesterol contributes ~15% and ~50% of total HDL and LDL mass, respectively, any cross-contamination with LDL could lead to gross overestimates of HDL. Additionally, cholesterol measures fail to account for variations in lipoprotein composition which are independent of variations in total lipoprotein mass or particle number. Nonetheless, since most epidemiological studies relating lipoproteins to CAD risk have been based on lipoprotein cholesterol and therapeutic action levels of the National Cholesterol Education Program (NCEP) were developed accordingly, HDL-C levels are currently the most appropriate means of HDL quantification for the clinician.

More recently, methods for measurement of apolipoproteins have been developed, and numerous studies have suggested that apolipoproteins are independent markers of CAD risk (Miller, 1987A). When analyzed in conjunction with lipoprotein cholesterol, apolipoprotein measurements provide information regarding lipoprotein particle size, and thus are useful for identifying several metabolic syndromes not apparent from cholesterol levels alone (Brunzell et al, 1983; Grundy et al, 1987; Sniderman et al, 1980). Apolipoproteins are measured using various immunochemical techniques which can be performed directly on plasma, thereby circumventing problems inherent lipoprotein separations. However, differences exist among methods in use with respect to technical ease, sensitivity, and susceptibility to variations in reagent and sample characteristics (see Albers et al, 1989). As a result, considerable variations in apolipoprotein estimates exist across laboratories (Smith et al, 1987). Efforts are currently being directed towards national and international standardization of apolipoprotein measurements and identification of age- and race-specific population-based reference values (Albers et al, 1989; Albers and Marcovina, 1989). Until such time, apoprotein estimates have limited clinical applicability.

Since small errors in lipoprotein estimates can result in misclassification of individual risk status, lipoprotein determinations should be performed in competent laboratories which participate

in appropriate standardization programs. To reduce misclassification due to methodological errors as well as intraindividual variations, multiple measurements should be performed on a single individual. In addition, attention should be given to minimizing other sources of variability by standardizing sample collection, processing and storage (see Bachorik, 1989).

4. HDL Metabolism

Multiple pathways have been identified for the synthesis, maturation and catabolism of HDL particles. This multiplicity contributes a metabolic flexibility and responsiveness which may be essential to the key role of HDL in plasma lipid metabolism.

A. Metabolic Origins of HDL

HDL apolipoproteins originate in both the liver and small intestine, and are secreted free or complexed with lipid in the form of 'nascent' HDL or in association with TG-rich lipoproteins (chylomicrons and VLDL) (Eisenberg, 1985). HDL particles are formed post-secretion upon interaction of free apolipoproteins and phospholipids, possibly in the aqueous phase, and release of lipid-apolipoprotein complexes from the surface of TG-rich lipoproteins. Free fatty acids may promote the formation of nascent HDL complexes on the lipoprotein surface (Musliner et al, 1991). Newly formed HDL appear as either spherical or discoidal particles (Green et al, 1976). The former are suggested to represent HDL which is produced intracellularly and secreted intact, and evidence of the presence of these particles in secretory organelles of hepatocytes and enterocytes has been demonstrated (Forester et al, 1983). Discoidal particles comprise lipid-poor complexes which arise from extracellular assembly and can be distinquished from spherical HDL by the absence of core lipids (i.e., CE). Maturation of nascent HDL particles involves uptake of UC with its conversion to CE by HDL-associated LCAT activity. These molecules are subsequently displaced to the hydrophobic domain of the PL bilayer forming the lipoprotein core. The central role of LCAT in discoidal to spherical transformations is indicated by the lack of mature HDL in patients with familial LCAT deficiency (Glomset and Norum, 1973).

B. Intravascular Remodeling of HDL

HDL lipids and apolipoproteins represent dynamic pools characterized by continuous entry followed by metabolic consumption or exchange, and thus, the HDL particle is subject to continuous modification while in circulation. Through the remodeling process, HDL serves as a repository of lipids and apolipoproteins from chylomicrons and VLDL, which appear to contribute the bulk of HDL mass (Eisenberg, 1985). Cells also serve as sources of HDL lipids, primarily UC, which is transferred to HDL in association with a process known as "reverse cholesterol transport". While in many cases this does not lead to net changes in lipoprotein composition, augmentation or shifts may occur, including those involved in the interconversions among HDL subclasses.

1. Interactions of HDL with Other Lipoproteins and Cells

TG-rich lipoproteins donate surface lipids to HDL during lipolysis. Net transfer of PL to HDL is driven by PL consumption, predominantly via the LCAT reaction, and to a lesser extent by the phospholipase activity of hepatic and lipoprotein lipase (HL and LPL) (Eisenberg, 1985; Krauss, 1982). PL exchange without net mass transfer may be facilitated by plasma PL exchange protein. HDL UC is derived from VLDL as well as LDL and may involve bidirectional exchange or unidirectional transfer with net mass cholesterol movement to HDL. Net cholesterol transfer is driven by HDL-associated LCAT activity, producing CE with the subsequent transfer of these molecules to the hydrophobic core. HDL TG also are contributed by TG-rich lipoproteins. HDL TG flux appears to be determined by the relative amounts of this component in HDL and donor lipoproteins. The fate of HDL TG is unknown although TG-enriched HDL may be subject to hydrolysis with removal of TG and PL by HL.

The contribution of cells to HDL PL turnover appears to be minor as indicated by the disparity between HDL and cell membrane PL composition. In contrast, cells are important donors of HDL UC. In addition to serving as a cholesterol acceptor, HDL plays a regulatory role in cellular cholesterol efflux by stimulating the translocation of intracellular cholesterol to an exchangable plasma membrane domain (Schroeder et al, 1991; Slotte et al, 1987). Both mobilization and transfer of cellular cholesterol to HDL are enhanced by its binding to specific cell surface

receptors (Oram et al, 1991). Stimulation by HDL appears to be mediated by a signal transduction process involving protein kinase C, as indicated by the ability of sphingosine to inhibit this process (Mendez et al, 1989).

HDL also may serve as lipid donors. Net mass transfer of CE from HDL to TG-rich lipoproteins and LDL is mediated by cholesteryl ester transfer protein (CETP) and involves exchange with TG (Barter et al, 1990). While CETP also may promote cellular uptake of HDL CE (Granot et al, 1987), this effect appears to require intermediate transfer of CE to LDL. HDL also may donate UC and PL under certain conditions. For example, HDL UC may be transferred to cells which consume high amounts, such as cells of steroidogenic tissues or liver cells for use in bile acid synthesis (Eisenberg, 1985; Krauss, 1982).

Although HDL apolipoproteins are not static, these particles generally remain associated with the HDL fraction while in circulation (Eisenberg, 1985). Apo A-I has been shown to exchange between HDL particles. This is suggested to involve dissociation from and reassociation with HDL particles, with intermediate residence in a rapidly turning over plasma 'free' apolipoprotein pool. HDL apolipoproteins appear to possess an avidity for the HDL surface, which may be related to the surface curvature, and are less likely to associate with other lipoproteins. Displacement of apo A-I by other apolipoproteins, such as apo A-II, apo C and apo E, also occurs. HDL serves as a repository for the latter two groups of apolipoproteins, with apo C-II providing a signal for lipolysis upon transfer to TG-rich lipoproteins.

2. Interconversions Among HDL Subclasses

Interconversions among HDL subclasses involve lipid and protein uptake or loss with concomitant enlargement or reduction of the HDL particle (Eisenberg, 1985). HDL enlargement occurs upon influx of PL and UC from TG-rich lipoproteins during lipolysis, UC from cell membranes, and apolipoproteins released from TG-rich lipoproteins and possibly directly from cells (Deckelbaum et al, 1982A). Conversion of HDL_3 to HDL_2 involves a 2- to 3-fold enrichment of core CE molecules, and addition of sufficient surface lipids to accommodate the expanded surface area as well as an additional apo A-I molecule. This process is suggested to occur in two

stages (Eisenberg, 1985). Conversion of HDL_3 to a lipid-enriched intermediate species, which is subsequently transformed into HDL_2 upon production of CE by LCAT. An apo A-I molecule associates with the lipid-enriched particle surface at some point in this process, and appears to accelerate CE accumulation. Intravascular conversion of HDL_2 to a larger particle, HDL_1, may occur as a result of further accumulation of CE and enrichment with apo E.

Evidence indicates that transitions from larger to smaller HDL species also occur (Deckelbaum et al, 1982B). Conversion of HDL_2 to HDL_3 is envisioned as a two-stage process with CETP-mediated exchange of HDL CE for TG. The TG-rich intermediate is a substrate for HL, which catalyzes the removal of both TG and PL (i.e., both core and surface lipids) (Hopkins et al, 1985; Hopkins and Barter, 1986). HDL_2 to HDL_3 conversion also must involve exclusion of an apo A-I molecule.

C. Plasma Clearance and Catabolism of HDL and HDL Constituents

An understanding of HDL catabolism is complicated by the dynamic nature of HDL constituent pools, each exhibiting unique kinetic parameters which do not reflect the kinetic behavior of the entire lipoprotein particle. As a result, none of the constituents can serve as a stable marker of catabolism of the HDL particle. This situation is further encumbered by multiple mechanisms for degradation of HDL constituents, which vary among cell types and are affected differentially by numerous metabolic factors.

