special report

# International Symposium on the Role of HDL in Disease Prevention: Report on a Meeting

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The International Symposium on The Role of HDL in Disease Prevention was held on November 7–9, 1996 in Fort Worth, TX. The symposium consisted of 24 lectures and 27 poster presentations.

## Therapeutic Applications of HDL and HDL Analogs

**Dr. Laurence Wong,** from Louisiana State University Medical Center, gave an overview of the biosynthesis and assembly of nascent HDL particles. Subsequently, he presented evidence that free apoA-I could be converted to HDLs through their interaction with fibroblasts. These HDLs have a pre-α electrophoretic mobility on agarose gel electrophoresis. He also described another potential HDL precursor particle containing only phospholipid and apoA-I with pre-α mobility.

**Dr. Jan Johansson,** of Pharmacia & Upjohn, gave a paper entitled "Biological properties and possible mechanism of action of apolipoprotein A-I-Milano (rapoAI-M)/phospholipid complexes." He pointed out that no known cases of coronary heart disease (CHD) have been diagnosed in apolipoprotein A-I Milano carriers. The reason for the extraordinary resistance to CHD is thought to reside in the unusual structural features of apoA-I Milano, as the cysteine residue at position 173 facilitates dimer formation that gives rise to an HDL particle with unique biological properties. Compared to native LpA-I, recombinant (r) apoA-IM dimer/PL complexes have enhanced fibrinolytic properties and improved capacity for cholesterol extraction from cells in vitro. In animal studies inhibition of neointima formation has been demonstrated in two rabbit models. Plaque stabilization and regression of atheroma of aortae was also demonstrated in apoE knockout mice. The rapoAI-M/PL complex is a promising preparation with regard to prevention of restenosis and regression of atherosclerosis.

**Dr. Mats Eriksson,** of the Karolinska Institutet, reviewed his work on the "Effects of intravenous proapoA-I/phosphatidylcholine discs on lipoproteins and fecal steroids in humans". During these studies, 4 g of artificial HDL particles consisting of a complex of recombinant human proapolipoprotein A-I with phosphatidylcholine were infused into patients with familial hypercholesterolemia. After a single infusion of proapoA-I liposomes, total fecal steroid excretion increased over the controls (liposomes without proapoA-I) but no increase in endogenous cholesterol synthesis was noted. The proapoA-I liposomes seemed to be active in mobilization of peripheral cholesterol via reverse cholesterol transport. The proapoA-I/phosphatidylcholine discs may thus provide a new tool for an effective treatment of ischemic coronary heart disease.

**Dr. Luisa Camoglio,** representing a research group from the Academic Medical Center of the University of Amsterdam, gave a paper on the "Antiinflammatory effects of reconstituted high density lipoproteins (HDL) during human endotoxemia." The LPS response in humans was investigated in a double-blind, randomized placebo-controlled, crossover study. Reconstituted HDL (rHDL) was administered to human subjects by a 4-h infusion at 40 mg/kg, starting 3.5 h prior to endotoxin challenge (4 ng/kg). The rHDL treatment reduced flu-like symptoms, but did not influence the febrile response. The endotoxin-induced release of TNF, IL-6, IL-8 was markedly reduced, while only modest attenuation in the synthesis of proinflammatory cytokine inhibitors and soluble TNF receptors was observed. The rHDL infusion, prior to LPS administration, resulted in down-regulation of CD14, the main LPS receptor, on monocytes. These results suggest that rHDL may inhibit LPS induced inflammatory events in humans not only by binding and neutralizing LPS but also by reducing CD14 expression in monocytes.

