special report

International Symposium on Basic Aspects of HDL Metabolism and Disease Prevention

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The "International Symposium on Basic Aspects of HDL Metabolism and Disease Prevention" was held on April 9, 2000 at the Renaissance Polat Hotel in Istanbul, Turkey. The scientific sessions were conducted in collaboration with the European Atherosclerosis Society (EAS) and the Turkish Cardiology Society and were preceded by an EAS workshop on "Low HDL in Cardiovascular Diseases." The symposium represented a continuation of a tradition carried on by previous conferences including the "International Symposium on High Density Lipoproteins and Atherosclerosis" (the third symposium, held in San Antonio, TX in 1992) and the "International Symposium on Reverse Cholesterol Transport and Coronary Heart Disease" (the second symposium, held in 1992 in Fort Worth, TX), "Molecular Basis of HDL Antiatherogenicity" (held in 1994 in Halifax, Nova Scotia, Canada), and the "Role of HDL in Disease Prevention" (held in 1996 in Fort Worth, TX). The meeting summarized in this communication was the first in this series held outside North America.

The symposium was originally intended to take place in September 1999; however, it had to be postponed because of the damage caused by the earthquakes in Turkey. The symposium consisted of 15 lecture presentations, organized under the following four topics.

HDL Functions Other Than Cholesterol Transport

Mohamad Navab (UCLA Medical Center, Los Angeles, CA) described his recent studies of the role of "seeding molecules" in low density lipoprotein (LDL) that promote oxidation and ultimately atherosclerosis and include 13-hydroperoxyoctadecadienoic acid and 15-hydroperoxyeicosatetraenoic acid. Apolipoprotein A-I (apoA-I), high density lipoprotein (HDL), or paraoxonase (PON) can remove/inactivate "seeding molecules" from LDL and render it resistant to oxidation by cells of the arterial wall. Injection of apoA-I into mice and infusion of apoA-I into humans render their LDL resistant to oxidation. Treatment of human artery wall cells with apoA-I, HDL, or paraoxonase renders the cells unable to oxidize LDL. Therefore: *a*) oxidation of LDL requires "seeding molecules"; *b*) normal HDL and its components can remove or inactivate lipids in fresh LDL that are required for oxidation; *c*) mildly oxidized LDL is formed in three steps, each of which can be inhibited by normal HDL; and *d*) HDL from at least some coronary artery disease patients is defective both in its ability to prevent LDL oxidation and to inhibit the biologic activity of oxidized phospholipids.

Richard W. James (University Hospital, Geneva, Switzerland) reported on his work on the epidemiological aspects of PON. His laboratory has identified several, distinct genetic and environmental factors that modulate serum PON levels. These include promoter polymorphisms of the *PON1* gene that are associated with strong variations in serum PON levels. They, in large measure, account for variations in serum PON previously associated with the coding region polymorphism L54M. They recently demonstrated that the L and M isoforms arising from polymorphism L54M differ in their proteolytic susceptibility and thus make an independent contribution to variations in serum PON. Among environmental factors, they have identified diabetes, smoking, and increased age as independent determinants of serum PON concentrations. The studies suggest a panoply of genetic and environmental factors that influence serum PON levels and the antioxidant capacity of HDL. These appear to modulate the ability of paraoxonase to offer a measure of protection against the risk of coronary disease.

Paul W. Baker (Institute of Medical and Veterinary Science, Adelaide, South Australia, Australia) reviewed his studies on the influence of reconstituted HDL (rHDL) phosphatidylcholine on endothelial cell adhesion molecule expression. Significant concentration-dependent inhibition of tumor necrosis factor α (TNF-α)-induced vascular cell adhesion molecule (VCAM-1) expression was observed after preincubation of human umbilical vein endothelial cells with rHDL containing either 1-palmitoyl-2-linoleoyl-phosphatidylcholine (PLPC) or 1-palmitoyl-2-arachidonyl-phosphati-

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dylcholine (PAPC). In contrast, rHDL containing either 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) or 7-dehydrocholesterol choline were found to be poor inhibitors of VCAM-1 expression. In all cases rHDL containing PLPC were significantly more inhibitory than those containing PAPC. This hierarchy of inhibition was evident whether the PLPC or PAPC rHDL were present or absent during activation of the cells with TNF-α. Investigation of the inhibition of VCAM-1 expression by rHDL with molar ratios of PLPC to POPC to apoA-I ranging from 100:0:1 to 0:100:1 showed that rHDL containing 100% PLPC were effective while those containing 100% POPC were poor inhibitors. As the molar ratio of POPC to PLPC increased the inhibitory activity of the rHDL decreased. These studies demonstrate that *i*) the content of PC is central to the inhibitory activity of rHDL; *ii*) the fatty acid composition of rHDL PC is an important determinant of the inhibition; *iii*) rHDL PC do not modulate inhibitory activity by affecting rHDL binding to the cells surface; and *iv*) inhibition is not due to interference by rHDL in the binding of TNF-α to the cells. These results suggest that the type of dietary fat consumed may influence the inhibitory activity of human HDL.