Cholesterol and PL from TG-rich lipoproteins and cells cycle through the HDL system, being converted to CE by LCAT, on route primarily to lower density lipoproteins from which greater than 80% of HDL-synthesized CE can be recovered (Nestel et al, 1979). The HDL CE pool completely turns over within about 24 hours (Glomset and Norum, 1973). The LCAT reaction utilizes an equivalent of 5-10 times the UC mass and essentially the total PL mass (i.e., 5-10 mmol) for this process, although with little effect on the HDL mass of these lipids due to continual replacement. In contrast, apolipoproteins do not undergo metabolic alterations and remain associated with the HDL pool while in circulation (Krauss, 1982). Thus, apolipoprotein kinetic behavior is suggested to provide a reasonable approximation of HDL turnover rates (Schaefer et al,

1978), although degradation of these constitutents may occur independently of intact HDL. HDL-associated lipoproteins exhibit a plasma half-life of several days with a greater plasma residence time noted for apo A-I. In a recent study, Rader et al (1991) showed that the plasma residence time of apo A-I associated with Lp A-I was substantially shorter than that associated with Lp A-I:A-II. Evidence is inconclusive regarding major tissue sites for apolipoprotein uptake and degradation.

Several examples of cellular uptake and degradation of intact HDL particles have been presented, although sites and mechanisms involved and the extent to which this process contributes to HDL turnover are poorly understood (Bradley and Gianturco, 1990). Examination of the accumulation of labelled HDL constituents suggests that liver and intestines are primary sites of HDL catabolism, although several reports have indicated a more modest hepatic contribution (Krauss, 1982). Hepatocytes isolated from rats and humans have a high capacity for HDL uptake and degradation (Nakai et al, 1976; Van Berkel et al, 1977; Shouten et al, 1989). Uptake and degradation of apo E-containing HDL_2 subspecies by a saturable process has been attributed to the interaction of these particles with receptors recognizing apolipoprotein B (Mahley et al, 1978). Shouten et al (1989) reported saturable high-affinity, binding and uptake of apo E-free HDL by hepatocytes which did not compete with LDL but did compete with VLDL binding suggesting a possible role for apo C. Tozuka and Fidge (1989) recently reported purification of two structurally distinct proteins from rat and human liver plasma membrane which recognize apo A-I and A-II. The role of these proteins in HDL turnover is presently unknown.

HDL bind to other cell types, and in some cases, evidence of uptake and degradation of intact particles has been presented. Rat aortic smooth muscle cells, human fibroblasts, and cultured rat adrenocortical cells are capable of binding and internalizing intact HDL, although in the latter tissue, preferential uptake of cholesterol for sterol synthesis without entry of the HDL particle may occur (see Krauss, 1982). HDL binding without internalization leading to partial degradation appears to represent an important means of clearance of HDL constituents (Pittman et al, 1987).

5. Regulation of Plasma HDL Concentrations

Levels of HDL and distributions among HDL subclasses reflect the interplay between synthesis and/or catabolism of HDL and HDL constituents and processes involved in HDL maturation and remodeling in plasma. The extent to which HDL and HDL subclass levels vary among and within individuals will depend on the relative contributions of these processes to HDL metabolism, and factors which influence HDL levels will operate through effects on these processes.

A. Metabolic Considerations

Rates of synthesis and catabolism of HDL apolipoproteins determine the amounts of these constituents available for formation of HDL. Although several exceptions have been presented, catabolic rates appear to exert greater control over plasma apolipoprotein and HDL levels than synthetic rates. The latter may be more important in determining responses to environmental factors, such as dietary fat intake. Regulation of plasma levels by catabolic rates is true primarily for apo A-I, whereas recent evidence in normolipemic females has shown that plasma apo A-II levels are correlated with the synthetic rate of this apolipoprotein (Brinton et al, 1989). Notable differences in the influence of apolipoprotein synthesis versus catabolism also exist among normolipemic and hypertriglyceridemic individuals. Reduced apo A-I synthetic rates have been observed in normolipemic subjects with low apo A-I and HDL C, as opposed to increased fractional catabolic rates (FCR) for apo A-I in hypertriglyceridemic subjects with low apo A-I and HDL C (Le and Ginsburg, 1988). Apolipoprotein kinetics also may influence HDL subclass distributions (Eisenberg, 1990). HDL apolipoprotein FCR have been shown to be associated with the degree of HDL lipid enrichment. Several molecular mechanisms are suggested to govern apolipoprotein turnover. In addition to direct regulation of synthesis and secretion, apolipoprotein catabolism may occur secondary to HDL lipid metabolism (Goldberg et al, 1989; see Tall, 1990).

HDL formation and remodeling are inexorably tied to the metabolism of TG-rich lipoproteins and, thus, HDL and HDL subclass levels are highly influenced by factors which affect the efficiency of TG hydrolysis, as indicated by the inverse relationships between plasma HDL and the concentrations (Chang et al, 1985), FCR, and metabolic fluxes of plasma triglycerides (Krauss,

1982). During hydrolysis of TG-rich lipoproteins, surface lipids and apo A-I are transferred to HDL. TG hydrolysis is catalyzed by LPL, and the activity of this enzyme in adipose tissue, skeletal muscle, and post-heparin plasma has been shown to correlate with plasma levels of HDL-C (Krauss, 1982). Plasma LPL levels also are correlated with HDL_2-cholesterol and HDL_2 mass, reflective of the possible conversion of HDL_3 to HDL_2 upon transfer of TG-rich lipoprotein surface lipids to HDL. Tissue LPL activity may be regulated at the transcriptional, translational, and post-translational level, and these processes may be variously affected by constitutive and environmental factors (Eckel, 1989; Olivecrona and Bengtsson-Olivecrona, 1990).

In contrast to LPL which acts on chylomicra and VLDL, hepatic lipase (HL) catalyzes the hydrolysis of both TG and PL in HDL and appears to play a major role in degradation of HDL constitutents, particularly those in HDL_2 (Clay et al, 1989). The activity of this enzyme is inversely correlated with levels of HDL and HDL_2, and is reduced in several conditions characterized by elevated HDL_2 levels (Krauss, 1982; Kuusi et al, 1989).

LCAT and CETP are two additional enzymes which play a role in the regulation of HDL and HDL subclass levels. LCAT, which is activated by apo A-I (Fielding et al, 1972), promotes CE enrichment of HDL. Smaller HDL particles are preferred substrates for LCAT and may be converted to HDL_2 by the activity of this enzyme (Barter et al, 1985). CETP promotes exchange of HDL CE for TG facilitating the redistribution of CE from HDL to VLDL and LDL. This leads to CE depletion and TG enrichment of HDL, and in the presence of HL which hydrolyzes HDL TG, results in a reduction in HDL particle size (Barter, 1990; Newnham and Barter, 1990). The ability of CETP to promote lipid transfers and changes in HDL particle size may be modulated by free fatty acids (Barter et al, 1990). The influence of CETP on HDL and HDL subclass levels is reflected in humans with an inherited CETP deficiency who exhibit markedly increased concentrations of HDL-C and enlarged CE-enriched and TG-depleted HDL particles (Brown et al, 1990).

B. Factors Influencing Plasma HDL and HDL Subclass Levels

Plasma levels of HDL and HDL subclasses are affected by numerous intrinsic or constituitive factors including genetic predisposition, gender and age, and extrinsic or environmental

factors such as dietary and exercise habits. Interactive effects have been noted, particularly between constituitive and environmental factors. Although all of these factors are expected to operate through the mechanisms described above, those operating under specific circumstances are still poorly defined.

1. Intrinsic Factors

Genetic Predisposition. Several relatively rare lipid disorders affecting HDL are genetically determined, including Tangier's disease, CETP and LCAT deficiency disorders, and familial hypercholesterolemia, and in some cases specific gene alterations have been identified (Thompson et al, 1989). However, the mild to moderate lipid and lipoprotein perturbations encountered in human populations have yet to be attributed to specific genetic factors. Nonetheless, a major role for heredity in determining intraindividual differences in plasma HDL-C concentrations and apo A-I levels has been indicated by numerous studies examining correlations of these variables between parents and offspring and among siblings (Moll et al, 1986; McGue et al, 1985; Burns et al, 1989; Namboodiri et al, 1985; Rao et al, 1987). Familial HDL transmission involves specific HDL sub-classes, with higher heritability estimates for HDL_2 than HDL_3 (Hasstedt et al, 1985; Kuusi et al, 1987). Both genetic and cultural heritability have been implicated, albiet with a greater contribution from the former (McGue et al, 1985; Hasstedt et al, 1984). Proposed genetic transmission schemes for HDL-C and apoplipoprotein levels variously include polygenic, additive major genes, recessive and dominant (Amos et al, 1986; Hasstedt et al, 1984; Moll et al, 1986).

Gender. Premenopausal females exhibit higher HDL-C levels than males, with elevations of the HDL_2 subclass (Nichols, 1967), absolute levels of apo A-I and the ratio of apo A-I to apo A-II (Cheung and Albers, 1977). Eunuchs have higher HDL-C levels than normal adult males (Furman et al, 1956), and in longitudinal studies of adolescent males, reductions in HDL-C accompany sexual maturation (Morrison et al, 1979). These gender differences are attributed to hormonal influences and evidence of the direct effects of estrogens and androgens has appeared. Exogenous estrogen leads to elevations in plasma HDL-C and HDL_2-C concentrations (Nichols, 1967; Krauss et al, 1979; Furman et al, 1967; Schaefer et al, 1983; Tikkanen et al, 1981). Conversely, androgen

administration lowers HDL-C concentrations in both hypogonal and normal men (Furman et al, 1958; Furman et al, 1967; Webb et al, 1984). While several cross-sectional studies have shown a positive association between testosterone and HDL-C concentrations in mature males (Nordoy et al, 1979; Gutai et al; 1981; Heller et al, 1981), this may actually be reflective of an underlying metabolic relationship between HDL and sex hormone-binding globulin (SHBG), which is related to testosterone levels (Stefanick et al, 1987A). HDL-C raising effects also have been suggested for follicle-stimulating hormone (FSH) and luteinizing hormone (LH) based on strong correlations between plasma levels of these hormones and HDL-C concentrations in post-menopausal women irrespective of estrogen administration (Krauss et al, 1979A).