## Roles of HDL in Disease Prevention, Alternative to Cholesterol Transport

**Dr. Alan Fogelman,** of UCLA Medical School, opened this session by providing an overview of the oxidative

processes impacting lipoproteins and the pathology of the arterial endothelium. Specific emphasis was placed on the antiinflammatory and antiatherogenic roles of HDL. Dr. Fogelman described subpopulations of HDL that carry enzymes with the ability to destroy the biologically active lipids, products of the mild oxidation of LDL in the artery wall. Two such enzyme systems are platelet activating factor acetylhydrolase and paraoxonase. Recent studies show that during an acute phase reaction these enzymes are lost from HDL and ceruloplasmin becomes associated with the HDL particles. The resulting HDL particles potentiate the oxidation of LDL instead of preventing LDL oxidation. Mice that are genetically susceptible to fatty streak formation show a decrease in their HDL paraoxonase activity when they are fed an atherogenic diet, and their HDL particles behave similarly to those obtained during an acute phase reaction that stimulates LDL oxidation. Thus, HDL are actually chameleon-like particles because in the basal state they are anti-inflammatory, but in an inflammatory milieu they become pro-inflammatory.

**Dr. Paul Durrington,** of the University of Manchester, discussed the work being carried out in collaboration with **Dr. Mackness** and other colleagues regarding the involvement of paraoxonase (PON) in the antioxidant properties of HDL. They have demonstrated that HDL associates with enzymatic activity that diminishes the accumulation of $Cu^{2+}$-induced lipid peroxides on LDL. Of the potential enzymes present, PON had a similar effect by decreasing lipid peroxidation in LDL. PON has two alloenzymes that differ in their substrate specificity. The B alloenzyme (arginine191) is more active towards paraoxon, whereas the A alloenzyme (glutamine191) is more active towards sarin. Case-control studies have shown that the B allele is associated with increased risk of coronary heart disease. In subsequent studies, HDL from individuals with different PON genotypes was examined for its ability to diminish the accumulation of lipid peroxides on LDL from the same individuals. Homozygotes for the A allele possessed HDL with the most protective effect, AB heterozygotes were intermediate and HDL from BB homozygotes was the least effective. Circulating PON is the product of the PON 1 gene. Another member of the PON multigene family that is widely expressed, but has no known intra- or extra-cellular gene product, is PON 2. Immunohistochemical studies using a PON antiserum have shown increasing immunolocalization of PON in the aortic wall with advancing atheroma. Further studies are in progress to determine whether this represents PON derived from circulating HDL or elaborated by cells in the advancing lesion.

**Dr. Carole L. Banka,** of the Scripps Research Institute, reported on her work on the role of estrogens in protecting lipoproteins from oxidation. Recent findings show that 17-β-estradiol preferentially protects HDL from oxidative modification under conditions that oxidize HDL and LDL to the same extent. Conjugated diene formation and lipid peroxide generation were inhibited during $Cu^{2+}$-mediated oxidation of HDL by physiologic concentrations (nM) of estradiol. Inhibition of LDL oxidation was seen only at micromolar concentrations. Furthermore, estradiol protected HDL from the protein modifications associated with lipid peroxidation. The preferential protection of HDL lipids and proteins by 17-β-estradiol has not been seen with other antioxidants or with non-estrogenic steroids. A specific interaction between estradiol and HDL is proposed that leads to enrichment of the HDL particle with estradiol and augmentation of the antioxidant potential of HDL. The resulting increased protection against oxidation facilitates the preservation of HDL function.

**Dr. Gillian Cockerill** reviewed her work conducted at the Royal Postgraduate Medical School in London and at the Hanson Centre for Cancer Research in Adelaide, Australia on the regulation of cytokine-induced gene expression by HDL. She and her coworkers have shown that HDL inhibited the cytokine-induced expression of endothelial cell adhesion molecules VCAM-1, ICAM-1, and E-selectin. In her more recent studies, she has demonstrated that HDL does not affect the cytokine-induced synthesis of GM-CSF. This observation is consistent with her earlier findings that HDL had no effect on the binding and translocation of NFkB, the common transcription factor shown to activate the proximal promoters of both the adhesion molecules and the growth factor in response to TNFα. As GM-CSF expression is regulated in part by an upstream enhancer element that is driven by the cyclosporin A-sensitive, $Ca^{2+}$-dependent transcription factor, NFAT, her ongoing studies are based on the hypothesis that HDL can confer differential gene regulation by altering the DNA binding of calcium-dependent transcription factors.