Regulation of HDL Levels

Bart Staels (Institut Pasteur de Lille, Lille, France) presented recent findings on the regulation of HDL metabolism by fibrates via peroxisome proliferator-activatored receptor α (PPARα). He has focused on studies of the transcriptional control of HDL metabolism, primarily the role of specific transcription factors of the nuclear receptor gene superfamily. The nuclear receptor PPARα has been identified as a transcriptional regulator of HDL metabolism in response to fenofibrate treatment. Fenofibrate via PPARα regulates the transcription of genes determining HDL levels, such as apoA-I and apoA-II. In addition, absence of PPARα expression profoundly perturbs HDL metabolism. This model is supported by the finding that polymorphisms in PPARα gene structure are associated with altered serum lipid and apoA-I levels in type II diabetic patients. Altogether, these results identify PPARα as the nuclear receptor mediating the actions of fenofibrate on HDL metabolism.

Wolfgang Patsch (Landeskliniken, Salzburg, Austria) described his laboratory's recent findings on the regulation of hepatic apoA-I gene expression by thyroid hormone. In rats, a single receptor-saturating dose of triiodothyronine (T_3) increased the transcriptional activity of the apoA-I gene as well as the abundance of nuclear and total cellular apoA-I ribonucleic acid (RNA) in liver. With chronic administration of T_3, the synthesis of apoA-I mRNA and the abundance of primary transcripts were reduced by half, but the abundance of nuclear and total cellular apoA-I RNA as well as the plasma apoA-I level remained elevated. Compartmental modeling of in vivo nuclear A-I transcript abundance data under steady state conditions and after the injection of actinomycin D suggested that T_3 enhanced apoA-I mRNA maturation 7-fold by protecting the mRNA precursor devoid of intron 2, but containing introns 1 and 3, from degradation or by facilitating splicing of intron 1 from this precursor. Measurement of transcription across the apoA-I gene revealed transcription elongation blocks that were twice as effective in T_3-treated rats in comparison with controls. Cell-free transcription studies with deletion constructs as templates identified two arrest sites as well as the respective arrest signals. These data are consistent with the model that in controls, a large portion of apoA-I transcripts does not mature because of degradation of a specific mRNA precursor that contains introns 1 and 3. In T_3-treated animals, intron 1 splicing is enhanced and/or degradation of the intron 1-containing RNA intermediate is reduced. As a result, mRNA maturation is increased, fewer exon 1-intron 1 fragments are created, and fewer transcripts are rescued from the elongation hindrance. Hence, transcriptional attenuation blunts the effects of enhanced mRNA maturation on apoA-I gene expression. Further elucidation of the processes linking RNA maturation and transcript elongation is necessary for the development of strategies that permit maximal increases of apoA-I levels in the circulation.

Arie van Tol (Erasmus University Rotterdam, Rotterdam, The Netherlands) presented his studies on the phospholipid transfer protein (PLTP)-lecithin:cholesterol acyltransferase (LCAT) cycle in reverse cholesterol transport, utilizing transgenic mice that overexpress human PLTP (huPLTPTg). Overexpression of PLTP resulted in a 30–40% decrease in plasma cholesterol levels, due to reduction of HDL of virtually normal size. On incubation in the presence of an LCAT inhibitor, plasma from transgenic mice showed increased rates of pre-βHDL formation. Plasma from huPLTPTg was more efficient in preventing acetylated LDL-induced cholesterol accumulation in macrophages than plasma from wild-type animals. Therefore PLTP may act as an antiatherogenic factor that enhances reverse cholesterol transport by generation of pre-βHDL. Together PLTP and LCAT catalyze a cyclic process through which conversion takes place between α-HDL and pre-βHDL.The PLTP-LCAT cycle is proposed to be important in reverse cholesterol transport, with PLTP-mediated formation of pre-βHDL as the rate-limiting (controlling) step. Plasma from human apoA-I overexpressing transgenic mice also prevented cholesterol accumulation in the macrophages, but the combination of human apoA-I and human PLTP genes did not show additive effects. Actually, the PLTP gene reversed the AI gene effect. So PLTP may either inhibit or stimulate reverse cholesterol transport, depending on the genetic background.