Metabolic events responsible for gender differences appear to involve apolipoprotein kinetics. Synthetic rates of apoproteins A-I and A-II are significantly greater in females than males. Schaefer et al (1982) reported respective values of 13.6 and 2.5 mg/kg/day for females and 11.1 and 2.1 mg/kg/day for males. Estrogen administration causes a 25% increase in the synthesis of apo A-I, but not apo A-II, in premenopausal females (Schaefer et al, 1983). In addition, higher activites of LPL and lower activities of HL have been observed in females (Krauss, 1982). Administration of estrogen reduces HL activity in post-heparin plasma and this is accompanied by elevations in HDL_2-C concentrations (Applebaum et al, 1977; Tikkanen et al, 1981).

Age. Population studies have indicated that HDL-C levels vary with age, with notable differences in age-effects among men and women (Heiss et al, 1980). In males populations, reductions in HDL-C levels occur during puberty and adolescence, followed by a steady decline through middle age (ages 55-60), after which an increase is noted. The latter may reflect an underrepresentation of older-aged individuals with low HDL-C levels due to cardiovascular mortality. In contrast, women exhibit a small, steady increase in HDL-C from pubescence through about age 60, which may be related to observations of linear increases in levels of LPL through the sixth decade in females (Krauss et al, 1974).

Adiposity and Fat Distribution. An inverse association between adiposity and HDL, HDL_2 and HDL_3 cholesterol levels has been observed in both children and adults (Aristimuno et al, 1984;

Lupien et al, 1985; Foster et al, 1987; Anderson et al, 1988; Despres et al, 1988; Terry et al, 1989). Individuals with centralized or upper body (android) obesity as compared with lower body (gynoid) obesity exhibit more marked dyslipoproteinemias including reduced HDL levels and hypertriglyceridemia (see Stern and Haffner, 1986). Measurements of upper body obesity (e.g., abdominal skinfold thickness, waist-to-hip ratio) are more strongly predictive of plasma HDL and HDL subclass levels than body weight or body mass index (Foster et al, 1987; Anderson et al, 1988; Despres et al, 1988; Terry et al, 1989). Both androgen excess and insulin resistance are suggested to mediate lipid and lipoprotein abnormalities including reduced HDL levels associated with upper body obesity (Stern and Haffner, 1986). Women exhibiting primarily upper body obesity have increased levels of free testosterone and SHBG relative to women of similar weight with predominantly lower body fat deposition (Evans et al, 1983). Upper body obesity is suggested to predispose to diabetes (Ohlson et al, 1985), a well-known CAD risk factor, and several reports have shown that subjects with upper as opposed to lower body obesity are more insulin-resistant (Stern and Haffner, 1986). Insulin-resistance has recently gained prominence as an important independent predictor of HDL levels (Laws et al, 1991; Laws and Reaven, 1992).

2. Extrinsic Factors

Diet. In addition to elevating atherogenic lipoproteins, high cholesterol intake increases plasma HDL-C and HDL_2-C, and elicits the formation of an apo E-containing HDL subspecies (HDL_c) which exhibits increased cell surface binding (Mahley et al, 1975, 1978; Beynen and Katan, 1985). High-fat diets enriched in saturated fatty acids (SFA) have been shown to raise HDL-C and apo A-I levels, and this may be related to observed increases in hepatic apo A-I synthesis (Tan et al, 1980). Isocaloric substitutions of SFA with either polyunsaturated fatty acids (PUFA) or carbohydrates bring about reductions in plasma HDL-C, HDL_2-C and apo A-I levels (Schaefer et al, 1981; Zanni et al, 1987; Enholm et al, 1982), although the HDL-lowering effects of PUFA substitution may not occur when total fat is relatively low (Dreon et al, 1990). Turnover studies have indicated that changes in the synthesis or fractional catabolic rate of HDL-associated apolipoproteins may account for reductions in HDL with PUFA or carbohydrate enrichment, respectively (Blum et al, 1977; Chong et al, 1987; Nestel et al, 1981; Brinton

et al, 1990). Thus, although beneficial effects have been noted with respect to total-C and LDL-C levels, assumption of PUFA-enriched or low-fat, high-carbohydrate diets produce less than optimal changes in plasma HDL.

Attention has recently focused on the possible cardiovascular benefits of monounsaturated (MUFA) and ω–3 fatty acids. When substituted for SFA within moderate to high fat diets, MUFA bring about reductions in plasma total- and LDL-C without concomitant reductions in HDL-C (Mattson and Grundy, 1985; Grundy, 1986; Mensink and Katan, 1987; Grundy et al, 1988). In contrast to ω–6 fatty acids in vegetable oils, ω–3 fatty acids in fish oils exert a mild HDL-C-elevating effect when moderate amounts are consumed (see Harris, 1989). This may be related to alterations in TG metabolism. Some reports have shown fish oil-induced reductions in HDL-C, albiet generally at higher intakes, and increased HDL turnover has been noted.

Total calorie intake, fiber and coffee consumption, and dietary carbohydrate and protein composition also are suggested to influence HDL levels. The effects of these and other dietary constituents on plasma lipid and lipoproteins including HDL are detailed in a recent review (Kris-Etherton et al, 1988).

Exercise. Numerous epidemiological studies have indicated a strong association between plasma levels of HDL-C, particularly HDL_2-C, and habitual physical activity level (see Krauss, 1989; Wood et al, 1985; Pelletier and Baker, 1987). Adoption of a moderate to strenuous exercise program leads to elevations in HDL in association with reduced TG levels, although such effects have been reported consistently only for longer-term studies (i.e., greater than 3 months) and with activity regimens unlikely to be achieved by most individuals. In many studies, exercise-induced elevations in HDL and HDL subfractions have been attributed to accompanying losses of body weight and/or fat mass (see Williams, 1990; Wood et al, 1988). Elevated adipose tissue LPL levels with weight loss may be involved in this relationship (Schwartz and Brunzell, 1981). Modest elevations in HDL and LPL activity in post-heparin plasma have been observed with exercise training in the absence of weight loss (Thompson et al, 1988), and elevations in HDL have been noted following short-term exercise (Kantor et al, 1987), indicating that exercise *per se*

can influence HDL metabolism. Measurement of HDL arteriovenous flux across exercising muscle have shown acute elevations in HDL_3 (Ruys et al, 1989). This response occurs in association with transient, localized elevations in muscle LPL activity (Kiens et al, 1989), and is dependent on the delivery of chylomicra and VLDL to LPL in the muscle vasculature (Kiens and Lithell, 1989; Ruys et al, 1989). Accordingly, intravenous fat clearance is greater after an exercise session (Annuzzi et al, 1987), and exercise-induced elevations of HDL-C have been shown to be greater after fat ingestion and in individuals consuming moderate- to high-fat diets (Ruys et al, 1989). Alterations in the clearance of HDL constituents resulting from lower levels of HL also may contribute to elevated HDL-C and HDL_2-C levels associated with regular physical activity (Krauss et al, 1979B; Stefanick et al, 1987B).

Alcohol. HDL elevations may be responsible for the reduced CAD risk associated with low to moderate alcohol intake (Crique et al, 1987). Alcohol raises plasma levels of HDL-C and apos A-I and A-II (Kannel et al, 1988), and epidemiological associations indicate a relationship between alcohol intake and HDL-C and apo A-I and A-II levels (Meilahn et al, 1988). In the LRC prevalence study, alcohol consumption was reported to account for as much as 4 to 6 % of the population variance in HDL-C levels (Ernst et al, 1980). Alcohol effects have been suggested variously to involve predominantly either HDL_3 or HDL_2 (Kris-Etherton et al, 1989). Elevated LPL activity has been noted with chronic alcohol intake and may be involved in elevating HDL-C (Belfrage et al, 1977). A possible role for alterations in hepatic synthesis of HDL constituents also has been suggested (Krauss, 1982). Despite the beneficial effects of alcohol on HDL levels and CAD risk, recommendations to increase consumption are contraindicated by the well-known adverse effects of this agent. Further, sustained heavy alcohol intake can induce dyslipoproteinemias, such as hypertriglyceridemia in susceptible subjects, which may increase CAD risk (Clark et al, 1988).

Smoking. Cigarette smoking is associated with an increased ratio of total- to HDL-C and reduced plasma HDL levels (Craig et al, 1989). The effect of smoking on HDL levels appears to be dose-dependent (Criqui et al, 1980) and is more pronounced in women (Bush et al, 1988).

Although smoking affects HDL levels independently of other factors, this relationship is confounded by differences in body fat mass, alcohol consumption, and dietary intake among smokers and nonsmokers (Simons et al, 1984; Quensel et al, 1989). Elevations in HDL-C levels have been noted within thirty days after smoking cessation (Mofatt, 1988; Quensel et al, 1989), although a recent report suggests that this may be partially attributable to accompanying changes in dietary habits (Quensel et al, 1989).

Drugs. Numerous drugs in common clinical use have been shown to influence HDL levels. Examples include steroid hormones with estrogenic, progestogenic, or androgenic activity, some of the effects of which were discussed in a previous section. For a detailed discussion of inadvertant drug-induced alterations in plasma lipids and lipoproteins including HDL, the reader is referred to a recent review by Henkin et al (1992).

