

High Density Lipoproteins and Protection from Coronary Heart Disease

From The HDLomics Consortium

Partner 1. Universität Zürich, Institute for Clinical Chemistry, Zurich, Switzerland (Coordinator):
Arnold von Eckardstein, Katharina M. Rentsch

Partner 2. Universiteit van Amsterdam, Academisch Medisch Centrum, Department of Vascular Medicine, Amsterdam, The Netherlands: **John J. P. Kastelein, Jan-Albert Kuivenhoven**

Partner 3, Rigshospitalet, University of Copenhagen, Dept. Clin. Biochemistry, Copenhagen, Denmark:
Anne Tybjaerg Hansen, Ruth Frikke-Schmidt

Partner 4, Linköping University, Occupational and Environmental Medicine, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping, Sweden: **Mats Lindahl**

Partner 5, National Centre of Scientific Research “Demokritos”, Institute of Biology, Athens, Greece:
Angeliki Chroni

Partner 6, Foundation for Research and Technology – Hellas, Institute of Molecular Biology and Biotechnology, Iraklion (Crete), Greece: **Vassilis Zannis*, Dmitri Kardasis**

Partner 7, Amsterdam Molecular Therapeutics BV, Amsterdam, The Netherlands: **Jaap Twisk**

*: Leading author

The Lipoprotein System

The transport of cholesterol and other water insoluble lipids in the circulation is achieved by packing them into water-soluble, mostly spherical particles that are called lipoproteins. These particles are complexes of various lipids and specific proteins. Plasma lipoproteins have traditionally been grouped into four major classes: the chylomicrons, the very low density lipoproteins (VLDL), the low density lipoproteins (LDL; that carries the “bad cholesterol”), and the high density lipoproteins (HDL; that carries the “good cholesterol”). Each lipoprotein has a distinct composition of lipids and specific structural proteins that are called apolipoproteins.

The HDL Particle

The plasma HDL is produced mainly (~80%) by the liver and to a lesser extent (~20%) by the intestine (1;2;2) and consists of spherical particles ranging from about 5 to 15 nm in diameter. The composition of HDL consists of approximately 50% apolipoprotein and 50% lipid moieties. The interior of HDL contains non-polar (water insoluble) lipids consisting of cholesteryl esters (CE) and a small amount of triglycerides (TG). On the surface relatively polar (water soluble) lipids like phospholipids, a portion of the free cholesterol and apolipoproteins are found. The major protein component of HDL is apolipoprotein A-I (apoA-I) followed by apoA-II, whereas other proteins are minor constituents (Fig. 1). As many as 60 other non-structural proteins have been reported to be associated with HDL particles but the roles of these proteins in concert with HDL are currently not known (3).

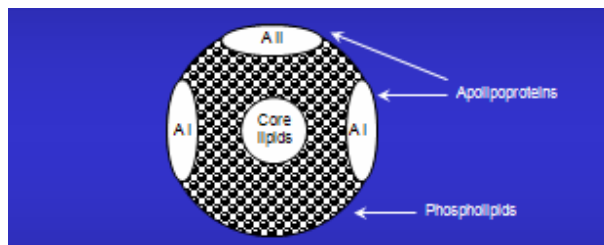


Figure 1. Schematic representation of an HDL particle.

HDL Levels and Risk for Coronary Heart Disease

Lipoproteins and apolipoprotein levels are associated with the risk for coronary heart disease (CHD); in fact, the momentum for the study of lipoproteins has been provided by epidemiological and genetic data which demonstrate clearly that increases in LDL cholesterol or apolipoprotein B (apoB) levels and decreases in HDL cholesterol or apoA-I levels are associated with an increased risk for CHD (4-6;6). In contrast, high HDL levels have been claimed to protect against CHD and confer longevity to humans (4;6;6;7;7;8;8;9;9). Other risk factors which contribute to CHD are age, blood pressure, cigarette smoking, diabetes, lack of physical activity, obesity and atherogenic diets (6). The contribution of the total plasma cholesterol and HDL cholesterol to the risk of CHD is shown in Tables IA and IB (6). Positive numbers indicate increased risk; negative numbers decreased risk. The tables show that increased levels of total cholesterol (or LDL cholesterol) contributes to increased risk while very low total cholesterol is beneficial. On the other hand, low HDL contributes to risk while high HDL is associated with protection. For example, as can be seen in Table 1B going from 25 to 90 mg/dl HDL, one reduces the risk by 14 points. Figure 2 illustrates that such a dramatic difference in risk points bears direct consequence with regards to the probability of developing CHD.

The probability of developing heart disease increases as the sum of the risk points from different causes increases (Fig. 2) (6;10;10).

TABLE IA: CONTRIBUTION OF PLASMA CHOLESTEROL TO CHD RISK

Plasma Cholesterol (mg/dl)	Risk Points	Plasma Cholesterol (mg/dl)	Risk Points
139-151	-3	220-239	2
152-166	-2	240-262	3
167-182	-1	263-288	4
183-199	0	289-315	5
200-219	1	316-330	6

TABLE IB: CONTRIBUTION OF HDL CHOLESTEROL TO CHD RISK

HDL Cholesterol (mg/dl)	Risk Points	HDL Cholesterol (mg/dl)	Risk Points
25-26	7	51-55	-1
27-29	6	56-60	-2
30-32	5	61-66	-3
33-35	4	67-73	-4
36-38	3	74-80	-5
39-42	2	81-87	-6
43-46	1	88-96	-7
47-50	0		

Modified from Anderson et al., 1991. *Circulation* 83, 356-362. (6)

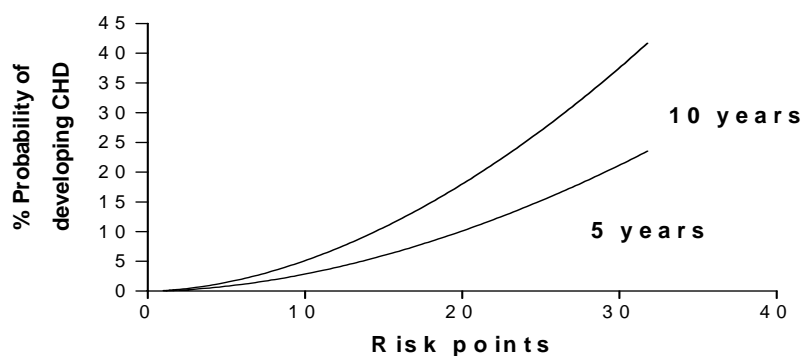


Figure 2. Graph showing the probability of developing CHD as a function of increases in risk points from different causes. Risk factors used, based on the Framingham study, include: age, total cholesterol, HDL cholesterol (<40 mg/dL), blood pressure, and cigarette smoking. (Adapted from (6;10).