Jonathan Cohen (Southwestern Medical School, Dallas, TX) reviewed his laboratory's recent findings on hepatic lipase polymorphisms and plasma HDL levels. A hepatic lipase allele (the −514T allele defined by four linked polymorphisms in the 5′ flanking sequence) has been associated with decreased hepatic lipase activity and increased plasma HDL-cholesterol (HDL-Chol) concentrations. However, the strength of this association has varied in different study populations. Hepatic lipase activity of −514T and −514C homozygotes was determined in African Americans, white Americans, Turks, and Chinese. The effect of this

polymorphism on hepatic lipase activity was markedly greater in Turks and white Americans (~10 mmol h^{-1} $liter^{-1}$ per copy of the −514C allele) than in African Americans and Chinese (~5 mmol h^{-1} $liter^{-1}$ per copy of the −514C allele). Stanozolol, a synthetic androgen, increased hepatic lipase activity to the same extent in men with normal, moderately elevated, and markedly elevated plasma triglyceride concentrations. However, the decrease in plasma HDL-C concentrations was greatest in men with low plasma triglyceride concentrations, and smallest in men with high plasma triglyceride concentrations. These data indicate that the association between the −514 polymorphism and plasma HDL-Chol concentrations is influenced by factors that modulate the effect of the polymorphism on hepatic lipase activity, and by factors that modulate the effect of hepatic lipase activity on HDL-Chol concentrations.

Omar L. Francone (Pfizer Central Laboratories, Groton, CT) reported on studies on the human ATP-binding cassette transporter 1 (ABC1) gene. ABC1 has been demonstrated to be mutated in patients with Tangier disease. To investigate the role of this ABC1 protein in an experimental in vivo model, gene targeting in DBA-1J ES cells was used to produce ABC1-deficient mice. Expression of the murine *Abc1* gene was ablated with a nonisogenic targeting construct that deletes six exons encoding the first nucleotide-binding fold. Lipid profiles from *Abc1* knockout (−/−) mice revealed an ~70% reduction in cholesterol, markedly reduced plasma phospholipids, and an almost complete lack of HDL when compared with wild-type littermates. Fractionation of lipoproteins by fast protein liquid chromatography (FPLC) demonstrated dramatic alterations in HDL-Chol including the near absence of apoA-I. LDL cholesterol (LDL-Chol) and apoB were also significantly reduced in +/− and −/− mice compared with their littermate controls. The inactivation of the *Abc1* gene led to an increase in the absorption of cholesterol in mice fed either chow or a high fat and cholesterol diet. Histopathologic examination of $Abc1^{-/-}$ mice at ages 7, 12, and 18 months, demonstrated a striking accumulation of lipid-laden macrophages and type II pneumocytes in the lungs. Taken together, these findings demonstrate that $Abc1^{-/-}$ mice display pathophysiologic hallmarks similar to human Tangier disease and highlight the capacity of ABC1 transporters to participate in the regulation of dietary cholesterol absorption.

Interaction of HDL with Plasma Components

Kerry-Anne Rye (Hanson Centre, Adelaide, Australia) presented her findings on interactions of HDL with hepatic lipase. To resolve conflicting data regarding the impact of different apolipoproteins on the hepatic lipase reaction, homogeneous preparations of spherical reconstituted HDL (rHDL) containing either apoA-I only, (A-I)rHDL, or apoA-II only, (A-II)rHDL, were incubated individually with hepatic lipase (HL). The results showed that HL has a greater affinity (K_m) for (A-II)rHDL than for (A-I)rHDL, but that the maximal rate (V_{max}) of phospholipid (PL) and triglyceride (TG) hydrolysis is greater in (A-I)rHDL than in (A-II)rHDL. When mixtures of (A-I)rHDL and (A-II)rHDL were both present in the incubation, the PL hydrolysis was two to three times greater than predicted from the hydrolysis of the individual rHDL preparations. This was due to increased PL hydrolysis in the (A-II)rHDL, not the (A-I)rHDL. This result could not be explained by the formation of rHDL containing apoA-I and apoA-II on the same particle, (A-I/A-II)rHDL. Anti-apoA-I immunoaffinity chromatography revealed that (A-I/A-II)rHDL constituted <3% of the total rHDL in the incubation. (A-I/A-II) rHDL were also prepared and incubated with HL. The K_m and V_{max} of the PL hydrolysis in (A-I/A-II)rHDL was intermediate between that of (A-I) rHDL and (A-II)rHDL These studies show that *i*) HL has a greater affinity (K_m) for (A-II)rHDL than for (A-I)rHDL; *ii*) V_{max} of phospholipid hydrolysis is greater in (A-I)rHDL than in (A-II)rHDL; *iii*) K_m and V_{max} of phospholipid hydrolysis in (A-I/A-II) rHDL is intermediate between that of (A-I) rHDL and (A-II)rHDL; *iv*) (A-II)rHDL are competitive inhibitors of phospholipid hydrolysis in (A-I)rHDL; and *v*) (A-I)rHDL enhance phospholipid hydrolysis in (A-II)rHDL.