Pharmacological treatments specific for dyslipidemias are designed for lipid-lowering (e.g., of hyperbetalipoproteinemia), although several are additionally effective in raising HDL levels. HDL elevations by various lipid-lowering agents in common use range from 5-30% (Tall, 1990). Nicotinic acid exerts the greatest and most consistent effects on HDL, which occur in association with decreased FCR and increased synthesis rates for apo A-I and apo A-II (Blum et al, 1977; Krauss, 1982; Miller, 1990). The effects of nicotinic acid are noted primarily within the HDL_2 subclass (Paoletti et al, 1983). Gemfibrozil results in fairly substantial elevations in HDL by increasing the synthesis of apo A-I and the activity of LPL (Glueck, 1983A). The latter property also has been demonstrated for fibrates (e.g., clofibrate and bezafibrate) which produce more modest HDL elevations concomitant with reductions in VLDL (Glueck, 1983B). Inhibition of endogenous cholesterol synthesis by HMG CoA reductase inhibitors (e.g., lovastatin) also reduces VLDL and TG and modestly elevates HDL-C levels. Modest HDL elevations have been acheived inconsistently with bile acid resins such as cholestyramine (Glueck, 1983B). In contrast, consistent and substantial reductions in HDL levels have been observed with Probucol, a drug with LDL-reducing properties, which has consequently been relegated to second line status (Glueck, 1983B; Miller, 1990). While most of these pharmacological treatments beneficially alter HDL,

most are associated with untoward side effects which prohibit their liberal use, and current NCEP guidelines do not advocate such treatments for individuals with low HDL levels independent of other lipid or lipoprotein abnormalities.

6. Epidemiological Evidence of a Relationship between HDL and Coronary Artery Disease

Numerous case-control and epidemiologic studies have indicated an inverse association between plasma HDL levels and cardiovascular disease endpoints in Western populations which generally remains significant following covariance adjustment (e.g., for TG levels, body mass index) (see reviews by Miller, 1987A; Gordon et al, 1989). Relative to age- and sex-matched controls, survivors of myocardial infarction (MI) exhibit significantly reduced levels of total HDL-C and often either or both HDL_2-C and HDL_3-C. In prospective studies, these parameters also have been shown to predict rates of future disease events, with a 1 mg/dl increment in HDL-C associated with a 2- to 3-percent decrement in risk (Gordon et al, 1989; Stampler et al, 1991). A greater percent decrease in risk has been noted among women, for whom HDL-C levels appear to be a more prominent CAD risk factor (see review by Bush et al, 1988).

In some studies, the HDL_2 subclass has been shown to be more predictive of risk than HDL_3 (Laakso et al, 1985; Hamsten et al, 1986; Miller, 1987A; Wallentine and Sundin, 1985), although significant associations between CAD and levels of HDL_3 also have been noted (Buring et al, 1992; Levy et al, 1984; Kempen et al, 1987). In the prospective Physician's Health Study, Stampfer et al (1991) found that although HDL-C and HDL_2-C predicted risk of myocardial infarction (MI) in men, HDL_3-C levels appeared to be the best risk indicator of the three. The basis for such discrepancies may become obvious with more precise characterization of subspecies within the HDL_2 and HDL_3 subclasses. For example, recent evidence suggests that the HDL_{3b} component may actually be associated with increased CAD risk (Wilson et al, 1991; Williams et al, in press), and thus unmeasured variations in this component may underlie conflicting results regarding the relative CAD predictive capacity of HDL_2 and HDL_3 obtained in earlier studies.

Measures of HDL-associated apolipoproteins appear to be useful for evaluating CAD risk, although it is unclear whether and the extent to which these are superior to HDL-C values (Miller,

1987A). Lower levels of apo A-I have been reported consistently in MI survivors, and in longitudinal studies have been found to predict future events. Results regarding apo A-II have been conflicting, with several reported observations of an inverse and no relationship of apo A-II levels to heart disease (Miller, 1987A). In a recent study, Lp A-I but not Lp A-I:A-II levels were shown to be inversely correlated with arteriographically proven CAD (Puchois et al, 1987). In the prospective Physician's Health Study, both HDL-associated apolipoproteins were found to be associated inversely with decreased risk of MI (Stampfer et al, 1991), although neither of these measurements added to the ability of standard risk factors and the ratio of total-C to HDL-C to predict risk. Conversely, Buring et al (1992) recently reported that apos A-I and A-II substantially added to the predictive information provided by standard lipid and lipoprotein measurements based on a case-control study of patients with a first MI versus neighborhood controls.

7. Direct Evidence of a Relationship between HDL and Coronary Artery Disease

Direct experimental testing of a protective role for HDL in humans has been precluded by the inability to manipulate HDL levels without simultaneously influencing other factors which may play a role in atherosclerosis. There is evidence, however, from studies of genetic variants in humans and animals, and from pharmacologic studies in humans, that HDL may reduce CAD independently of other known risk factors.

A. Animal models

Several major genetic loci have been identified in mice which appear to influence susceptibility to development of aortic and coronary atherosclerosis on high-fat, high-cholesterol diets supplemented with cholic acid (Ishida et al, 1989). One such locus, designated *ath-1*, has been mapped to a chromosomal site near the apo A-II gene (Paigen et al, 1987A,B). Atherosclerosis susceptibility in affected mouse strains is strongly associated with reduced levels of HDL on high-fat diets, whereas resistant strains have normal HDL levels, and comparable levels of apo B-containing lipoproteins (Paigen et al, 1987A,B; LeBeoeuf et al, 1990). A second gene determining atherosclerosis susceptibility and HDL levels in mice *(ath-2)*, also has been described by Paigen at al (1989). Recently, Rubin et al (1991A) generated a transgenic strain of atherosclerosis-suscep-

tible C57/BL6 mice expressing human apo A-I. These animals have approximately twice-normal levels of HDL which are similar in size to human apo A-I-containing HDL subclasses. HDL levels remain elevated on high-fat diets, and the extent of aortic fatty streaks is markedly reduced in comparison with nontransgenic littermates (Rubin et al, 1991B). Metabolic studies in other mouse strains expressing similar levels of human apo A-I have demonstrated increased apo A-I production rates (Rubin et al, 1991B). Thus, while further studies will be required to establish the relevance of these studies to CAD in humans, it appears that increased synthesis of human apo A-I in this genetic model directly retards atherogenesis.

B. Human genetic diseases

Premature atherosclerosis has been found to characterize individuals affected with rare mutations in the apo A-I-apo C-III-apo A-IV locus which result in undetectable plasma apo A-I levels (Norum et al, 1986; Schaefer et al, 1982). Other uncommon genetic disorders resulting in altered HDL metabolism and reduced plasma HDL levels are not clearly associated with increased risk of CAD. These include $apoAI_{Milano}$, Tangier's disease , and "fish-eye" disease (Breslow et al, 1988). Recently, a dominant mutation in the apo A-I gene resulting in reduced HDL levels has been described (Deeb ett al, 1991), again with no evidence of increased risk of disease. In one family, the absence of apo A-II due to a gene defect was not found to be associated with any clinical abnormalities (Takata et al, 1990). The rarity of these disorders makes it difficult to reach firm conclusions regarding the relation of particular genetic defects to CAD. Nevertheless, the available information supports the notion that apo A-I may have a direct protective effect.

While less extreme reductions in HDL levels have been reported to aggregate in families with increased prevalence of heart disease (Third et al, 1984), the available data do not establish that the reductions in HDL are primary or independent of alterations in metabolism of TG-rich lipoproteins that may have a direct relationship to CAD (Austin et al, 1991).

C. Pharmacologic interventions in humans

Variable increases in HDL levels have been observed in interventional trials for primary or secondary prevention of CAD. However, the therapeutic effects have involved reductions in levels

of atherogenic lipoproteins, and a specific benefit due to increased HDL levels cannot be determined with certainty. In the LRC Primary Prevention Trial (Lipid Research Clinics Program, 1984), as well as in the arteriographic NHLBI Type II Intervention Trial (Levy et al, 1984), small changes in HDL-C in subjects on diet ± cholestyramine therapy were associated with reduced disease risk. In the Cholesterol Lowering Atherosclerosis Study, the strongest lipoprotein index of reduced angiographic progression in patients treated with colestipol and nicotinic acid was a relative increase in the amount of apo C-III in HDL vs. the non-HDL lipoprotein fraction (Blankenhorn et al, 1990). This result again emphasizes the reciprocity of changes in levels or composition of HDL and apo B-containing lipoproteins, and the difficulty of dissociating their effects on CAD risk. A similar problem arises in assessing the benefit ascribed to gemfibrozil-induced increases in HDL in the Helsinki Heart Trial, in which there were also major reductions in TG levels (Manninen et al, 1988). A recent reanalysis of the results of this trial has indicated that a lipoprotein profile consisting of TG levels greater than 2.3 mM and an LDL-C/HDL-C ratio greater than 5 identified a subgroup of 10% of moderately hypercholesterolemic men that had a relative risk for coronary disease of 3.8 and accounted for 71% of the benefit of drug therapy (Manninen et al, 1991). Statistical manipulations of the relation of such highly intercorrelated features of lipoprotein metabolism to coronary disease risk (Austin et al, 1988; Campos et al, 1992) are of minimal value in identifying primary pathophysiologic mechanisms (Austin, 1991). Nevertheless, the consistency of HDL-C as an index of CAD risk in cross-sectional, observational, and interventional trials argues for its utility and importance in identifying high-risk individuals. While the benefits and optimal means of raising HDL *per se* remain open to question, it is reasonable to assume that increases in HDL may be desirable in conjunction with other approaches to lipid-lowering therapy.