Formation of HDL in the Body and Genetic Mutations that Lead to Low HDL Levels

The synthesis of HDL goes through a series of steps where apoA-I and a variety of other proteins present in the surface of cells or in plasma interact to form the HDL particle. Initially a poorly lipidated HDL precursor particle is formed (frequently called pre β HDL) that contains apoA-I and a small amount of cholesterol and phospholipids which are subsequently converted to discoidal and spherical HDL particles as shown in Figure 3.

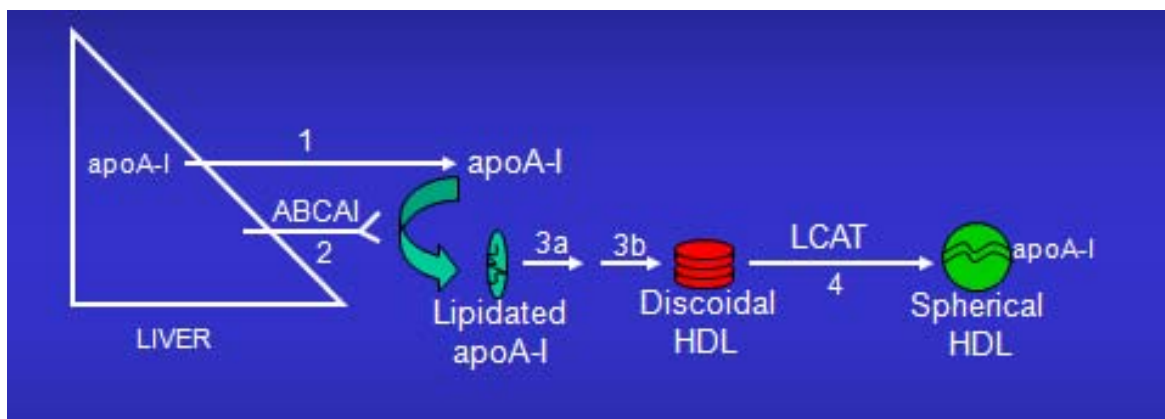


Figure 3. Schematic representation showing the various steps that lead to the formation of spherical HDL. (Adapted from (11))

More precisely for this de novo assembly of the HDL particle, apoA-I that is secreted by the liver or the intestine (Fig. 3, step 1) receives phospholipids and cholesterol from cells. This transfer of lipids from the cells to apoA-I is performed by a very important protein present on the cell surface, the ATP binding cassette transporter A1 (ABCA1) (Fig. 3, step 2) (12). If ABCA1 is missing HDL is not formed and this results in a human disease known as Tangier Disease, which was first observed in patients on the island of Tangier, off the east coast of the US (13). Similarly, HDL is not formed in humans who lack apoA-I (13). The lipidated apoA-I produced as a result of the interaction of apoA-I with ABCA1 goes through one or more steps (Fig. 3, steps 3a,3b), that are not fully understood, and reaches the stage of discoidal HDL particles. The cholesterol present on these particles is converted to an ester (cholesteryl ester) by the action of the plasma enzyme lecithin:cholesterol acyltransferase (LCAT). This conversion changes the discoidal HDL into spherical particles because the newly formed hydrophobic cholesteryl esters sequester in the core of HDL (Fig. 3, step 4). If LCAT is missing or rendered inactive by mutations, the discoidal HDL particles accumulate in plasma but HDL cholesterol levels are very low (13).

Laboratory experiments and studies of human subjects also showed that specific mutations in apoA-I may result in very low HDL levels due to the fast removal of the lipidated apoA-I from the circulation by the kidney or accumulation of discoidal HDL particles (13-15;15;16;16;17;17).

Both these conditions can be corrected in experimental animals by treatment with LCAT. Finally, it has been found that other mutations in apoA-I may cause high plasma triglyceride levels and these conditions could be corrected by treatment with lipoprotein lipase (17;17-19;19).

Remodeling of HDL by Lipid Transfer Proteins, Lipolytic Enzymes and Cellular Factors

Once formed, the initially small HDL particles (denoted as HDL3) interact with various proteins present on the cell surface or in plasma. For example discoidal and spherical HDL3 particles interact with the ABCG1 transporter which is predominantly expressed in lipid laden macrophages for example in the arterial wall and causes efflux of cholesterol (Fig. 4, step 6) (12). Another example is the transfer of phospholipids from VLDL and chylomicrons onto HDL3 by the phospholipid transfer protein (PLTP) (20). By these acquisitions of lipids and also proteins and by ongoing esterification of cholesterol by LCAT small HDL3 particles mature to large HDL2 particles.

The cholesterol moiety of mature HDL can be directly or indirectly transferred to the liver or steroidogenic organs and used for the synthesis of bile acids and steroid hormones, respectively.

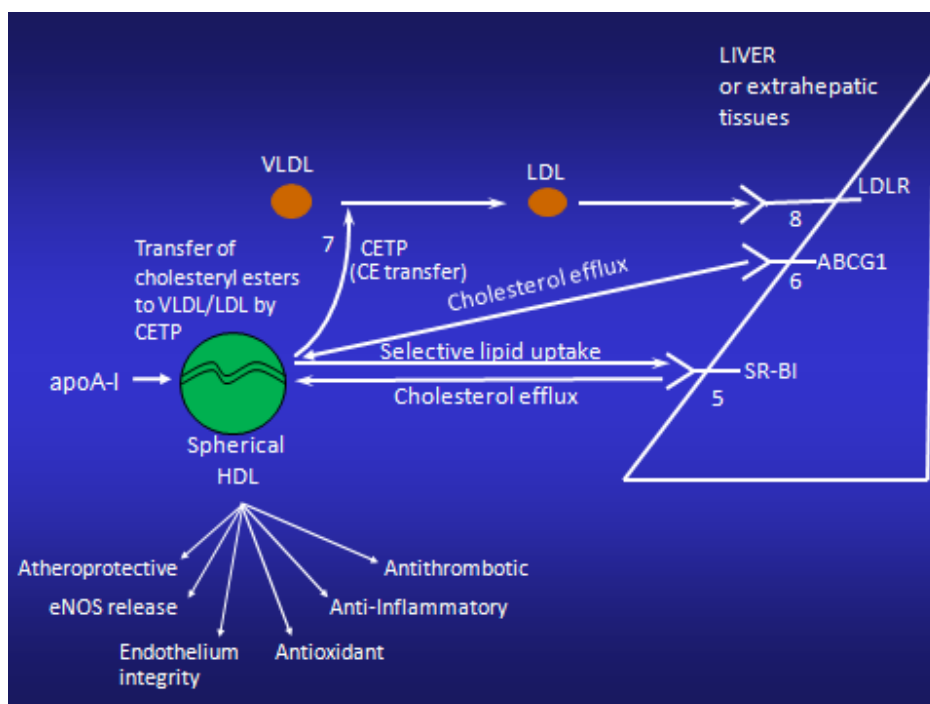


Figure 4. Schematic representation showing the remodeling of HDL in the plasma and the various reported functions of HDL. Not shown in the scheme is the transfer of phospholipids from VLDL into HDL and the hydrolysis of phospholipids of HDL by HL and EL.

