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Functionally Defective High-Density Lipoprotein: A New Therapeutic Target at the Crossroads of Dyslipidemia, Inflammation, and Atherosclerosis

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Abstract*——High-density lipoproteins (HDL) possess key atheroprotective biological properties, including cellular cholesterol efflux capacity, and antioxidative and anti-inflammatory activities. Plasma HDL particles are highly heterogeneous in physicochemical properties, metabolism, and biological activity. Within the circulating HDL particle population, small, dense HDL particles display elevated cellular cholesterol efflux capacity, afford potent protection of atherogenic low-density lipoprotein against oxidative stress and attenuate inflammation. The antiatherogenic properties of HDL can, however be compromised in metabolic diseases associated with accelerated atherosclerosis. Indeed, metabolic syndrome and type 2 diabetes are characterized not only by elevated cardiovascular risk and by low HDL-cholesterol (HDL-C) levels but also by defective HDL function. Functional HDL deficiency is intimately associated with alterations in intravascular HDL metabolism and structure. Indeed, formation of HDL particles with attenuated antiatherogenic activity is mechanistically related to core lipid enrichment in triglycerides and cholesteryl ester depletion, altered apolipoprotein A-I (apoA-I) conformation, replacement of apoA-I by serum amyloid A, and covalent modification of HDL protein components by oxidation and glycation. Deficient HDL function and subnormal HDL-C levels may act synergistically to accelerate atherosclerosis in metabolic disease. Therapeutic normalization of attenuated antiatherogenic HDL function in terms of both particle number and quality of HDL particles is the target of innovative pharmacological approaches to HDL raising, including inhibition of cholesteryl ester transfer protein, enhanced lipidation of apoA-I with nicotinic acid and infusion of reconstituted HDL or apoA-I mimetics. A preferential increase in circulating concentrations of HDL particles possessing normalized antiatherogenic activity is therefore a promising therapeutic strategy for the treatment of common metabolic diseases featuring dyslipidemia, inflammation, and premature atherosclerosis.

I. Introduction

According to the recent estimates of the World Health Organization, approximately one-third of all deaths (16.7 million people) around the globe resulted from cardiovascular (CV[1]) disease in 2002 (World Health Organization, 2004). As shown in the recent INTERHEART study, which enrolled 29,972 subjects in 52 countries worldwide, the most strongly predictive CV risk factors for myocardial infarction were dyslipidemia, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, consumption of fruits, vegetables, and alcohol, and lack of regular physical activity (Yusuf et al., 2004). Collectively, these factors accounted for most (≥90%) of the risk of myocardial infarction in both sexes and at all ages in all regions.

Atherosclerosis represents the pathological process that typically underlies CV morbidity and mortality, formation of plaques in the intima and media of the arterial wall. Such atherosclerotic plaques result from the progressive accumulation of cholesterol and diverse lipids in native and oxidized forms, extracellular matrix material, and inflammatory cells. Atherogenic dyslipidemia, a highly prominent CV risk factor, is intimately associated with premature atherosclerosis and corresponds to an imbalance between excess circulating levels of cholesterol in the form of pro-atherogenic apolipoprotein (apo) B-containing lipoproteins compared with subnormal levels of antiatherogenic apoA-I-containing lipoproteins (Fig. 1). Indeed, apoB is the predominant protein component of proatherogenic, cholesterol-rich low-density lipoprotein (LDL), triglyceride (TG)-rich very-low density lipoproteins (VLDL), VLDL remnants