8. Proposed Mechanisms Underlying the Antiatherogenic Properties of HDL

Much attention has been given to the reverse cholesterol transport (RCT) hypothesis as a plausable explanation for the apparent antiatherogenic properties of HDL (see review by Miller, 1987B). RCT refers to the cycle of tissue cholesterol uptake by HDL and its subsequent transfer

to liver, either directly from HDL, or following transfer to VLDL or LDL, which are subsequently taken up by liver via receptor-mediated endocytosis. Through this process, HDL plays a critical role in cellular cholesterol homeostasis. Mobilization of lysozomal cholesterol is suggested to prevent the formation of cholesterol-laden foam cells whereas mobilization of cholesterol from cholesteryl ester stores appears to reverse formation of these cells. Through these processes, HDL may play a key in preventing progression and stimulating regression of atherosclerotic plaques.

Although an attractive hypothesis, evidence that variations of HDL-C levels within physiologic ranges determine the rate and/or amount of tissue cholesterol removal is equivocal. Several small studies have indicated inverse associations between body cholesterol pool sizes and HDL cholesterol (Miller, 1987B). However, Blum et al (1985) were unable to demonstrate a relationship between HDL cholesterol or apolipoprotein levels and the tissue mass of exchangable cholesterol in a major physiological study in man. It appears possible that HDL may mediate selective tissue cholesterol removal, such as from lipid-rich cells in the arterial intima, and this may not be reflected in whole body studies.

Recent reports have indicated that HDL may protect LDL against atherogenic oxidative changes (Ohta et al, 1989; Parthasarathy et al, 1990), although the basis of these antioxidant effects is unknown. Given the compelling evidence of a critical role for LDL oxidation in atherogenesis, this promises to be an area of intense research activity in the coming years.

As discussed above, in addition to a direct antiatherogenic role, levels of HDL and HDL subclasses may provide an index of other metabolic factors which influence atherogenesis. HDL levels are related to the proficiency of metabolism of TG-rich lipoproteins, and thus may indirectly reflect the extent to which these lipoproteins are available to promote disease. Although the atherogenicity of TG-rich lipoproteins has been the subject of considerable controversy, these lipoproteins have been shown to perturb endothelial cell metabolism and cause massive lipid accumulation in macrophages even when unmodified (in contrast to LDL) (see Gianturco and Bradley, 1991; Gianturco et al, 1986; Huff et al, 1991; Booyse et al, 1988). Low HDL-C levels also occur in association with a lipoprotein profile characterized by a predominance of small, dense

LDL particles, along with elevated TG and IDL levels, and thus, may represent a marker of this atherogenic lipoprotein phenotype and associated pathophysiologic processes. Affected individuals exhibit a three-fold increased risk of MI (Austin et al, 1988).

9. Concluding Remarks

Of the major plasma lipoprotein classes, HDL uniquely show an inverse association with CAD. Some evidence is available to suggest that this may be indicative of direct antiatherogenic properties of HDL. Although much is known about the metabolic behavior of HDL, specific properties which may underlie the protective effects are still in question. Further characterization of the metabolic and preventive features of individual HDL subclasses may be particularly useful for discerning the antiatherogenic properties of HDL. Elevations in plasma HDL and HDL subclasses, either independently of or in addition to other beneficial lipoprotein changes, are expected to reduce CAD risk. Numerous factors, including modifiable behaviors such as diet, exercise or smoking cessation, are known to influence HDL and thereby are suggested to influence CAD risk, although direct evidence of the effects of specific interventions is still sparse. Nonetheless, promotion of behaviors which exert a beneficial influence on HDL levels represents a key strategy for reducing CAD risk and should be encouraged, particularly in individuals with initially low HDL levels.

Acknowledgements:

The authors wish to thank Timi Mannion for assisting with the computer literature searches. This work was supported by NIH Grants HL 18574 and HL 33577 from the National Heart, Lung, and Blood Institute of the National Institutes of Health, a grant from the National Dairy Promotion and Research Board, and was conducted at the Lawrence Berkeley Laboratory (Department of Energy contract DE-AC03-76SF00098 to the University of California.)

REFERENCES

Albers JJ, Brunzell JD, Knopp RH. Apoprotein measurements and their clinical application. Clin Lab Med 1989; 9:137-152.

Albers JJ, Chen CH, Aladjem F. Human serum lipoproteins. Evidence for three classes of lipoproteins in Sf 0-2. Biochemistry 1972; 11:57-63.

Albers JJ, Marcovina M. Standardization of apolipoprotein B and A-I measurements. Clin Chem 1989; 7:1357-1361.

Amos CI, Wilson AF, Rosenbaum PA, et al. An approach to the multivariate analysis of high density lipoprotein cholesterol in a large kindred: The Bogalusa Heart Study. Genet Epidemiol 1986; 3:255-268.

Anderson AJ, Sobocinski KA, Freedman DS, et al. Body fat distribution, plasma lipids, and lipoproteins. Arteriosclerosis 1988; 8:88-94.

Annuzzi G, Jansson E, Kaijser L, et al. Increased removal rate of exogenous triglycerides after prolonged exercise in man: Time course and effect of exercise duration. Metabolism 1987; 36:438-443.

Applebaum D, Goldberg AP, Pykalisto OJ, et al. Effect of estrogens on post-heparin lipolytic activity. Selective decline in hepatic triglyceride lipase. J Clin Invest 1977; 59:601-608.

Aristimuno GG, Foster TA, Voors AW, et al. Influence of persistent obesity in children on cardiovascular risk factors: The Bogalusa Heart Study. Circulation 1984; 69:895.

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

Austin MA, Breslow JL, Hennekens CH, et al. Low density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 1988; 260:1917-1921.

Austin MA, King MC, Vranizan KM, et al. Atherogenic lipoprotein phenotype: A proposed genetic marker for coronary heart disease risk. Circulation 1990 82: 495-506.

Bachorik, PS. Measurement of total cholesterol, HDL cholesterol, and LDL cholesterol. Clin Lab Med 1989; 9:61-72.

Barbaras, R, Puchois, P, Fruchart, JC, et al. Cholesterol efflux from cultured adipose cells is mediated by LpA-I particles but not by LpA-I:A-II particles. Biochem Biophys Res Commun 1987; 142:63-69.

Barter PJ. Enzymes involved in lipid and lipoprotein metabolism. Curr Opin Lipidol 1990; 1:518-523.

Barter, PJ, Hopkins, GJ, Gorjatschko, L. Lipoprotein substrates for plasma cholesterol esterification. Influence of particle size and composition of the high density lipoprotein subfraction 3. Atherosclerosis 1985; 58:97-107.

Barter PJ, Chang LBF, Rajaram OV. Sodium oleate promotes a redistribution of cholesteryl esters from high to low density lipoproteins. Atherosclerosis 1990; 84:13-24.

Belfrage P, Berg B, Hagerstrand I, et al. Alterations of lipid metabolism in healthy volunteers during long-term ethanol intake. Eur J Clin Pharmacol 1977; 7:127-131.

Beynen AC, Katan MB. Effect of egg yolk feeding on the concentration and composition of serum lipoprotein in man. Atherosclerosis 1985; 54:157-166.

Blanche PJ, Gong EL, Forte TM, et al. Characterization of human high density lipoproteins by gradient gel electrophoresis. Biochim Biophys Acta 1981; 65:408-419.

Blankenhorn DH, Alaupovic P, Wickham E, et al. Prediction of angiographic change in native human coronary arteries and aortocoronary bypass grafts: Lipid and nonlipid factors. Circulation 1990; 81:470-476.

Blum CB, Levy RI, Eisenberg S, et al. High density lipoprotein metabolism in man. J Clin Invest 1977; 60:795-807.

Blum CB, Dell RB, Palmer RH, et al. Relationship of the parameters of body cholesterol metabolism with plasma levels of HDL cholesterol and the major HDL apoproteins. J Lipid Res 1985; 26:1079-1088.

Bradley WA, Gianturco SH. Lipoprotein receptors. Curr Opin Lipidol 1990; 1:216-221.

Breslow JL. Familial disorders of HDL metabolism. In: The Metabolic Basis of Inherited Disease. McGraw-Hill Book Co., Shriver AL, Beaudet, Sly WS, et al, eds. New York. 1988 Pp. 1251-1266.

Brinton EA, Eisenberg S, Breslow JL. Elevated high density lipoprotein cholesterol levels correlate with decreased apolipoprotein A-I and A-II fractional catabolic rate in women. J Clin Invest 1989; 84:262-269.

Brinton EA, Eisenberg S, Breslow JL. A low fat diet decreases high density lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. J Clin Invest 1990; 85:144-151.

Brown ML, Hesper C, Tall AR. Plasma enzymes and transfer proteins in cholesterol metabolism. Curr Opin Lipidol 1990; 1:122-127.

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

Brunzell JD, Albers JJ, Chait A, et al. Plasma lipoproteins in familial combined hyperlipidemia and monogenic familial hypertriglyceridemia. J Lipid Res 1983; 24:147-155.

Buring JE, O'Conner GT, Goldhaber SZ, et al. Decreased HDL2 and HDL3 cholesterol, apo A-I and apo A-II, and increased risk of myocardial infarction. Circulation 1992; 85:22-29.

Burns TL, Moll PP, Lauer RM. The relation between ponderosity and the coronary risk factors in children and their relatives. Am J Epidemiol 1989; 129:973-987.