**Dr. Thomas S. Parker** reported on studies carried out with **Dr. Daniel M. Levine** at The Rogosin Institute, Cornell Medical Center. He stated that the reverse cholesterol transport hypothesis may be extended to include the binding and neutralization of water-insoluble toxins by HDL. Gram negative bacteria release a lipopolysaccharide (LPS) endotoxin that activates production of a cascade of inflammatory cytokines including tumor necrosis factor (TNF-α) and interleukin 6 (IL6) by monocyte/macrophages. These cytokines orchestrate the immune response to infection. However, during severe sepsis, over-production of cytokines can lead to shock and death. By incubating LPS with HDL in whole blood, LPS was observed to bind to the phospholipid surface monolayer of HDL and other lipoproteins.

Binding to HDL prevented binding of LPS to CD14, the LPS receptor on monocyte/macrophages, and lowered TNF-α levels in mice. Transgenic Hu-AI mice with high HDL and mice given intravenous HDL were resistant, and Hu-CETP mice with low HDL showed increased sensitivity to LPS. In a second study, low plasma cholesterol and HDL were related to plasma TNF-α and IL6 levels in patients with chronic inflammatory disease. These data and results from other laboratories suggest that lipoproteins (including HDL) bind and neutralize endotoxin in blood. Inflammatory cytokines may cause low cholesterol and HDL in patients with acute and/or chronic disease, and thus expose them to increased risk of endotoxemia.

### Reverse Cholesterol Transport

**Dr. Michael C. Phillips** of the Allegheny University of the Health Sciences, gave an overview of this area followed by a report of his research group's recent findings on HDL and cholesterol efflux from cells. Dr. Phillips discussed the bidirectional flux of unesterified cholesterol molecules that occurs between the plasma membrane and HDL particles in the extracellular medium; net efflux of cholesterol mass occurs via passive diffusion from the cells, through the aqueous phase and down a concentration gradient between the plasma membrane and HDL. Fully lipidated apoA-I is important in promoting this "aqueous diffusion" mechanism because it *1*) acts as a cofactor for LCAT and *2*) solubilizes phospholipid into small HDL-sized particles that are efficient at absorbing cholesterol molecules diffusing away from the cell surface. ApoA-I also exists in an incompletely lipidated state in plasma and in this state it is able to solubilize phospholipid and cholesterol from the plasma membrane of cells. This "membrane-microsolubilization" process is enhanced by enrichment of the plasma membrane with cholesterol, and it is the mechanism whereby pre-β-HDL particles in the extracellular medium remove cholesterol and phospholipid from cells. The relative contributions of the "aqueous diffusion" and "membrane-microsolubilization" mechanisms of apoA-I-mediated cell cholesterol efflux in vivo are not readily predicted from cell culture experiments. Confounding issues are the variation with cell type and the dependence on the degree of cholesterol loading of various domains of the plasma membrane.

**Dr. Phoebe Fielding,** of the Cardiovascular Research Institute at the University of California San Francisco, described a novel pathway that is a major contributor to cholesterol homeostasis in confluent fibroblasts, smooth muscle cells, and the vascular endothelium. Free cholesterol (FC) is selectively internalized from LDL by coated pits at a rate independent of the presence of high affinity LDL receptors. This step is sensitive to cytochalasin and N-ethylmaleimide (NEM) but resistant to nocodazole. Density gradient analysis of cells, pulse-labeled with [$^3$H]FC-containing LDL, showed that the label was internalized first in clathrin-coated pits. Label was subsequently detected in uncoated vesicles and then, together with markers of the trans Golgi network (TGN), in an intermediate density vesicle fraction. At temperatures below 20°C, label was retained with the TGN. At higher temperatures, FC was returned to the cell surface by a nocodazole-dependent, cytochalasin- and brefeldin A-resistant pathway. Cell surface label was then sensitive to cholesterol oxidase in unfixed cells, regulated by okadaic acid and co-migrated with caveolin in a density gradient. These properties are characteristic of FC within the cell surface caveolae of these cells. Cell surface label was rapidly released by extracellular HDL, but was completely resistant to release by extracellular LDL. Most cell-derived [$^3$H]FC label was recovered in the prebeta migrating fraction of HDL. This pathway appears well adapted to regulate cellular FC content at the level of FC efflux in general, and may be of greatest significance in quiescent cells including those of the vascular bed.