The direct pathway involves an HDL receptor (HDLr), commonly known as SR-B1 (Fig. 4, step 5). Through these interactions, the cells selectively take up cholesteryl esters. The indirect pathway of cholesterol removal from HDL appears quantitatively more important in humans and involves the action of a plasma protein, the cholesteryl ester transfer protein (CETP) (Fig. 4, step 7). This protein transfers cholesteryl esters of HDL to the VLDL and LDL lipoproteins, which ultimately are taken up by liver and other cells via another membrane protein, the LDL receptor (LDLr) (Fig. 4, step 8).

The phospholipid moiety of HDL is metabolized by lipases predominantly produced in the liver and endothelium, namely the hepatic lipase (HL) and the endothelial lipase (EL) respectively (21).

The remodeling of HDL is an important phenomenon for two reasons. It serves to provide or remove cholesterol from cells (Fig. 4, steps 5,6,7). It also serves as a carrier of proteins and lipids that contribute to atheroprotection by mechanisms beyond reverse cholesterol transport (11;11;22). Interestingly the remodeling of HDL by PLTP, CETP, SR-B1, HL and EL as well as the degradation of triglyceride rich lipoproteins by lipoprotein lipase (LPL) liberate lipid-free or lipid-poor apoA-I molecules that are either cleared by the kidney or restart the formation of HDL by interaction with ABCA1 not only in the liver or intestine but also in other organs. For the protection from atherosclerosis the interaction of lipid-free apoA-I with lipid laden macrophages of the arterial wall, which express high levels of ABCA1 and ABCG1, is currently anticipated to convey one of the most anti-atheroprotective actions of HDL. This is indicated by the massive cholesterol accumulation in macrophages of patients or mice which lack ABCA1 and/or ABCG1 (12).

The interactions of HDL with the HDL receptor and CETP appears to be important for atheroprotection. One apparent paradox is that of mice that lack the HDL receptor have high levels of HDL, but are more prone to atherosclerosis which is related to the accumulation of abnormal HDL particles in plasma (23;24;24). Another apparent paradox is the recent attempt to increase HDL cholesterol levels in humans by the drug Torcetrapid that inhibits the protein CETP and thus prevents transfer of the cholesteryl esters to VLDL-LDL. This treatment indeed increased plasma levels of HDL but also increased the risk for heart disease in humans. It is still however possible that this side effect is caused by unanticipated off-target effects that result in increased blood pressure. As a result clinical trials using this drug were discontinued. In addition, few human subjects were identified with high HDL levels and documented coronary heart disease that was attributed to the atherogenic rather than atheroprotective properties of HDL that was formed in these patients (25).

These at first sight paradoxical findings clearly demonstrate that high levels of HDL cholesterol are not synonymous with atheroprotection. All this new knowledge suggests that what is important for protection from CHD is not necessarily a high level of HDL cholesterol, but more importantly the presence of functional HDL. The functionality of HDL can be influenced by the abundance of various bioactive proteins and lipids, that exert anti-inflammatory, anti-oxidative, anti-coagulative and other atheroprotective functions, as well as by the acquisition of atypical

proteins, for example serum amyloid A in the course of inflammation, which interfere with regular HDL function (see below).

Regulation of HDL levels.

HDL cholesterol levels are regulated by many genetic and non-genetic factors which are only partially resolved.

Genetic factors account for about 50% of the variability in HDL cholesterol levels among individuals (26). Both monogenic and polygenic factors are involved. Heterozygosities for individual mutations in the apoA-I, ABCA1, LCAT and LPL genes explain about 10 to 15% of HDL cholesterol levels below the 10th percentile (27;28). Polymorphisms in CETP, LPL, hepatic lipase or apoE explain considerably less than 10% mean differences between carriers and non-carriers (26). Most recently, genome-wide linkage analysis identified most of the genes mentioned above as well as endothelial lipase and four other genes of which the role in HDL metabolism is as of yet unknown (29).

Important life style and environmental factors influencing HDL cholesterol include gender, body fat, physical activity, smoking and alcohol intake as well as several drugs (30). On average, men have 15 mg/dL (0.4 mmol/L) lower HDL cholesterol levels than women because HDL cholesterol levels are increased by estradiol and decreased by testosterone. Overweight and obesity as well as smoking decrease HDL cholesterol whereas regular aerobic exercise and moderate alcohol intake increase HDL cholesterol levels. In addition to some lipid modifying drugs described below, there are several medications which as side effects alter HDL cholesterol levels. Notably androgens, progestins and anabolics, some diuretics and beta blockers decrease HDL cholesterol whereas estrogens, niacin, and some anti-convulsives are known to increase HDL cholesterol.

Clinically most relevant is the frequent association of low HDL cholesterol with overweight or obesity, hypertriglyceridemia, latent or overt type 2 diabetes mellitus, and hypertension, (i.e., with components of the metabolic syndrome). Therefore and because low HDL cholesterol is a risk factor of future diabetes mellitus, insulin resistance and metabolic sequelae thereof appear to play an important role in the determination of HDL cholesterol levels in the population. In fact, insulin and free fatty acids, which are found at increased concentrations in the metabolic syndrome, regulate several important steps of HDL metabolism at the transcriptional and post-translational level (31).

Protective Functions of HDL

The ability of HDL and apoA-I to remove excess cholesterol from macrophages, which are blood-borne cells that invade into the arterial wall, is beneficial. The removal of the excess cholesterol from macrophage prevents or reverts overloading of these cells with cholesterol, their subsequent death and thereby the extracellular deposition of cholesterol in the artery wall (12;32).

In addition HDL and its lipid and protein components have been reported to have antioxidant, anti-inflammatory and anti-thrombotic properties. HDL also protects the integrity of the endothelial cells that line the blood vessels and promote the release of nitric oxide (NO) (Fig. 4) (33;34;34;34;35;35;35;36;36;36;37;37;37;38;38;38;39;39;39;40;40;41;41;42;42;43;43;44;44;45;45;46;46;47;47;48;48;49). In this regard it is important to note that mass spectrometric analyses of HDL identified the presence of more than 50 different proteins that are associated with HDL many of which are not typical apolipoproteins but proteins involved in the regulation of inflammation (3). Also the lipid moiety of HDL is very heterogeneous and includes not only inert cargo but also bioactive lipids such as sphingosine-1-phosphate (49).