Bush TL, Fried LP, Barrett-Conner E. Cholesterol, lipoproteins, and coronary heart disease in women. Clin Chem 1988; 34:B60-70.

Campos H., Genest Jr JJ, Blijlevens E, et al. Low density lipoprotein particle size and coronary artery disease. Arteriosclerosis 1992; 12: 187-195.

Chang LBF, Hopkins GJ, Barter PJ. Particle size distribution of high density lipoproteins as a function of plasma triglyceride concentration in human subjects. Atherosclerosis 1985; 56:61-70.

Cheung MC, Albers JJ. The measurement of apolipoprotein A-I and A-II levels in men and women by immunoassay. J Clin Invest 1977; 60:43-50.

Cheung MC, Albers JJ. Characterization of lipoprotein particles isolated by immunoaffinity chromatography. Particles containing A-I and A-II and particles containing A-I but no A-II. J Biol Chem 1984; 259:12201-12209.

Cheung MC, Nichols AV, Blanche PJ, et al. Characterization of A-I-containing lipoproteins in subjects with A-I milano variant. Biochim Biophys Acta 1988; 960:73-82.

Chong KS, Nicolosi RJ, Rodger RF, et al. Effect of dietary fat saturation on on plasma lipoproteins and high density lipoprotein metabolism of the rhesus monkey. J Clin Invest 1987; 79:675.

Clark SA, Bramwell Cook H, Oxner RBG, et al. Defenestration of hepatic sinusoids as a cause of hyperlipoproteinaemia in alcoholics. Lancet 1988; 2:1225-1227.

Clay HA, Hopkins GJ, Ehnholm C, et al. The rabbit as an animal model of hepatic lipase deficiency. Biochim Biophys Acta 1989; 1002:173-181.

Craig WY, Palomaki GE, Haddow JE. Cigarette smoking and serum lipid and lipoprotein concentrations: an analysis of published data. Br Med J 1989; 298:784-788.

Criqui MH, Cowan L, Tyroler HA, et al. Lipoproteins as medaitors for the effects of alcohol consumption and cigarette smoking on cardiovascular mortality: results from the Lipid Research Clinics Follow-up Study. Am J Epidemiol 1987; 126:629-637.

Crique MH, Wallace RB, Heiss G, et al. Cigarette smoking and plasma high-density lipoprotein cholesterol. The Lipid Research Clinics Program Prevalence Study. Circulation 1980; 62(Suppl IV):70-76.

Deckelbaum RJ, Eisenberg S, Oschry Y, et al. Reversible modifications of human plasma high density lipoproteins toward triglyceride-rich precursors. J Biol Chem 1982A; 257:6509-6517.

Deckelbaum R, Eisenberg S, Granot, Y, et al. Core lipid exchange and lipoprotein lipase in modeling human high density lipoproteins. Arteriosclerosis 1982B; 2:437a.

Deeb SS, Cheung MC, Peng R, et al. A mutation in the human apolipoprotein A-I gene. Dominant effect on the level and characteristics of plasma high density lipoproteins. J Biol Chem 1991; 266: 13654-13660.

Despres JP, Tremblay A, Perusse L, et al. Abdominal adipose tissue and serum HDL-cholesterol: Association independent from obesity and serum triglyceride concentration. Int J Obesity 1988; 12:1.

Dreon DM, Vranizan KM, Krauss RM, et al. The effects of polyunsaturated vs monounsaturated fat on plasma lipoproteins. JAMA 1990; 263:2462-2466.

Eckel RH. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. N Engl J Med 1989; 320:1060-1068.

Eisenberg S. High density lipoprotein metabolism. J Lipid Res 1985;25:1017-1058.

Eisenberg S. Metabolism of apolipoproteins and lipoproteins. Curr Opin Lipidol 1990; 1:205-215.

Ernst N, Fisher M, Smith W, et al. The association of plasma high-density lipoprotein cholesterol with dietary intake and alcohol consumption. The Lipid Research Clinics Progarm Prevalance Study. Circulation 1980; 62(Suppl IV):41.

Evans DJ, Hoffmann RG, Kalkhoff RK, et al. Relationship of androgenic activity to body fat topography, fat cell morphology, and metabolic aberrations in premenopausal women. J Clin Endocrinol Metab 1983; 57:304-310.

Fielding CJ, Shore VG, Fielding PE. A protein cofactor of lecithin:cholesterol acyltransferase. Biochem Biophys Res Commun 1972; 46:1493-1498.

Foster CJ, Weinsein RL, Birch R, et al. Obesity and serum lipids: An evaluation of the relative contribution of body fat and fat distribution and lipid levels. Int J Obesity 1987; 11:151.

Forester GP, Tall AR, Bisgaier CL, et al. Rat intestine secretes spherical high density lipoproteins. J Biol Chem 1983; 258:5938-5943.

Furman RH, Howard RP, Imagawa R. Serum lipid and lipoprotein concentrations in castrate and noncastrate institutionalized male subjects. Circulation 1956; 14:490.

Furman RH, Howard RO, Nocia LN, et al. The influence of androgens, estrogens and related steroids on serum lipids and lipoproteins. Am J Med 1967; 24:80.

Gianturco SH, Bradley WA. Triglyceride-rich lipoproteins and their role in atherogenesis. Curr Opin Lipidol 1991; 2:324-328.

Gianturco SH, Brown SA, Via DP, et al. The β-VLDL receptor pathway in murine P388D1 macrophages. J Lipid Res 1986; 27:412-420.

Gidez LI, Miller GJ, Burstein M, et al. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. J Lipid Res 1982; 23:1206-1223.

Glomset JA, Norum KR. The metabolic role of lecithin:cholesterol acyltransfersase: prospectives of pathology. Adv Lipid Res 1973; 11:1-65.

Glueck C. Influence of gemfibrozil on high-density lipoproteins. Am J Cardiol 1983A; 52:31B-34B.

Glueck C. Influence of clofibrate, bile-sequestering agents and probucol on high-density lipoprotein levels. Am J Cardiol 1983B; 52:28B-30B.

Goldberg I, Blaner S, Vanni TM, et al. Arteriosclerosis 1989; 9:695a.

Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease: four prospective American studies. Circulation 1989; 79:8-15.

Granot E, Tabas I, Tall AR, et al. Human plasma cholesteryl ester transfer protein enhances the transfer of cholesteryl ester from high density lipoproteins into cultured HepG2 cells. J Biol Chem 1987; 262:3482-3487.

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

Grundy SM, Chait A, Brunzell JD. Familial combined hyperlipidemia workshop. Arteriosclerosis 1987; 7:203-.

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

Gutai J, LaPorte R, Kuller L, et al. Plasma testosterone, high density lipoprotein cholesterol and other lipoprotein fractions. Am J Cardiol 1981; 48:897-902.

Hamsten A, Walldius G, Dahlen G, et al. Serum lipoproteins and apolipoproteins in young male survivors of myocardial infarction. Atherosclerosis 1986; 59:223-235.

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

Hasstedt SJ, Kuida H, Ash O, et al. Effects of household sharing on high density lipoprotein and its subfractions. Genet Epidemiol 1985; 2:339-348.

Heiss G, Tamir I, Davis CE, et al. Lipoprotein cholesterol distributions in selected North American populations: The Lipid Research Clinics Program Prevalence Study. Circulation 1980; 61:302-315.

Heller RF, Miller NE, Lewis B, et al. Associations between sex hormones, thyroid hormones and lipoproteins. Clin Sci 1981; 48:897.

Henkin Y, Como JA, Oberman A. Secondary dyslipidemia. Inadvertant effects of drugs in clinical practice. JAMA 1992; 267:961-968.

Huff MW, Evans AJ, Sawtez CG, et al. Cholesterol accumulation in J774 macrophages induced by triglyceride-rich lipoproteins. Comparison of very low density lipoprotein from subjects with Type III, IV and V hyperlipoproteinemias. Arteriosclerosis 1991; 11:221-223.

Ishida BY, Paigen B. Atherosclerosis in the mouse. Monographs in Human Genetics 1991; 12:189-222.

Kannel et al. Proc Nutr Soc 1988; 47:99-110.

Kantor MA, Cullinane EM, Sady SP, et al. Exercise acutely increases high density lipoprotein-cholesterol and lipoprotein lipase activity in trained and untrained men. Metabolism 1987; 36:188-192.

Kempen HJ, Van Gent CM, Buytenhek R, et al. Association of cholesterol concentrations in low density lipoprotein, high density lipoprotein, and high density lipoprotein subfractions, and of apolipoprotein A-I and A-II, with coronary stenosis and left ventricular function. J Lab Clin Med 1987; 109:19-26.

Kiens B, Lithell H. Lipoprotein metabolism influenced by training-induced changes in human skeletal muscle. J Clin Invest 1989; 83:558-564.

Kiens B, Lithell H, Mikines KJ, et al. Effects of insulin and exercise on muscle lipoprotein lipase activity in man and its relation to insulin action. J Clin Invest 1989; 84:1124-1129.

Koren E, Puchois P, Alaupovic P, et al. Quantification of two different types of apolipoprotein A-I-containing lipoprotein particles in plasma by enzyme-linked differential-antibody immunosorbent assay. Clin Chem 1987; 33:38-43.

Kostner GM, Holasek, A. The separation of human serum high density lipoproteins by hydroxy apatite column chromatography. Evidence for the presence of discrete subfractions. Biochim Biophys Acta 1977; 488:417-431.

Krauss RM. Regulation of high density lipoprotein levels. Med Clin North Amer 1982; 66:403-430.