**Dr. Shinji Yokoyama,** of Nagoya City University Medical School in Japan, described his studies on "Selective inhibition of apolipoprotein-mediated cell cholesterol efflux." Findings from his laboratory indicate that cellular cholesterol efflux is carried out by two independent mechanisms that are complimentary to one another. The first, consistent with the classical view of the HDL-mediated cell cholesterol efflux, is mediated by cholesterol diffusion through an aqueous phase, and facilitated by a cholesterol gradient including the LCAT reaction. The other involves a lipid-free or lipid-poor helical apolipoprotein that presumably dissociates from HDL surface. Extracellular helical apolipoproteins such as apoA-I, A-II, E, and A-IV interact with the cell surface and generate prebeta-HDL by combining with cellular phospholipids and cholesterol. This interaction requires a selective site on the cell membrane and a specific number (2–4) amphiphilic helical segment of apolipoproteins. The cellular interaction site for apolipoproteins is blocked by probucol, resulting in complete inhibition of apolipoprotein-mediated cellular lipid efflux without influencing the other pathway mediated by the physicochemical mechanism. These findings are consistent with studies using cells from Tangier disease patients, and suggest that the generation of HDL, initiated by lipid-free/lipid-poor apolipoproteins, is a major source of plasma HDL. Cholesterol is selectively mobilized by this pathway from the intracellular pool that is also available for ACAT. This process is apparently regulated by signal transduction.

**Dr. Silvia Santamarina-Fojo** presented her studies car-

ried out with the staff of the Molecular Disease Branch of the NHLBI, titled "Transgenic animals models overexpressing human LCAT". Transgenic mice that overexpress the human LCAT gene have increased plasma concentrations of HDL cholesterol and apoA-I but paradoxically, have enhanced diet-induced atherosclerosis. This finding contrasts with those recently reported for LCAT-transgenic rabbits that have markedly reduced atherosclerosis. One of the major differences in the lipoprotein metabolism of these two animal models is in the expression of cholesteryl ester transfer protein (CETP). Based on the findings presented, Dr. Santamarina-Fojo introduced a hypothesis stating that in the absence of CETP, the LCAT transgenic mouse HDL cannot transfer newly generated cholesterol esters from HDL to apoB-containing lipoproteins, resulting in an HDL with abnormal composition and function. These studies also emphasize the importance of using different animal models to evaluate the atherogenic properties of potential candidate genes for gene therapy.

**Dr. Ruth McPherson,** of the University of Ottawa Heart Institute, presented a paper on studies of the plasma kinetics of recombinant human cholesteryl ester transfer protein (rCETP) in six rabbits before and after cholesterol feeding (0.5% w/w). After intravenous infusion, the labeled rCETP associated with rabbit lipoproteins similar to endogenous rabbit CETP (62–64% HDL-associated). The plasma kinetics of CETP conformed to a two-pool model, likely representing free and loosely HDL-associated CETP (fast pool) and tightly apoA-I-associated (slow pool) CETP. The plasma residency time (chow diet) of the fast pool averaged 7.1 h and of the slow pool, 76.3 h. The production rate (PR) and fractional catabolic rate (FCR) of the fast pool were 20 and 10 times the PR and FCR, respectively, of the slow pool. In response to cholesterol feeding, CETP-PR, FCR, and plasma mass increased by 416%, 60%, and 230%, respectively. There was a strong correlation between the increase in rabbit plasma CETP ($r = 0.95$, $P = 0.003$) and the modeled increase in CETP-PR in response to cholesterol feeding, suggesting that labeled rCETP is catabolized via distinct pools, corresponding to an apoA-I-associated (slow) and a free and/or loosely HDL-associated (fast) pool. Factors that alter the affinity of CETP for HDL would be predicted to result in altered CETP catabolism. The effect of dietary cholesterol on plasma CETP mass can be largely explained by its effects on CETP synthesis, consistent with the observed effects of cholesterol feeding on tissue mRNA levels