The antioxidant activity of HDL protects and reverts oxidation of LDL, which is an early event in the formation of atherosclerotic lesions and promotes inflammation in the artery wall (34;34;44;44;45;45;50). HDL promotes the production of NO in endothelial cells by the enzyme endothelial nitric oxide synthase (eNOS) through different mechanisms. These mechanisms involve the interaction of HDL with the HDL receptor SR-BI (41;43) and sphingosine-1-phosphate receptors (51). Through the anti-inflammatory properties, HDL prevents the release of pro-inflammatory mediators (such as interleukin-1 β or the synthesis of serum amyloid protein A by the liver, macrophages and other leukocytes (40;52;52-54). Through its anti-thrombotic properties, HDL promotes blood flow, inhibits formation of thrombin and prevents activation of platelets and alterations of the surface of the endothelial cells that could lead to the formation of thrombus (41;52;52). HDL also helps maintain the integrity of the endothelial cells by promoting the migration of the endothelial cells to the denuded areas of the vessel wall and this process leads to re-endothelialization (41;42;42). All these properties may contribute to atheroprotection by HDL through mechanisms that are only partially understood at the present time (55) (Fig. 5).

The beneficial effect of HDL on the arterial wall was also demonstrated in subjects with heterozygous deficiency of ABCA1. Low HDL levels in these subjects were associated with impairment in NO bioactivity and this defect could be corrected by infusion of artificially reconstituted HDL particles (56).

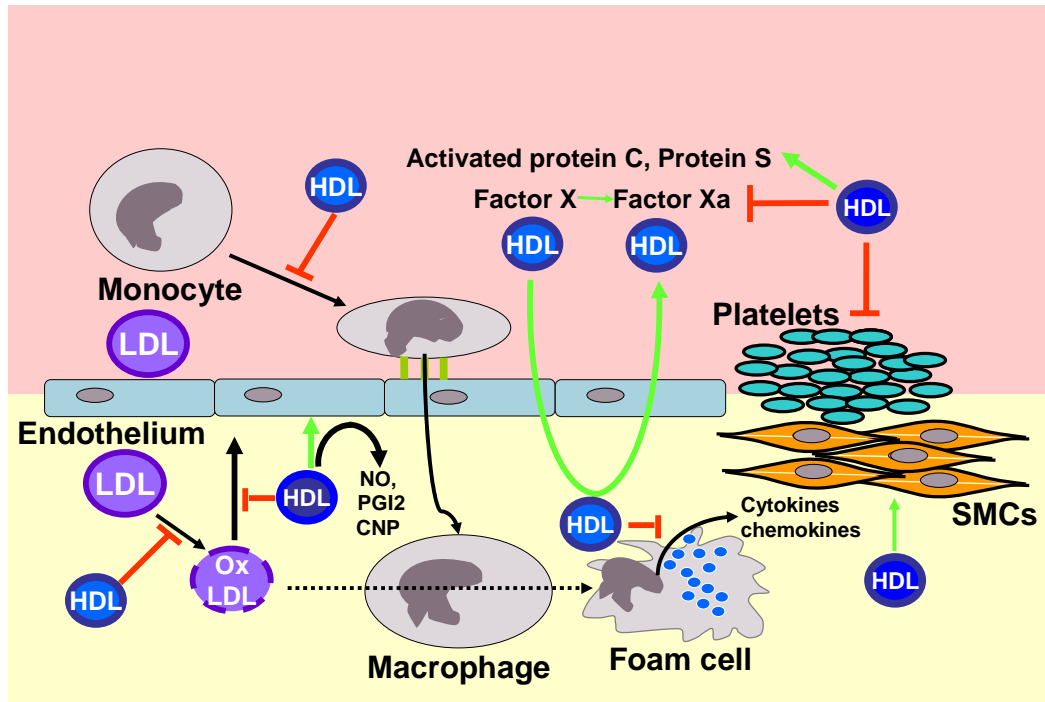


Figure 5: Anti-atherogenic properties of HDL

HDL and Atheroprotection

HDL has been implicated in the inhibition or the regression of atherosclerosis in humans and experimental animals (57-59). Initial studies showed that transgenic mice that overexpress the apoA-I gene in the atherosclerosis-susceptible mouse lines protected the mice from atherosclerosis in response to a high fat diet, as compared to non-transgenic control mice placed on the same diet (60). The same effect was observed in atherosclerosis-prone, apoE-deficient mice, in which the expression of the apoA-I gene significantly reduced lesion formation of atherosclerotic lesions (61). Similar results were obtained in other atherosclerosis-prone mouse (62;63) and rabbit (64) models, as well as by apoA-I gene therapy experiments in atherosclerosis-prone mouse strains (65-68).

It remains a paradox that apoA-I deficient mice that lack HDL did not develop atherosclerosis on normal or atherogenic diets (69). It is possible that apoE and apoE containing HDL (11) may assume some of the anti-atherogenic functions of apoA-I and HDL. Findings in humans of HDL deficiency syndromes have however also been inconclusive, and defects in major HDL genes such as *APOA1*, *LCAT* and *ABCA1* in individual subjects, have not consistently translated into the expected increased risk of atherosclerosis (70-72)(73).

The Beneficial Effects of Raising HDL

The Framingham Study in the United States, the PROCAM study in Germany and several other population studies have shown an inverse correlation between HDL cholesterol levels and the risk of developing premature coronary heart disease (57;74-77). On this basis, other authors calculated that an elevation of HDL cholesterol by 1 mg/dL (about 0.025 mmol/L), reduces the risk of having a CHD event by 2.5%. The risk is independent of the LDL cholesterol level. Low HDL cholesterol increases the risk for heart disease in people with low intermediate or high LDL cholesterol levels. The existing evidence suggest that low concentration of HDL cholesterol is a predictor of CHD, independent of LDL cholesterol, plasma triglyceride, body weight or the presence of diabetes.

The beneficial effects of raising HDL to protect from coronary artery disease has been suggested by several clinical intervention studies. An early clinical treatment study showed that raising HDL with the drug niacin decreased significantly the major coronary heart disease events by 14% and decreased mortality. In another study, treatment of survivors of myocardial infarction (heart attack) with niacin reduced the coronary heart disease mortality by 36% and the total mortality by 26% (78).

In the *HDL Atherosclerosis Treatment Study* (HATS), patients with coronary disease and low plasma HDL levels were treated with simvastatin, a cholesterol-lowering drug, and niacin. This treatment reduced LDL cholesterol (bad cholesterol) by 42% and increased HDL cholesterol (good cholesterol) by 26%. More importantly, the average coronary stenosis progressed by 3.9% with placebo and regressed by 0.4% in the treated group. The frequency of a clinical cardiovascular end point was 24% in the placebo group and 3% in the treated group. All these beneficial changes were statistically highly significant (79). In another study, treatment of patients with a combination of statins and extended release niacin (Niaspan) increased HDL cholesterol by 21% and caused a statistically significant decrease in the thickening of the carotid arteries in the group of treated subjects within 24 months (79;80;80;81;81;82). It may be noted, however, that both these studies were carried out with a rather limited number of patients and that larger studies are needed to confirm these results.