Krauss RM. Exercise, lipoproteins, and coronary artery disease. Circulation 1989; 79:1143-1145.

Krauss RM, Levy RI, Fredrickson DS. Selective measurement of two lipase activities in post-heparin plasma from normal subjects and patients with hyperlipoproteinemia. J Clin Invest 1974; 54:1107-1124.

Krauss RM, Wood PD, Giotas C, et al. Heparin-released plasma lipase activities and lipoprotein levels in distance runners. Circulation (Suppl II) 1979; 50:73a.

Krauss RM, Lindgren FT, Williams PT, et al. Intermediate density lipoproteins and progression of coronary artery disease in hypercholesterolaemic men. Lancet 1987; 2:62-65.

Krauss RM, Lindgren FT, Wingerd J, et al. Effects of estrogens and progestins on high density lipoproteins. Lipids 1979; 14:113.

Kris-Etherton PM, Krummel D, Russell ME, et al. The effect of diet on plasma lipids, lipoproteins, and coronary heart disease. J Amer Diet Assoc 1988; 88:1373-1400.

Kuusi T, Kesaniemi A, Vuoristo M, et al. Inheritance of high-density lipoprotein and lipoprotein lipase and hepatic lipase activity. Arteriosclerosis 1987; 7:421-425.

Kuusi T, Ehnholm C, Viikari J, et al. Postheparin plasma lipoprotein and hepatic lipase are determinants of hypo- and hyperalphalipoproteinemia. J Lipid Res 1989; 30:1117-1126.

Laakso M, Voutilainen E, Pyorala K, et al. Association of low HDL and HDL2 cholesterol with coronary heart disease in non-insulin dependent diabetics. Arteriosclerosis 1985; 5:653-658.

Laws A, King AC, Haskell WL, Reaven GM. Relation of fasting plasma insulin concentration to high density lipoprotein cholesterol and triglyceride concentrations in men. Arteriosclerosis 1991; 11:1636-1642.

Laws A, Reaven GM. Evidence for an independent relationship between insulin resistance and fasting plasma HDL-cholesterol, triglyceride and insulin concentrations. J Intern Med 1992; 231:25-30.

Le N, Ginsburg HN. Heterogeneity of apolipoprotein A-I turnover in subjects with reduced concentrations of plasma high density lipoprotein cholesterol. Metab Clin Exp 1988; 37:614-617.

LeBoeuf RC, Doolittle MH, Montcalm A, et al. Phenotypic characterization of the Ath-1 gene controlling high density lipoprotein levels and susceptibility to atherosclerosis.J Lipid Res 1990; 31:91-101.

Levy RI, Brensike JF, Epstein SE, et al. The influence of changes in lipid values induced by cholestyramine and diet on progression of coronary artery disease: results of the NHLBI Type II Coronary Intervention Study. Circulation 1984; 69:325-337.

Lipid Research Clinics Program. The Lipid Research Clinics Coronary Primary Prevention Trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA 1984; 251:365-74.

Lupien PJ, Ronchon J, Dagenais GR, et al. Lipid profile of a French-Canadian population. I. Association of plas-lipid and lipoprotein levels with age, relative body-weight, and education. Can Med Assoc J 1985; 133:1127.

Mackenzie SL, SUndaram GS, Sodhi HS.Heterogeneity of human serum high-density lipoprotein. Clin Chim Acta1973; 43:223-229.

Mahley RW, Weisgraber KH, Innerarity TL, et al. Swine lipoproteins and atherosclerosis. Changes in the plasma lipoproteins and apoproteins induced by cholesterol feeding. Biochemistry 1975; 14:2817-2823.

Mahley RW, Bersot TP, Innerarity TL, et al. Alterations in human high density lipoproteins, with or without increased plasma-cholesterol, induced by diets high in cholesterol. Lancet 1978; 2:807.

Manninen V, Elo MO, Frick MH, et al. Lipid alterations and decline in the incidence of coronary heart disease in the Helsinki heart study. JAMA 1988; 260:641-651.

Manninen V, Tenkanen L, Koskinen P, et al. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart diesease risk in the Helsinki heart study. Circulation 1991; 85:37-45.

Mattson FH, Grundy SM. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J Lipid Res 1985; 26:194-202.

Mensink RP, Katan MB. Effect of monounsaturated fatty acids versus complex carbohydrates on high density lipoproteins in healthy men and women. Lancet 1987; 1:122-124.

Meilahn EN, Kuller LH, Stein EA, et al. Characteristics associated with apoprotein and lipoprotein lipid levels in middle-aged women. Arteriosclerosis 1988; 8:515-520.

Mendez AJ, Oram JF, Bierman EL. Sphingosine inhibits HDL-mediated efflux of intracellular sterols. Arteriosclerosis 1989; 9:720a.

Miller NE. Associations of high density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atheroslcerosis. Am Heart J 1987A; 113:589-597.

Miller NE. On the associations of body cholesterol pool size with age, HDL cholesterol and plasma total cholesterol concentration in humans. Atherosclerosis 1987B; 67:163-172.

Miller NE. Pharmacotherapy of disorders of plasma lipoprotein metabolism. Am J Cardiol 1990; 66:16A-19A.

Miller NE, Weinstein DB, Steinberg D. Binding, internalization, and degradation of high density lipoprotein by cultured human fibroblasts. J Lipid Res 1977; 18: 438-450.

Mofatt RJ. Effects of cessation of smoking on serum lipids and high density lipoprotein-cholesterol. Atherosclerosis 1988; 74:85-89.

Moll PP, Sing CF, Williams RR, et al. The genetic determination of plasma apolipoprotein A-I levels measured by radioimmunoassay: A study of high risk pedigrees. Am J Hum Genet 1986; 38:361-372.

Morrison JA, Laskarzewski PM, Rauh JL, et al. Lipids, lipoproteins, and sexual maturation during adolescence: The Princeton Maturation Study. Metabolism 1979; 28:264.

Musliner, TA, Krauss, RM. Lipoprotein subspecies and risk of coronary disease. Clin Chem 1988; 33:B78-B83.

Musliner TA, Long MD, Forte TM, et al. Dissociation of high density lipoprotein precursors from apolipoprotein B-containing lipoproteins in the presence of unesterified fatty acids and a source of apolipoprotein A-I. J Lipid Res.1991; 32:917-933.

McGue M, Rao DC, Iselius I, et al. Resolution of genetic and cultural inheritance in twin families by path analyses: Application to HDL-cholesterol. Am J Hum Genet 1985; 37: 998-1014.

Nakai T, Otto PS, Kennedy DL, et al. Rat high density lipoprotein subfraction (HDL3) uptake and catabolism by isolated rat liver parenchymal cells. J Biol Chem 1976; 251:4914-4921.

Namboodiri KK, Kaplan EB, Heuch I, et al. The collaborative Lipid Research Clinics Family Study: biological and cultural determinants of familial resemblance for plasma lipids and lipoproteins. Genet Epidemiol 1985; 2:227-254.

Newnham NN, Barter PJ. Synergistic effects of lipid transfers and hepatic lipase in the formation of very small high-density lipoproteins during incubation of human plasma. Biochim Biophys Acta 1990; 1044:57-64.

Nestel PJ, Billington T, Smith B. Low density and high density lipoprotein kinetics and sterol balance in vegetarians. Metab Clin Exp 1981; 30:941-945.

Nestel PJ, Reardon M, Billington T. In vivo transfer of cholestryl esters from high density lipoproteins to very low density lipoproteins in man. Biochim Biophys Acta 1979; 573:403-407.

Nichols AV. Human serum lipoproteins and their interrelationships. In: Lawrence JH, Gofman, JW, eds.: Advances in Biological and Medical Physics, Vol II. Academic Press (New York), 1967, Pp. 109-158.

Nordoy A, Aakvaag A, Thelle D. Sex hormones and high-density lipoproteins in healthy males. Atherosclerosis 1979; 34:431.

Norum RA, Forte TM, Alaupovic P, et al. Clinical syndrome and lipid metabolism in hereditary deficiency of apolipoproteins A-I and C-III, variant 1. In: Proceedings of Symposium on Lipoprotein Deficiency Syndromes, Vancouver, B.C., May 13-14, 1985. Angel A, ed. Plenum Press, New York. 1987 137-149.

Ohlson LO, Larsson B, Svardsudd K, et al. The influence of body fat distribution on the incidence of diabetes mellitus. 13.5 year follow-up of the participants in the study of men born in 1913. Diabetes 1985; 34:1055-1058.

Ohta T, Takata K, Horiuchi S, et al. Protective effect of lipoproteins containing apoprotein A-I on Cu2+-catalyzed oxidation of human low density lipoprotein. FEBS Lett 1989; 257:435-438.

Olivecrona T, Bengtsson-Olivecrona G. Lipoprotein lipase and hepatic lipase. Curr Opin Lipidol 1990; 1:222-230.

Oram JF. Cholesterol trafficking in cells. Curr Opin Lipidol 1990; 1:416-421.

Paigen B, Mitchell D, Reue K, et al Ath-1, a gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. Proc Natl Acad Sci USA 1987A; 84: 3763-3767.

Paigen B, Mitchell D, Holmes P, et al. Genetic analysis of strains C57BL/6J and BALB/cJ for Ath-1, a gene determining atherosclerosis susceptibility in mice. Biochem Genet 1987B; 25: 881-892.

Paigen B, Nesbitt MN, Mitchell D, et al.. Ath-2, a second gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. Genetics 1989; 122:163-168.

Paoletti R, Franceschini G, Sirtori CR. Influence of bezafibrate, fenofibrate, nicotinic acid and etofibrate on plasma high-density lipoprotein levels. Am J Cardiol 1983; 52:21B-27B.