### Regulation of HDL Levels

**Dr. Aldons Lusis,** of UCLA Medical Center, discussed his current research on the dissection of genetic factors controlling HDL in vivo. Although human studies of candidate genes have revealed the causes of certain relatively rare syndromes, the genetic factors contributing to common variations in HDL metabolism are poorly understood. Analysis of complex traits is greatly simplified in the mouse model. Over the years, a large number of variations in HDL metabolism have been noted among inbred strains of mice. Through analysis of extensive genetic crosses between these strains by complete linkage mapping, over a dozen genetic loci have been identified to contribute to the regulation of HDL levels or structural features. Meanwhile, known candidate genes for HDL have been mapped in the mouse. Because most of the loci controlling HDL metabolism do not coincide with the locations of known candidate genes, many of the genes or pathways contributing to HDL metabolism remain to be identified. Using a candidate gene approach, some genes representing a number of loci impacting HDL levels were identified, including the genes of apoA-II (chromosome 1) and LCAT (chromosome 8). Many of the loci exhibited strong genetic–dietary interactions. For example, a decrease in HDL levels in response to an atherogenic diet in strain C57BL/6J mice was shown to be due to multiple loci, associated with decreased cholesterol-7α-hydroxylase activity. Clear interactions with body fat and HDL levels were also observed. Two of these loci were associated with hepatic lipase activity.

**Dr. Jonathan Cohen,** of the University of Texas Southwestern Medical Center, discussed the application of linkage analysis to the determination of factors impacting on HDL-C levels in humans. The use of linkage analysis to evaluate candidate genes is particularly applicable to the HDL-C system because many of the factors that control HDL levels have been identified and several of the relevant genes have been cloned. Dr. Cohen focused his studies on polymorphism in the hepatic lipase gene that, based on initial findings, accounts for approximately 25% of the variation in HDL levels. A detailed analysis of an extended sample provided greatly increased power for this analysis. Sibling pair linkage analysis of the observed HDL-C levels using all possible pairs (n = 1995) in the data set revealed significant linkage ($P = 0.01$) between hepatic lipase and plasma HDL-C. After adjusting the data for the effects of age and sex and excluding individuals with confounding factors (such as high triglyceride levels, diabetes, hormone or lipid-lowering drug use) the sample size was reduced to 1100 pairs, but the $P$ value remained significant ($P = 0.03$). Variance components analysis indicated that polymorphism in the hepatic lipase gene still accounted for approximately one quarter of the variation in HDL-C levels. These data provide strong evidence that polymorphism in the hepatic lipase gene affects plasma HDL-C levels.

**Dr. Omar Francone,** of Pfizer Research Laboratories,

reported his studies on the molecular mechanism(s) responsible for the generation of pre-β-HDL. To study the effects of LCAT, CETP, and PLTP in the speciation of HDL in vivo, HuapoA-I transgenics were crossed with HuLCAT, HuCETP, and HuPLTP transgenics, resulting in mice expressing different combinations of these genes. The increase in HDL-cholesterol levels, observed in transgenic mice expressing the HuLCAT and apoA-I transgenes, was also reflected in the HDL particle size distribution. HDL isolated from HuapoA-I transgenics showed a bimodal distribution with the peak diameter corresponding to the $HuHDL_2$ and $HDL_3$. On the other hand, HDL isolated from transgenic mice expressing the HuLCAT and HuapoA-I showed a predominant peak of larger particles. The simultaneous expression of the CETP and the apoA-I transgenes resulted in a 2-fold increase in the proportion of HuapoA-I in the pre-β-HDL fraction and enhancement of efflux and esterification of cell-derived cholesterol, suggesting that CETP could contribute to reverse cholesterol transport not only by delivering cholesteryl esters to the liver via VLDL + LDL but also by generating small HDL species that stimulate the efflux of cholesterol. The simultaneous expression of PLTP and HuapoA-I also resulted in changes in the HDL species including increases, suggesting that PLTP increases the influx of phospholipid and, secondarily, cholesterol into HDL, leading to an increase in potentially antiatherogenic pre-β-HDL particles.