Treatment of 4,081 subjects with coronary heart disease and relatively high plasma cholesterol (270 mg/dl) in the *Helsinki Heart Study* (HHS) with gemfibrozil increased HDL cholesterol by 11%, decreased plasma cholesterol and triglycerides by 10 and 35%. The combined improvement of the lipid profile reduced by 34% the incidence of total coronary heart disease events over a five-year period. Similarly, in the *Veteran's Affairs High Density Lipoprotein Intervention Trial* (VA-HIT), treatment of 2,531 subjects with low HDLc and LDLc with gemfibrozil increased HDL by 6%, reduced triglycerides by 31% and reduced the incidence of myocardial infarction and death by 22% (83).

Based on the above data, the most effective available treatment for raising HDL levels appear to be a combination of niacin with statins and the different brands of fibrates alone or in combination with niacin.

It has been suggested that acute administration of artificially constructed HDL containing apoA-I and phospholipids can stabilize unstable plaques and reduce inflammation. This hypothesis was

tested in two studies. In a small scale experimental treatment, intravenous administration in five doses at weekly intervals of 15 mg/kg of one preparation containing apoA-I Milano in patients with acute coronary syndrome caused small, but statistically significant reduction of coronary atherosclerosis, as determined by intravascular ultrasound (59). However, the small number of patients studied and the absence of a dosage effect necessitates that these suggestive findings are confirmed in larger studies. Another study of similar design using another type of artificial HDL did not demonstrate any protective effect (84).

Potential Therapeutic Targets to Increase HDL Levels Without Affecting the Functions of HDL

Based on our current understanding of the HDL field, one can envision the following avenues for increasing the HDL levels in ways that produce functional HDL that promotes atheroprotection:

- 1- **Increase the expression of apoA-I or ABCA1 genes, or both.** *Based on genetic data of humans and studies of transgenic mice, increases in either apoA-I or ABCA1 generate functional and atheroprotective HDL and support cholesterol removal from lipid-laden macrophages.*
- 2- **Increase the expression of LCAT.** *Based on our data, increased expression of LCAT can correct low HDL syndromes that result from LCAT insufficiency.*
- 3- **Increase the expression of lipoprotein lipase.** *Numerous studies have established an inverse correlation between plasma triglyceride levels (which result from a decrease in lipoprotein lipase activity) and HDL levels. Based on our data, hypertriglyceridemia in experimental animals and low HDL levels that result from specific structural alterations in apoA-I can be corrected by treatment with lipoprotein lipase.*

Existing data suggest that inhibition of other steps of the HDL pathway (i.e., CETP, SR-BI) may increase HDL cholesterol levels but may not provide the desired atheroprotective effects.

References

1. Timmins, J. M., Lee, J. Y., Boudyguina, E., Kluckman, K. D., Brunham, L. R., Mulya, A., Gebre, A. K., Coutinho, J. M., Colvin, P. L., Smith, T. L., Hayden, M. R., Maeda, N., and Parks, J. S. (2005) Targeted inactivation of hepatic Abca1 causes profound hypoalphalipoproteinemia and kidney hypercatabolism of apoA-I. *J. Clin. Invest* 115, 1333-1342.
2. Singaraja, R. R., Van Eck, M., Bissada, N., Zimetti, F., Collins, H. L., Hildebrand, R. B., Hayden, A., Brunham, L. R., Kang, M. H., Fruchart, J. C., van Berkel, T. J., Parks, J. S., Staels, B., Rothblat, G. H., Fievet, C., and Hayden, M. R. (2006) Both hepatic and extrahepatic ABCA1 have discrete and essential functions in the maintenance of plasma high-density lipoprotein cholesterol levels in vivo. *Circulation* 114, 1301-1309.
3. Vaisar, T., Pennathur, S., Green, P. S., Gharib, S. A., Hoofnagle, A. N., Cheung, M. C., Byun, J., Vuletic, S., Kassim, S., Singh, P., Chea, H., Knopp, R. H., Brunzell, J., Geary, R., Chait, A., Zhao, X. Q., Elkon, K., Marcovina, S., Ridker, P., Oram, J. F., and Heinecke, J. W. (2007) Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. *J. Clin. Invest* 117, 746-756.
4. Castelli, W. P., Doyle, J. T., Gordon, T., Hames, C. G., Hjortland, M. C., Hulley, S. B., Kagan, A., and Zukel, W. J. (1977) HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. *Circulation* 55, 767-772.
5. Heiss, G. and Tyroler, H. Are apolipoproteins useful for evaluating ischemic heart disease? A brief overview of the literature. [83-1266], 7-24. 1982. Bethesda, MD, U.S. Department of Health and Human Services, National Institutes of Health. Proceedings of the Workshop on Apolipoprotein Quantification.
6. Anderson, K. M., Wilson, P. W., Odell, P. M., and Kannel, W. B. (1991) An updated coronary risk profile. A statement for health professionals. *Circulation* 83, 356-362.
7. Glueck, C. J., Gartside, P., Fallat, R. W., Sielski, J., and Steiner, P. M. (1976) Longevity syndromes: familial hypobeta and familial hyperalpha lipoproteinemia. *J. Lab Clin. Med.* 88, 941-957.
8. Glueck, C. J., Fallat, R. W., Millett, F., and Steiner, P. M. (1975) Familial hyperalphalipoproteinemia. *Arch. Intern. Med.* 135, 1025-1028.
9. Patsch, W., Kuisk, I., Glueck, C., and Schonfeld, G. (1981) Lipoproteins in familial hyperalphalipoproteinemia. *Arteriosclerosis* 1, 156-161.
10. National Cholesterol Education Program (NCEP) Expert Panel on Detection, E. a. T. o. H. B. C. i. A. A. T. P. I. (2002) Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 106, 3143-3421.
11. Zannis, V. I., Koukos, G., Drosatos, K., Vezeridis, A., Zanni, E. E., Kypreos, K. E., and Chroni, A. (2008) Discrete roles of apoA-I and apoE in the biogenesis of HDL species: lessons learned from gene transfer studies in different mouse models. *Ann. Med.* 40 Suppl 1, 14-28.
12. von Eckardstein, A. (2006) Differential diagnosis of familial high density lipoprotein deficiency syndromes. *Atherosclerosis* 186, 231-239.
13. Cavelier, C., Lorenzi, I., Rohrer, L., and von Eckardstein, A. (2006) Lipid efflux by the ATP-binding cassette transporters ABCA1 and ABCG1. *Biochim. Biophys. Acta* 1761, 655-666.
14. Chroni, A., Duka, A., Kan, H. Y., Liu, T., and Zannis, V. I. (2005) Point mutations in apolipoprotein a-I mimic the phenotype observed in patients with classical lecithin:cholesterol acyltransferase deficiency. *Biochemistry* 44, 14353-14366.
15. Koukos, G., Chroni, A., Duka, A., Kardassis, D., and Zannis, V. I. (2007) Naturally occurring and bioengineered apoA-I mutations that inhibit the conversion of discoidal to spherical HDL: the abnormal HDL phenotypes can be corrected by treatment with LCAT. *Biochem. J.* 406, 167-174.
16. Koukos, G., Chroni, A., Duka, A., Kardassis, D., and Zannis, V. I. (2007) LCAT can rescue the abnormal phenotype produced by the natural ApoA-I mutations (Leu141Arg)Pisa and (Leu159Arg)FIN. *Biochemistry* 46, 10713-10721.