Parthasarathy S, Barnett J, Fong LG. High-density lipoprotein inhibits the oxidative modification of low-density lipoprotein. Biochim Biophys Acta 1990; 1044:275-283.

Patsch W, Schonfeld G, Gotto Jr AM, et al. Characterization of high density lipoproteins by zonal ultracentrifugation. J Biol Chem 1980; 255:3178-3185.

Patsch W, Brown SA, Morriset JD, et al. A dual precipitation method evaulated for measurement of cholesterol in high-density lipoprotein subfractions HDL2 and HDL3 in human plasma. Clin Chem 1989; 35:265-270.

Pelletier DL, Baker PT. Physical activity and plasma total- and HDL-cholesterol levels in Western Samoan men. Am J Clin Nutr 1987; 46:577-585.

Phillips MC, Johnson WJ, Rothblat GH. Mechanisms and consequences of cellular cholesterol exchange and transfer. Biochim Biophys Acta 1987; 906:223-276.

Pittman RC, Knecht TP, Rosenbaum MS, et al. A nonendocytotic mechanism for the selective uptake of high-density lipoprotein-associated cholesterol esters. J Biol Chem 1987; 262:2443-2450.

Puchois P, Kandoussi A, Fievet P, et al. Apolipoprotein A-I-containing lipoproteins in coronary artery disease. Atherosclerosis 1987; 68:35-40.

Quensel M, Soderstrom A, Agardh CD, et al. High density lipoprotein concentrations after cessation of smoking: the importance of alterations in diet. Atherosclerosis 1989; 75:189-193.

Rader DJ, Castro G, Zech LA, et al. In vivo metabolism of apolipoprotein A-I on high density lipoprotein particles LpA-I and LpA-I,LpA-II. J Lipid Res 1991; 32:1849-1859.

Rao DC, Volger GP, McGue M, et al. Maximum likelihood estimation of familial correlations from multivariate quantitative data on pedigrees: A general method and examples. Am J Hum Genet 1987; 41:1104-1116.

Rinninger F, Pittman RC. Mechanism of the cholesteryl ester transfer protein-mediated uptake of high density lipoprotein cholesteryl esters by HepG2 cells. J Biol Chem 1989; 264:6111-6118.

Rubin EM, Ishida BY, Clift SM, et al. Expression of human apolipoprotein A-I in transgenic mice results in reduced plasma levels of murine apolipoprotein A-I and the appearance of two new high density lipoprotein size subclasses. Proc Natl Acad Sci. 1991A; 88:

Rubin EM, Krauss RM, Spangler EA, et al. Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. Nature 1991B; 353:265-267.

Ruys T, Shaikh M, Nordestgaard BG, et al. Effects of exercise and fat ingestion on high density lipoprotein production by peripheral tissues. Lancet 1989; 2:1119-1122.

Schaefer EJ, Eisenberg S, Levy RI. Lipoprotein apoprotein metabolism. J Lipid Res 1978; 19:667-687.

Schaefer EJ, Foster DM, Zech LA, et al. The effects of estrogen administration on plasma lipoprotein metabolism in premenopausal females. J Clin Endocrinol Metab 1983; 57:262-267.

Schaefer EJ, Heaton WH, Wetzel MG, et al. Plasma apolipoprotein A-I absence associated with a marked reduction of high density lipoproteins and premature coronary artery disease. Arterioscl Thromb 1982; 2: 16-26.

Schaefer EJ, Zech LA, Jenkins LL, et al. Human apolipoprotein A-I and A-II metabolism. J Lipid Res 1982; 23: 850-862.

Schroeder F, Jefferson JR, Kier AB, et al. Membrane cholesterol dynamics: Cholesterol domains and kinetic pools. Proc Soc Exp Biol Med 1991; 195:235-252.

Schwartz RS, Brunzell JD. Increase of adipose tissue lipoprotein lipase activity with weight loss. J Clin Invest 1981; 67:1425-1430.

Shouten D, Kleinherenbrink-Stins M, Brouwer A, et al. Interaction in vivo and in vitro of apolipoprotein E-free high-density lipoprotein with parenchymal, endothelial and Kupffer cells from rat liver. Biochem J 1989; 256:615-621.

Shwartz LC, Zech LA, Vandenbroek JM, et al. In: High Density Lipoprotein and Atherosclerosis.Vol. II. N. Miller, ed. Elsevier Science Publishing Co., Inc., New York. Pp. 321-329.

Slotte JP, Oram JF, Bierman EL, et al. Binding of high density lipoproteins to cell receptors promotes translocation of cholesterol from intracellular membranes to the cell surface. J Biol Chem 1987; 262:12904-12907.

Smith SJ, Cooper GR, Henderson LO, et al. An international collaborative study on standardization of apolipoproteins A-I and B. Part I. Evaluation of a lyophilized candidate reference and calibration material. Clin Chem 1987; 33:2240-2249.

Sniderman A, Shapiro S, Marpole D, et al. Association of coronary atherosclerosis with hyperapobetalipoproteinemia [increased protein but normal cholesterol levels in human plasma low density (β) lipoproteins]. Proc Natl Aca Sci USA 1980; 77:604-608.

Stampfer MJ, Sacks FM, Salvini S, et al. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. N Engl J Med 325:373-381.

Stefanick ML, Williams PT, Krauss RM, et al. Relationships of plasma estradiol, testosterone, and sex hormone-binding globulin with lipoproteins, apolipoproteins, and high density lipoprotein subfractions in men. J Clin Endocrinol Metab 1987; 64:723-729.

Stefanick ML, Terry RB, Haskell WL, et al. Relationships of changes in post-heparin hepatic and lipoprotein lipase activity to HDL-cholesterol changes following weight loss acheived by dieting versus exercise, in Gallo L (ed): Cardiovascular Disease: Molecular and Cellular Mechanisms, Prevention, and Treatment. New York. Plenum Publishing, 1987B. Pp 61-68.

Stern MP, Haffner SM. Body fat distribution and hyperinsulinemia as risk factors for diabetes and cardiovascular disease. Arteriosclerosis 1986; 6:123-130.

Takata K., Watanabe T, Kawamura H, et al. Apolipoprotein A-II not necessary for antiatherogenic function of HDL. Circulation 1990. 82 Supp.III: III-425.

Tall AR. Plasma high density lipoproteins. Metabolism and relationship to atherogenesis. J Clin Invest 1990; 86:379-384.

Tan MH, Dickinson MA, Albers JJ, et al. The effect of s high cholesterol and saturated fat diet on serum high-density lipoprotein-cholesterol, apoprotein A-I and apoprotein E levels in normolipidemic humans. Am J Clin Nutr 1980; 33:2559-.

Terry RB, Wood PD, Haskell WL, et al. Regional adiposity in relation to plasma lipids, lipoproteins and subfraction mass in men. J Clin Endocrinol and Metab 1989; 68:191-195.

Third JHC, Montag J, Glynn M, et al. Primary and familial hypoalphalipoproteinemia. Metabolism 1984; 33: 136-146.

Thompson GR, Seed M, Niththyananthan S, et al. Genotypic and phenotypic variations in familial hypercholesterolemia. Arteriosclerosis 1989; (Suppl 1) 9: I75-I80.

Thompson PD, Cullinane EM, Sady SP, et al. Modest changes in high density lipoprotein concentration and metabolism with prolonged exercise training. Circulation 1988; 78:25-34.

Tikkanen MJ, Nikkila EA, Kuusi T, et al. High density lipoprotein-$_2$ and hepatic lipase: reciprical changes produced by estrogen and norgestrol. J Clin Endocrinol Metab 1981; 54:1113.

Tozuka M, Fidge N. Purification and characterization of two high-density lipoprotein binding proteins from rat and human liver. Biochem J 1989; 261:239-244.

Van Berkel TJ, Koster JF, Hulsmann WC. High density lipoprotein and low density lipoprotein catabolism by human and parenchymal and nonparenchymal cells from rat liver. Biochim. Biophys. Acta 1977; 486:586-589.

Wallentine L, Sundin B. HDL2 and HDL3 lipid levels in coronary artery disease. Atherosclerosis 1985; 59:131-136.

Warnick GR, Dominiczak MH. Separation and quantitation of lipoproteins. Curr Opin Lipidol 1990; 1:493-499.

Warnick GR, Packard CJ, Shepherd J. Plasma lipid measurement. Curr Opin Lipidol 1990; 1:500-507.

Webb OL, Laskarzewski PM, Glueck CJ. Severe depression of high-density lipoprotein cholesterol levels in weight lifters and body builders by self-administered exogenous testosterone and anabolic-androgenic steroids. Metabolism 1984; 33:971-975

Williams PT. Weight set-point theory and the high-density lipoprotein concentrations of long distance runners. Metabolism 1990; 39:460-467.

Williams PT, Krauss RM, Vranizan KM, et al. Associations of lipoproteins and apolipoproteins with gradient gel electrophoresis estimates of high density lipoprotein subfractions in men and women. Arteriosclerosis, in press.

Wood PD, Stefanick ML, Dreon DM, et al. Changes in plasma lipids and lipoproteins in overweight men during weight loss through dieting as compared with exercise. N Engl J Med 1988; 319:1173-1179.

Wood PD, Terry RB, Haskell WL. Metabolism of substrates: diet, lipoprotein metabolism, and exercise. Fed Proc 1985; 44:358-363.

LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
TECHNICAL INFORMATION DEPARTMENT
BERKELEY, CALIFORNIA 94720