**Dr. Helen Hobbs,** of the University of Texas Southwestern Medical Center, discussed the results of studies performed in her laboratory, in collaboration with **Dr. Monty Krieger,** of MIT, to explore the regulation of the most recently cloned and characterized member of the scavenger receptor family SR-BI. SR-BI is a transmembrane protein (~82 kDa) that binds native LDL, acetylated LDL, as well as anionic phospholipids. The expression of SR-B1 in Chinese hamster ovary (CHO) cells results in high affinity association and the selective transfer of cholesteryl esters to the cells, but no degradation of $^{125}$I-labeled HDL. The highest tissue levels of SR-BI in rats, mice, and hamsters are in the adrenal gland and ovary, with the next highest levels of expression in the liver, preputial gland, and mammary gland of the pregnant animal. The tissue distribution of expression suggested that this receptor may mediate the well-characterized pathway by which HDL-cholesterol is selectively delivered to steroidogenic tissues and the liver. To probe the regulation of the receptor, rats were made profoundly hypolipidemic with high-dose estrogen to up-regulate the hepatic LDL receptor. These animals showed a dramatic increase in immunodetectable SR-BI in the zona fasciculata of the adrenal gland and in the corpus luteum of the ovary, while the levels of the receptor fell to nearly undetectable levels in the liver. Thus, SR-BI is likely to play a central role in the delivery of HDL-associated lipids to steroidogenic tissues as well as to the liver, breast, and preputial gland. The signaling mechanisms by which the levels of expression of this receptor are regulated remain to be elucidated.

**Dr. Peter H. Weinstock,** of The Rockfeller University, reported on studies of triglyceride and HDL metabolism in lipoprotein lipase (LPL)-induced mutant mice (LPL). LPL has long been thought to regulate HDL levels by two potential mechanisms: *1*) post-hydrolytic surface components of triglyceride-rich lipoproteins either add to circulating HDL or form nascent HDL, and *2*) LPL regulates the amount of TG substrate available for CETP-mediated transfer. LPL knockout mice were produced via gene targeting in embryonic stem cells. Disruption of both LPL alleles proved lethal within 18 h after the initiation of suckling. Prior to suckling, knockout mice had a 400% increase in triglycerides, yet had normal amounts of HDL when compared with controls. At 18 h of life, after suckling, knockout mice became severely hypertriglyceridemic (HTG, >15,000 mg/dl) and entirely deficient in HDL-C. Heterozygotes lived to adulthood and demonstrated a mild HTG (2-fold increase) with normal HDL levels as compared with controls. In the presence of CETP, the HTG caused further reductions in HDL as compared to CETP alone.

The goals of these studies were to rescue the knockout mice, produce animals that express human LPL only, and to determine the potential tissue-specific effects of LPL on HDL metabolism. The expression of a human LPL transgene, driven by a muscle-specific promoter (MCK), was superimposed via crossbreedings onto either the normal (L2-MCK) or LPL-null (L0-MCK) background. Inhibition of human LPL in mice expressing human LPL only (L0-MCK), caused a 4-fold increase in TG within 6 h of injection of antibody. In the absence and presence of CETP, antibody inhibition of LPL in these mice caused a 15% and 45% reduction in HDL, respectively. When compared with controls, both L2-MCK and L0-MCK mice showed reduced triglyceride levels that correlated with the amount of muscle LPL activity rather than PHP-LPL activity. Despite reduced triglyceride levels, these mice showed reductions in HDL-C, particularly in the absence of LPL expression in non-muscle tissues. Findings from LPL-induced mutant mice demonstrate for the first time the relative contributions of CETP-dependent and -independent mechanisms of LPL regulation of HDL, and begin to shed light on tissue-specific effects of the enzyme on HDL metabolism.