17. Zannis, V. I., Chroni, A., and Krieger, M. (2006) Role of apoA-I, ABCA1, LCAT, and SR-BI in the biogenesis of HDL. *J. Mol. Med.* *84*, 276-294.
18. Chroni, A., Kan, H. Y., Kypreos, K. E., Gorshkova, I. N., Shkodrani, A., and Zannis, V. I. (2004) Substitutions of glutamate 110 and 111 in the middle helix 4 of human apolipoprotein A-I (apoA-I) by alanine affect the structure and in vitro functions of apoA-I and induce severe hypertriglyceridemia in apoA-I-deficient mice. *Biochemistry* *43*, 10442-10457.
19. Chroni, A., Kan, H. Y., Shkodrani, A., Liu, T., and Zannis, V. I. (2005) Deletions of helices 2 and 3 of human apoA-I are associated with severe dyslipidemia following adenovirus-mediated gene transfer in apoA-I-deficient mice. *Biochemistry* *44*, 4108-4117.
20. Huuskonen, J., Olkkonen, V. M., Jauhiainen, M., and Ehnholm, C. (2001) The impact of phospholipid transfer protein (PLTP) on HDL metabolism. *Atherosclerosis* *155*, 269-281.
21. Brown, R. J. and Rader, D. J. (2007) Lipases as modulators of atherosclerosis in murine models. *Curr. Drug Targets.* *8*, 1307-1319.
22. Zannis, V. I., Zanni, E. E., Papapanagiotou, A., Kardassis, D., and Chroni, A. (2006) in *High-Density Lipoproteins. From Basic Biology to Clinical Aspects* (Fielding, C. J., Ed.) pp 237-265, Wiley-VCH, Weinheim.
23. Trigatti, B., Rayburn, H., Vinals, M., Braun, A., Miettinen, H., Penman, M., Hertz, M., Schrenzel, M., Amigo, L., Rigotti, A., and Krieger, M. (1999) Influence of the high density lipoprotein receptor SR-BI on reproductive and cardiovascular pathophysiology. *Proc. Natl. Acad. Sci. U. S. A* *96*, 9322-9327.
24. Huszar, D., Varban, M. L., Rinninger, F., Feeley, R., Arai, T., Fairchild-Huntress, V., Donovan, M. J., and Tall, A. R. (2000) Increased LDL cholesterol and atherosclerosis in LDL receptor-deficient mice with attenuated expression of scavenger receptor B1. *Arterioscler. Thromb. Vasc. Biol.* *20*, 1068-1073.
25. Ansell, B. J., Navab, M., Hama, S., Kamranpour, N., Fonarow, G., Hough, G., Rahmani, S., Mottahedeh, R., Dave, R., Reddy, S. T., and Fogelman, A. M. (2003) Inflammatory/antiinflammatory properties of high-density lipoprotein distinguish patients from control subjects better than high-density lipoprotein cholesterol levels and are favorably affected by simvastatin treatment. *Circulation* *108*, 2751-2756.
26. Holleboom, A. G., Vergeer, M., Hovingh, G. K., Kastelein, J. J., and Kuivenhoven, J. A. (2008) The value of HDL genetics. *Curr. Opin. Lipidol.* *19*, 385-394.
27. Frikke-Schmidt, R., Nordestgaard, B. G., Jensen, G. B., and Tybjaerg-Hansen, A. (2004) Genetic variation in ABC transporter A1 contributes to HDL cholesterol in the general population. *J. Clin. Invest* *114*, 1343-1353.
28. Cohen, J. C., Kiss, R. S., Pertsemlidis, A., Marcel, Y. L., McPherson, R., and Hobbs, H. H. (2004) Multiple rare alleles contribute to low plasma levels of HDL cholesterol. *Science* *305*, 869-872.
29. Kathiresan, S., Musunuru, K., and Orho-Melander, M. (2008) Defining the spectrum of alleles that contribute to blood lipid concentrations in humans. *Curr. Opin. Lipidol.* *19*, 122-127.
30. Ashen, M. D. and Blumenthal, R. S. (2005) Clinical practice. Low HDL cholesterol levels. *N. Engl. J. Med.* *353*, 1252-1260.
31. Rohrer, L., Hersberger, M., and von Eckardstein, A. (2004) High density lipoproteins in the intersection of diabetes mellitus, inflammation and cardiovascular disease. *Curr Opin Lipidol.* *15*, 269-278.
32. Maxfield, F. R. and Tabas, I. (2005) Role of cholesterol and lipid organization in disease. *Nature* *438*, 612-621.
33. Mineo, C., Yuhanna, I. S., Quon, M. J., and Shaul, P. W. (2003) High density lipoprotein-induced endothelial nitric-oxide synthase activation is mediated by Akt and MAP kinases. *J. Biol. Chem.* *278*, 9142-9149.
34. Navab, M., Hama, S. Y., Cooke, C. J., Anantharamaiah, G. M., Chaddha, M., Jin, L., Subbanagounder, G., Faull, K. F., Reddy, S. T., Miller, N. E., and Fogelman, A. M. (2000) Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1. *J. Lipid Res.* *41*, 1481-1494.
35. Mackness, M. I., Arrol, S., and Durrington, P. N. (1991) Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. *FEBS Lett.* *286*, 152-154.