Matti Jauhiainen (National Public Health Institute, Helsinki, Finland) reported on his recent findings regarding the interactions of HDL with PLTP. Recent in vivo data from PLTP knockout mice indicate that transfer of phospholipids from triglyceride-rich lipoproteins to HDL during lipolysis is facilitated by PLTP and that this transfer plays a major role in regulating plasma HDL levels. Adenovirus-mediated overexpression of PLTP (AdPLTP) in mice indicated that PLTP in this model also caused the interconversion of HDL, leading to the appearance of large HDL particles as well as pre-βHDL. There was a clear positive correlation between AdPLTP mouse serum PLTP activity and the ability to form pre-βHDL, which strongly suggests that one function of PLTP in vivo is to generate pre-βHDL during the HDL conversion process. PLTP was shown to bind to both apoA-I and apoA-II in a solid ligand-binding assay, and PLTP binding was localized to the amino-terminal region of apoA-I. Recent data derived from molecular modeling and site-directed mutagenesis of PLTP demonstrated the importance of the lipid-binding pockets in PL transfer and HDL interaction. The structure model and PLTP mutants will be used to clarify the precise mechanism of PLTP-mediated PL transfer and HDL interconversion.

Arnold von Eckardstein (Institute of Clinical Chemistry and Laboratory Medicine and Institute of Arteriosclerosis Research, Munich, Germany) reviewed his laboratory's recent efforts in the area of the role of HDL in reverse cholesterol transport. The presentation focused on the individual roles of the respective minor HDL components in this system. Pulse-chase experiments identified the quantitatively minor HDL subclasses pre-β_1-LpA-I, LpA-IV, and γ-LpE as initial and fast acceptors of cell-derived cholesterol and α-HDL as a late and slow acceptor. Likewise, apolipoproteins A-I, A-IV, and E induce fast and saturable cholesterol efflux from various cells including fibroblasts and macrophages whereas HDL induced slow and nonsaturable cholesterol efflux. In addition, HDL has been shown to stimulate multiple cellular signaling pathways.

In fibroblasts, both HDL_3 and lipid-free apoA-I activate phosphatidylcholine (PC)-specific phospholipases C and D whereas HDL_3 but not lipid-free apoA-I activate phosphatidylinositol (PI)-specific phospholipase C and, as a consequence, mobilization of intracellular Ca^{2+}. Stimulation of PC breakdown increases cholesterol efflux. In contrast, pharmacological inhibition of HDL-mediated PI biphosphate breakdown and Ca^{2+} mobilization had no effect on cholesterol efflux but inhibited HDL-induced proliferation of fibroblasts and smooth muscle cells. PI biphosphate turnover and Ca^{2+} mobilization were mimicked by lipids extracted from HDL. Thus, HDL triggers cellular signaling by at least two agonists: apoA-I stimulates PC breakdown and thereby induces cholesterol efflux, whereas an as yet unknown lipid of HDL activates PI-specific phospholipase C and thereby can induce proliferation of fibroblasts and smooth muscle cells. These findings, taken together with data from studies in transgenic animals, indicate that pharmacological modulation of HDL cholesterol levels may not necessarily imply equidirectional modifications of the antiatherogenic potential of HDL.

HDL Receptors and Clinical Implications

Marisa Vinals (Massachusetts Institute of Technology, Cambridge, MA) has discussed her laboratory's recent findings on the role of scavenger receptor class B type I (SR-BI) in regulating HDL. To study the effect of SR-BI on atherosclerosis, SR-BI mutant mice were crossed with apoE mutant mice. The plasma cholesterol increased in the double knockout (KO) relative to the apoE single KO and the normal-sized HDL cholesterol peak disappeared. There were no differences in plasma levels of apoA-I but there was a shift of apoA-I to larger particles. The study of the size distribution of HDL showed that, in the double KO, almost all the cholesterol was in abnormally large particles, [very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL)/LDL]. Further analysis of IDL/LDL-sized particles suggested the presence of large HDL-like particles that contained apoA-I but not apoB. The disappearance of the HDL peak was attributed to the formation of abnormally large HDL-like particle due to the absence of SR-BI and apoE-mediated cholesterol uptake. Atherosclerosis was assessed by analyzing the aortic root atherosclerotic plaque formation. There were no detectable lesions in the single-KO mice at this relatively young age. However, there was a substantial, statistically significant lesion development in the double-KO mice in the aortic root region. Thus, SR-BI plays a protective role against atherosclerosis formation in apoE single-KO mice.