**Dr. Matti Jauhiainen,** of the National Public Health Institute, Helsinki, Finland, reported on his work on phospholipid transfer protein (PLTP) remodeling of HDL. They have recently determined the phospholipid

acyl chain and headgroup specificity of PLTP and have demonstrated that PLTP can promote the conversion of HDL (diameter of 8.5 nm) into populations of larger (11–12 nm) and smaller particles (7.0–7.4 nm). In addition, they have examined the mechanism of this conversion process that involves fusion of HDL particles. The smaller particles generated by PLTP treatment contain apolipoprotein A-I as their only protein component and some phospholipids. These particles have pre-β-HDL characteristics and they can function as primary acceptors of cell membrane cholesterol from fibroblasts in culture. Therefore, when considering the role of HDL (pre-β-HDL) in reverse cholesterol transport, the function of PLTP in HDL conversion is physiologically relevant. PLTP-mediated HDL conversion is regulated by the composition of HDL apolipoproteins and core neutral lipids. ApoA-II is inhibitory in this conversion process, whereas elevated levels of core triglycerides increase the conversion. These findings suggest that PLTP action favors the utilization of specific HDL subpopulations in the HDL fusion process.

### Diagnosis and Treatment of Low HDL Syndromes

**Dr. Gloria Vega,** of the University of Texas Southwestern Medical Center, reviewed the strategies for the treatment of low HDL cholesterol. Although treatment of high LDL with potent cholesterol-lowering drugs has led to reduction in morbidity and mortality from coronary heart disease (CHD), the patients enrolled in clinical trials with low HDL did not have the same benefits as those with normal HDL levels. Low HDL cholesterol thus remains an unresolved clinical problem, particularly because low HDL is an independent high risk condition for CHD, whether it presents as isolated low HDL or in association with hypertriglyceridemia or combined hyperlipidemia. Recent studies have shown that 75% of patients with low HDL exhibit abnormal LDL metabolism resulting from defective clearance of VLDL+IDL. Treatment of isolated low HDL with gemfibrozil results in a reduction of cholesterol and triglyceride levels in VLDL+IDL, and small increments in HDL and LDL. Levels of plasma triglycerides are also reduced. Statins have a more desirable effect on the reduction of non-HDL lipoproteins than gemfibrozil and also reduce the ratios of LDL to HDL and of total cholesterol to HDL without normalizing HDL-C per se. In contrast, nicotinic acid at a dose of 4.5 g/day is very effective in normalizing HDL-cholesterol, in addition to lowering VLDL+IDL. However, unlike statins, nicotinic acid does not lower LDL cholesterol levels in isolated low HDL. Unfortunately, nicotinic acid has a number of untoward side effects that make long-term compliance difficult. For this reason, two different doses of crystalline nicotinic acid were tested for efficacy in raising HDL and lowering VLDL+IDL cholesterol levels in isolated low HDL. A dose of 1.5 g per day was effective in lowering VLDL+IDL and in raising HDL, but not as effective as the 3.0 g dose. A low dose of nicotinic acid, however, failed to reduce LDL levels effectively. Therefore, a combination of low dose nicotinic acid and simvastatin was tested for efficacy and tolerability in a pilot study. This drug combination proved very effective in normalizing the lipoprotein profile in isolated low HDL. Levels of VLDL, IDL, and LDL were reduced optimally, and HDL concentrations were increased to normal levels. There were no untoward side effects observed during this trial. Thus, combined drug therapy may be the most effective way to treat patients with combined lipoprotein abnormalities.