36. Watson, A. D., Navab, M., Hama, S. Y., Sevanian, A., Prescott, S. M., Stafforini, D. M., McIntyre, T. M., Du, B. N., Fogelman, A. M., and Berliner, J. A. (1995) Effect of platelet activating factor-acetylhydrolase on the formation and action of minimally oxidized low density lipoprotein. *J. Clin. Invest* 95, 774-782.
37. Watson, A. D., Berliner, J. A., Hama, S. Y., La Du, B. N., Faull, K. F., Fogelman, A. M., and Navab, M. (1995) Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J. Clin. Invest* 96, 2882-2891.
38. Theilmeier, G., De Geest, B., Van Veldhoven, P. P., Stengel, D., Michiels, C., Lox, M., Landeloos, M., Chapman, M. J., Ninio, E., Collen, D., Himpens, B., and Holvoet, P. (2000) HDL-associated PAF-AH reduces endothelial adhesiveness in apoE^{-/-} mice. *FASEB J.* 14, 2032-2039.
39. Shih, D. M., Xia, Y. R., Wang, X. P., Miller, E., Castellani, L. W., Subbanagounder, G., Cheroutre, H., Faull, K. F., Berliner, J. A., Witztum, J. L., and Lusis, A. J. (2000) Combined serum paraoxonase knockout/apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. *J. Biol. Chem.* 275, 17527-17535.
40. Kontush, A. and Chapman, M. J. (2006) Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. *Pharmacol. Rev.* 58, 342-374.
41. Mineo, C., Deguchi, H., Griffin, J. H., and Shaul, P. W. (2006) Endothelial and antithrombotic actions of HDL. *Circ. Res.* 98, 1352-1364.
42. Seetharam, D., Mineo, C., Gormley, A. K., Gibson, L. L., Vongpatanasin, W., Chambliss, K. L., Hahner, L. D., Cummings, M. L., Kitchens, R. L., Marcel, Y. L., Rader, D. J., and Shaul, P. W. (2006) High-density lipoprotein promotes endothelial cell migration and reendothelialization via scavenger receptor-B type I. *Circulation Research* 98, 63-72.
43. Assanasen, C., Mineo, C., Seetharam, D., Yuhanna, I. S., Marcel, Y. L., Connelly, M. A., Williams, D. L., Llera-Moya, M., Shaul, P. W., and Silver, D. L. (2005) Cholesterol binding, efflux, and a PDZ-interacting domain of scavenger receptor-BI mediate HDL-initiated signaling. *J. Clin. Invest* 115, 969-977.
44. Navab, M., Berliner, J. A., Subbanagounder, G., Hama, S., Lusis, A. J., Castellani, L. W., Reddy, S., Shih, D., Shi, W., Watson, A. D., Van Lenten, B. J., Vora, D., and Fogelman, A. M. (2001) HDL and the inflammatory response induced by LDL-derived oxidized phospholipids. *Arterioscler. Thromb. Vasc. Biol.* 21, 481-488.
45. Navab, M., Hama, S. Y., Hough, G. P., Subbanagounder, G., Reddy, S. T., and Fogelman, A. M. (2001) A cell-free assay for detecting HDL that is dysfunctional in preventing the formation of or inactivating oxidized phospholipids. *J. Lipid Res.* 42, 1308-1317.
46. Shaul, P. W. (2003) Endothelial nitric oxide synthase, caveolae and the development of atherosclerosis. *J. Physiol* 547, 21-33.
47. Navab, M., Imes, S. S., Hama, S. Y., Hough, G. P., Ross, L. A., Bork, R. W., Valente, A. J., Berliner, J. A., Drinkwater, D. C., Laks, H., and . (1991) Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. *J. Clin. Invest* 88, 2039-2046.
48. Bart, D. G., Stengel, D., Landeloos, M., Lox, M., Le Gat, L., Collen, D., Holvoet, P., and Ninio, E. (2000) Effect of overexpression of human apo A-I in C57BL/6 and C57BL/6 apo E- deficient mice on 2 lipoprotein-associated enzymes, platelet-activating factor acetylhydrolase and paraoxonase. Comparison of adenovirus-mediated human apo A-I gene transfer and human apo A-I transgenesis. *Arterioscler. Thromb. Vasc. Biol.* 20, E68-E75.
49. Nofer, J. R., Kehrel, B., Fobker, M., Levkau, B., Assmann, G., and von Eckardstein, A. (2002) HDL and arteriosclerosis: beyond reverse cholesterol transport. *Atherosclerosis* 161, 1-16.
50. Zannis, V. I., Kypreos, K. E., Chroni, A., Kardassis, D., and Zanni, E. E. (2004) in *Molecular Mechanisms of Atherosclerosis* (Loscalzo, J., Ed.) pp 111-174, Taylor & Francis, New York, NY.
51. Nofer, J. R., van der, G. M., Tolle, M., Wolinska, I., von Wnuck, L. K., Baba, H. A., Tietge, U. J., Godecke, A., Ishii, I., Kleuser, B., Schafers, M., Fobker, M., Zidek, W., Assmann, G., Chun, J., and Levkau, B. (2004) HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. *J. Clin. Invest* 113, 569-581.

52. Barter, P. J., Nicholls, S., Rye, K. A., Anantharamaiah, G. M., Navab, M., and Fogelman, A. M. (2004) Antiinflammatory properties of HDL. *Circulation Research* 95, 764-772.
53. Nofer, J. R., Bot, M., Brodde, M., Taylor, P. J., Salm, P., Brinkmann, V., van Berkel, T., Assmann, G., and Biessen, E. A. (2007) FTY720, a synthetic sphingosine 1 phosphate analogue, inhibits development of atherosclerosis in low-density lipoprotein receptor-deficient mice. *Circulation* 115, 501-508.
54. Gomasrachi, M., Basilico, N., Sisto, F., Taramelli, D., Eligini, S., Colli, S., Sirtori, C. R., Franceschini, G., and Calabresi, L. (2005) High-density lipoproteins attenuate interleukin-6 production in endothelial cells exposed to pro-inflammatory stimuli. *Biochim. Biophys. Acta* 1736, 136-143.
55. Rader, D. J. (2002) High-density lipoproteins and atherosclerosis. *Am. J. Cardiol.* 90, 62i-70i.
56. Bisoendial, R. J., Hovingh, G. K., Levels, J. H., Lerch, P. G., Andresen, I., Hayden, M. R., Kastelein, J. J., and Stroes, E. S. (2003) Restoration of endothelial function by increasing high-density lipoprotein in subjects with isolated low high-density lipoprotein. *Circulation* 107, 2944-2948.
57. Gordon, D. J., Probstfield, J. L., Garrison, R. J., Neaton, J. D., Castelli, W. P., Knoke, J. D., Jacobs, D. R., Jr., Bangdiwala, S., and Tyroler, H. A. (1989) High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 79, 8-15.
58. Miyazaki, A., Sakuma, S., Morikawa, W., Takiue, T., Miake, F., Terano, T., Sakai, M., Hakamata, H., Sakamoto, Y., Natio, M., and . (1995) Intravenous injection of rabbit apolipoprotein A-I inhibits the progression of atherosclerosis in cholesterol-fed rabbits. *Arterioscler. Thromb. Vasc. Biol.* 15, 1882-1888.
59. Nissen, S. E., Tsunoda, T., Tuzcu, E. M., Schoenhagen, P., Cooper, C. J., Yasin, M., Eaton, G. M., Lauer, M. A., Sheldon, W. S., Grines, C. L., Halpern, S., Crowe, T., Blankenship, J. C., and Kerensky, R. (2003) Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *JAMA* 290, 2292-2300.
60. Rubin, E. M., Krauss, R. M., Spangler, E. A., Verstuyft, J. G., and Clift, S. M. (1991) Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. *Nature* 353, 265-267.
61. Paszty, C., Maeda, N., Verstuyft, J., and Rubin, E. M. (1994) Apolipoprotein AI transgene corrects apolipoprotein E deficiency-induced atherosclerosis in mice. *J. Clin. Invest* 94, 899-903.
62. Hughes, S. D., Verstuyft, J., and Rubin, E. M. (1997) HDL deficiency in genetically engineered mice requires elevated LDL to accelerate atherogenesis. *Arterioscler. Thromb. Vasc. Biol.* 17, 1725-1729.
63. Voyiaziakis, E., Goldberg, I. J., Plump, A. S., Rubin, E. M., Breslow, J. L., and Huang, L. S. (1998) ApoA-I deficiency causes both hypertriglyceridemia and increased atherosclerosis in human apoB transgenic mice. *J. Lipid Res.* 39, 313-321.
64. Duverger, N., Tremp, G., Caillaud, J. M., Emmanuel, F., Castro, G., Fruchart, J. C., Steinmetz, A., and Deneffe, P. (1996) Protection against atherogenesis in mice mediated by human apolipoprotein A-IV. *Science* 273, 966-968.
65. Benoit, P., Emmanuel, F., Caillaud, J. M., Bassinet, L., Castro, G., Gallix, P., Fruchart, J. C., Branellec, D., Deneffe, P., and Duverger, N. (1999) Somatic gene transfer of human ApoA-I inhibits atherosclerosis progression in mouse models. *Circulation* 99, 105-110.
66. Tangirala, R. K., Tsukamoto, K., Chun, S. H., Usher, D., Pure, E., and Rader, D. J. (1999) Regression of atherosclerosis induced by liver-directed gene transfer of apolipoprotein A-I in mice. *Circulation* 100, 1816-1822.
67. Boisvert, W. A., Black, A. S., and Curtiss, L. K. (1999) ApoA1 reduces free cholesterol accumulation in atherosclerotic lesions of ApoE-deficient mice transplanted with ApoE-expressing macrophages. *Arterioscler. Thromb. Vasc. Biol.* 19, 525-530.
68. Belalcazar, L. M., Merched, A., Carr, B., Oka, K., Chen, K. H., Pastore, L., Beaudet, A., and Chan, L. (2003) Long-term stable expression of human apolipoprotein A-I mediated by helper-dependent adenovirus gene transfer inhibits atherosclerosis progression and remodels atherosclerotic plaques in a mouse model of familial hypercholesterolemia. *Circulation* 107, 2726-2732.