Noel Fidge (Baker Medical Institute, Melbourne, Australia) presented his work on the cloning and properties of an HDL receptor. A candidate HDL receptor, HB_2, that is homologous to several adhesion molecules, has recently been cloned. Because some adhesion proteins have signaling properties, experiments were carried out to identify HDL-binding domains on the extracellular region of HB_2. Four peptides including residues 1–527 (entire extracellular domain) and truncates 1–415, 1–334, and 1–247 were expressed in a baculovirus vector and subjected to ligand blotting. The proteins encompassing amino acids 1–415 revealed no binding whereas amino acids 1–527 bound HDL, apoA-I, and apoA-II but not LDL, indicating that a region proximal to the membrane domain contains a specific HDL-binding site. Because HB_2 is not involved in lipid transfers, but possesses a specific extracellular HDL-binding site, justifies further investigation of its role as a signaling receptor.

H. Bryan Brewer, Jr. (Molecular Disease Branch, NHLBI, NIH, Bethesda, MD) provided an overview on new insights into HDL metabolism, reverse cholesterol transport, and the development of atherosclerosis. Although high and low HDL levels are associated with decreased and increased risk of cardiovascular disease (CVD), respectively, elevated plasma levels of HDL, which exhibit abnormal properties, do not appear to protect against CVD and they are also dysfunctional, as they do not effectively transport cholesterol from peripheral cells back to the liver. Dysfunctional HDL are present in patients with cholesteryl ester transfer protein deficiency and the LCAT transgenic mouse model system.

Of particular interest has been the molecular mechanism responsible for the efflux and translocation of cellular cholesterol, which is mediated by apoA-I. Recent studies of patients with Tangier disease revealed that the genetic defect in these patients was a structural mutation in the ABC1 transporter. Several lines of evidence have now established that ABC1 is the key receptor in apoA-I efflux and is efficiently regulated by cholesterol and cyclic AMP. The identification of the genetic defect in Tangier disease has now permitted a definitive evaluation of patients with low plasma HDL levels to determine which kindreds have a genetic defect in ABC1. Identifying those patients with low HDL who require drug treatment continues to be a challenge for the physician. Clinical features that are useful in identifying those patients with decreased HDL that should be considered for drug treatment include those patients with established CVD, a family history of premature CVD where there is cosegregation of CVD and decreased HDL, and patients with persistently elevated plasma LDL-Chol levels on drug therapy.

Philip Barter (University of Adelaide, South Australia, Australia) reported on the preparation and design of the FIELD Study. Patients with non-insulin-dependent diabetes mellitus (NIDDM) have a substantially increased risk of developing premature atherosclerotic coronary heart disease (CHD). Each component of the dyslipidemia associated with NIDDM, mild to moderate elevation of plasma triglyceride, a low level of HDL-Chol, and an LDL fraction containing particles that are smaller and denser than normal has the potential to contribute to premature CHD. The most appropriate treatment of the typical dyslipidaemia in patients with NIDDM is with fibrates that effectively reduce plasma triglyceride, raise the concentration of HDL-Chol, and increase the size and density of the LDL particles. Indeed, the use of fibrates in large-scale, endpoint studies of nondiabetic patients with this lipid

profile substantially reduces the incidence of future coronary events. Moreover, the benefit is in addition to that attributed to the reduction of LDL-Chol and can be largely explained in terms of the effects of the drug on levels of triglyceride and HDL-Chol. It is logical therefore to consider such therapy for NIDDM patients with this lipid profile. The specific hypothesis that treating NIDDM patients with fenofibrate reduces their coronary risk is currently being tested in the FIELD Study. This study of 8,000 patients with NIDDM is a double-blind, placebo controlled, 5-year mean follow-up end-point study that is under way in Australia, New Zealand, and Finland. The primary end point for this study is coronary mortality. Recruitment for the study will be completed by June 2000, with the study due to conclude toward the end of 2004 or early in 2005.

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