**Dr. Ernst J. Schaefer** reported on his studies of "Lifestyle and pharmacologic strategies for raising low HDL levels" conducted at Tufts University and New England Medical Center, Boston, MA. Low levels of high density lipoprotein (HDL) cholesterol (<35 mg/dl or 0.9 mmol/L) have been shown to be an independent risk factor for premature coronary heart disease (CHD). Factors related to a low HDL cholesterol include male gender, obesity, sedentary lifestyle, and elevated triglyceride levels. Common genetic disorders associated with low HDL cholesterol include familial combined hyperlipidemia, familial dyslipidemia, and familial hypoalphalipoproteinemia. Exercise and weight reduction will increase HDL cholesterol, especially when subjects lose a substantial amount of weight and then weight-stabilize. Diets low in saturated fat, cholesterol, and total fat are the most efficacious for lowering low density lipoprotein (LDL) cholesterol when studied under ad libitum circumstances, because they promote weight loss. In the isoweight situation, however, such diets lower HDL, and for this reason some investigators have recommended a high monounsaturated fat diet. However, such a diet is more calorically dense because it tends to exceed 30% of calories as fat. Medications that are effective in raising HDL cholesterol include estrogen therapy in postmenopausal women, which can raise HDL cholesterol 20–25% and raise apoA-I levels by as much as 50%; niacin treatment of 3 grams per day which can raise HDL cholesterol levels by as much as 30%; gemfibrozil therapy, which raises HDL cholesterol levels 15% in patients with decreased HDL cholesterol levels; and HMG-CoA reductase inhibitors, which raise HDL cholesterol only modestly but markedly lower LDL cholesterol. No guidelines have been formulated for pharmacological management of decreased HDL cholesterol because of lack of prospective studies in patients selected for having a low HDL cholesterol. However, data from the Helsinki Heart Study, the Lipid Research Clinics Trial, and other studies do indicate that HDL

cholesterol raising is of benefit in reducing CHD risk. Guidelines for therapy can only be formulated after prospective studies are completed.

**Dr. Sander Robins,** of Boston University Medical Center, reported on the Veterans Administration gemfibrozil intervention trial that he is co-directing with **Dr. Hanna Rubins.** The VA-HDL Intervention Trial (HIT) was initiated in 1991 by the Veterans Affairs Cooperative Studies Program with the financial support of Parke-Davis to determine whether lipid intervention with the fibric acid derivative, gemfibrozil, reduces the combined incidence of coronary heart disease death and non-fatal myocardial infarction in men with known CHD, low HDL-C, and a desirable LDL-C. This is a randomized, double-blind, placebo-controlled trial in which 2531 men have been enrolled and are being followed for 5–7 years. Baseline lipids (in mg/dL) of the subjects in this study are very different from previous or ongoing large clinical trials with mean HDL-C of 32, LDL-C of 111, triglycerides of 161, and total cholesterol of 175. This lipid pattern is representative of a sizable segment of the male population with CHD in the U.S. at present. Baseline features of the HIT study population are representative of patients with CHD and a low HDL-C, and are notable for a high prevalence of type II diabetes (25%), hypertension (57%), and obesity (55% with $>27.8$ kg/m$^2$) with high fasting plasma insulin (76% with insulin $> 24$ μU/ml). The study is projected to end in September 1998 and, if gemfibrozil treatment reduces the incidence of CHD events, would be the first clinical trial to demonstrate the benefits of lipid drug therapy in patients with a distinctly low HDL-C.

**Dr. Abhimanyu Garg,** of the University of Texas Southwestern Medical Center, presented a paper titled "HDL, insulin resistance and non-insulin-dependent diabetes mellitus". Several recent studies have shown that insulin resistance may be a factor in causing low HDL cholesterol levels. Low HDL cholesterol levels in patients with non-insulin-dependent diabetes mellitus (NIDDM) is likely to develop because most of them have severe insulin resistance. Insulin resistance may cause low HDL cholesterol by several mechanisms. Diminished activity of LPL may result in excessive transfer of triglycerides from triglyceride-rich chylomicrons and VLDL particles in exchange for cholesteryl esters from HDL particles, thus reducing levels of HDL cholesterol. The resulting triglyceride-rich $HDL_2$ particles are quickly converted to $HDL_3$ particles because of elevated HTGL activity. Insulin stimulates production and secretion of apoA-I in primary cultures of rat hepatocytes. Whether insulin affects apoA-I production and secretion from intestinal cells is not clear. Thus, in insulin-resistant states, synthesis and secretion of apoA-I in the liver and perhaps in the intestine may be reduced. Kinetic studies, however, report increased catabolic rate of apoA-I to be the primary mechanism for reduced HDL levels in patients with NIDDM. Additionally, reduced HDL levels in insulin-resistant states may be due to reduced LCAT activity or increased CETP activity. Recently, increased CETP activity and mass have been reported in obese subjects. The precise involvement of insulin action in the regulation of the activities of LCAT, CETP, and PLTP is not yet clear.

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