69. Li, H., Reddick, R. L., and Maeda, N. (1993) Lack of apoA-I is not associated with increased susceptibility to atherosclerosis in mice. *Arterioscler. Thromb.* *13*, 1814-1821.
70. Qasim, A. and Rader, D. J. (2006) Human genetics of variation in high-density lipoprotein cholesterol. *Curr. Atheroscler. Rep.* *8*, 198-205.
71. Kuivenhoven, J. A., Pritchard, H., Hill, J., Frohlich, J., Assmann, G., and Kastelein, J. (1997) The molecular pathology of lecithin:cholesterol acyltransferase (LCAT) deficiency syndromes. *J. Lipid Res.* *38*, 191-205.
72. Frikke-Schmidt, R., Nordestgaard, B. G., Stene, M. C. A., Sethi, A. A., Remaley, A. T., Schnohr, P., Grande, P., and Tybjaerg-Hansen, A. (2008) Association of loss-of-function mutations in the ABCA1 gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. *Jama-Journal of the American Medical Association* *299*, 2524-2532.
73. Tall, A. R., Breslow, J. L., and Rubin, E. M. (2001) in *The Metabolic & Molecular Bases of Inherited Disease* (Scriver, C. R., Beaudet, A. L., Valle, D., and Sly, W. S., Eds.) pp 2915-2936, McGraw-Hill, New York.
74. Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B., and Dawber, T. R. (1977) High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am. J. Med.* *62*, 707-714.
75. Barter, P. and Rye, K. A. (2005) *High density cholesterol: The new target. A Handbook for clinicians* Sherborne Gibbs Limited, Birmingham.
76. Gordon, D. J. and Rifkind, B. M. (1989) High-density lipoprotein--the clinical implications of recent studies. *N. Engl. J. Med.* *321*, 1311-1316.
77. Barter, P., Kastelein, J., Nunn, A., and Hobbs, R. (2003) High density lipoproteins (HDLs) and atherosclerosis; the unanswered questions. *Atherosclerosis* *168*, 195-211.
78. Canner, P. L., Berge, K. G., Wenger, N. K., Stamler, J., Friedman, L., Prineas, R. J., and Friedewald, W. (1986) Fifteen year mortality in Coronary Drug Project patients: long-term benefit with niacin. *J. Am. Coll. Cardiol.* *8*, 1245-1255.
79. Brown, B. G., Zhao, X. Q., Chait, A., Fisher, L. D., Cheung, M. C., Morse, J. S., Dowdy, A. A., Marino, E. K., Bolson, E. L., Alaupovic, P., Frohlich, J., and Albers, J. J. (2001) Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N. Engl. J. Med.* *345*, 1583-1592.
80. Chapman, M. J., Assmann, G., Fruchart, J. C., Shepherd, J., and Sirtori, C. (2004) Raising high-density lipoprotein cholesterol with reduction of cardiovascular risk: the role of nicotinic acid--a position paper developed by the European Consensus Panel on HDL-C. *Curr. Med. Res. Opin.* *20*, 1253-1268.
81. Taylor, A. J., Sullenberger, L. E., Lee, H. J., Lee, J. K., and Grace, K. A. (2004) Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2: a double-blind, placebo-controlled study of extended-release niacin on atherosclerosis progression in secondary prevention patients treated with statins. *Circulation* *110*, 3512-3517.
82. McGovern, M. E. (2004) Use of nicotinic acid in patients with elevated fasting glucose, diabetes, or metabolic syndrome. *Br. J. Diabetes Vasc. Dis.* *4*, 78-83.
83. Rubins, H. B., Robins, S. J., Collins, D., Fye, C. L., Anderson, J. W., Elam, M. B., Faas, F. H., Linares, E., Schaefer, E. J., Schectman, G., Wilt, T. J., and Wittes, J. (1999) Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. *N. Engl. J. Med.* *341*, 410-418.
84. Tardif, J. C., Gregoire, J., L'Allier, P. L., Ibrahim, R., Lesperance, J., Heinonen, T. M., Kouz, S., Berry, C., Basser, R., Lavoie, M. A., Guertin, M. C., and Rodes-Cabau, J. (2007) Effects of reconstituted high-density lipoprotein infusions on coronary atherosclerosis: a randomized controlled trial. *JAMA* *297*, 1675-1682.