[1] Abbreviations: CV, cardiovascular; apo, apolipoprotein; LDL, low-density lipoprotein(s); TG, triglyceride(s); VLDL, very low-density lipoprotein(s); IDL, intermediate-density lipoprotein(s); HDL, high-density lipoprotein(s); LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol; CHD, coronary heart disease; MetS, metabolic syndrome; CAD, coronary artery disease; LOOH, lipid hydroperoxide(s); PL, phospholipid(s); NOS, nitric oxide synthase; oxLDL, oxidized LDL; CRP, C-reactive protein; RCT, reverse cholesterol transport; CE, cholesteryl ester; LCAT, lecithin/cholesterol acyltransferase; PAF-AH, platelet-activating factor-acetyl hydrolase; PON 1, paraoxonase 1; GSPx, glutathione selenoperoxidase; SAA, serum amyloid A; LpA-I, lipoprotein particles containing only apoA-I; LpA-I/A-II, lipoprotein particles containing both apoA-I and apoA-II; ABC, ATP-binding cassette transporter; PLTP, phospholipid transfer protein; CETP, cholesteryl ester transfer protein; SR-BI, scavenger receptor type BI; HL, hepatic lipase; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; IL, interleukin; rHDL, reconstituted HDL; S1P, sphingosine-1-phosphate; hs, high sensitivity; FH, familial hypercholesterolemia; NEFA, nonesterified fatty acid(s); IMT, intima-media thickness; VA-HIT, Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial; AFREGS, Armed Forces Regression Study; BIP, Bezafibrate Infarction Prevention Trial; HATS, HDL-Atherosclerosis Treatment Study; ARBITER, Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol; JTT-705, *S*-[2-([[1-(2-ethylbutyl)cyclohexyl]carbonyl]-amino)phenyl]2-methylpropanethioate; FIELD, Fenofibrate Intervention and Event Lowering in Diabetes; REVERSAL, Reversal of Atherosclerosis with Aggressive Lipid Lowering.

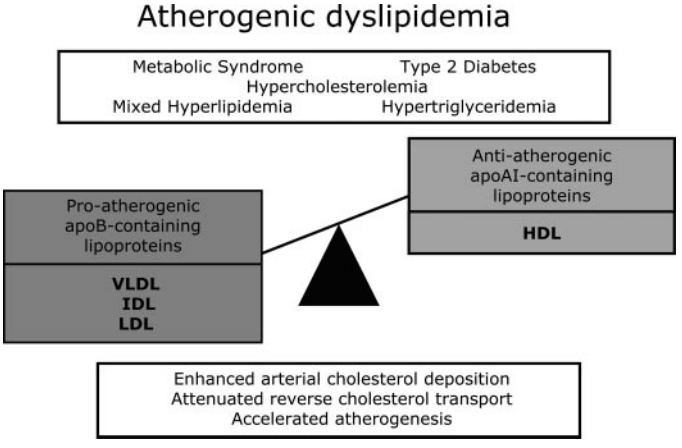


FIG. 1. Atherogenic dyslipidemia as an imbalance between circulating levels of proatherogenic apoB-containing lipoproteins and antiatherogenic apoA-I-containing lipoproteins.

and intermediate-density lipoprotein (IDL), whereas apoA-I is the major protein component of antiatherogenic high-density lipoprotein (HDL). In the INTERHEART study, dyslipidemia was assessed as an elevated ratio of plasma levels of proatherogenic apoB to antiatherogenic apoA-I (≥5:1) (Yusuf et al., 2004), and as such, represented a direct estimate of atherogenic potential in any individual.

Elevated circulating concentrations of LDL-cholesterol (LDL-C) occur frequently as hypercholesterolemia, a common form of atherogenic dyslipidemia (Wilson, 1990). LDL is the major vehicle for transport of cholesterol not only to peripheral tissues but also to the arterial wall (Lusis, 2000); indeed, ionic interaction of positively charged domains of apoB with negatively charged proteins of the extracellular matrix, including proteoglycans, collagen, and fibronectin, leads to intimal retention of apoB-containing lipoproteins, a major initiating factor in atherogenesis (Khalil et al., 2004).

Among factors other than LDL-C that are associated with dyslipidemia, a low level of HDL-cholesterol (HDL-C) is now most recognized (Gotto and Brinton, 2004). Several prospective epidemiological studies, including the Framingham Heart Study, US Physicians' Health Study, Prospective Cardiovascular Münster (PROCAM) Study, and Atherosclerosis Risk in Communities (ARIC) Study, have found that low serum HDL-C concentrations (defined as <40 mg/dl in both sexes or as <40 mg/dl in men and <50 mg/dl in women) (Chapman et al., 2004)) constitute an independent risk factor for coronary heart disease (CHD) in both nondiabetic and diabetic subjects (Maron, 2000; Sharrett et al., 2001; Gotto and Brinton, 2004). Moreover, low HDL-C is characteristic of atherogenic dyslipidemia and increased CV risk in patients with metabolic diseases such as type 2 diabetes and metabolic syndrome (MetS). In this context, it is of special relevance that the World Health Organization has estimated that the population of individuals with type 2 diabetes will have increased worldwide to 250 millions or more by 2025 (World Health Organization, 2004).

Prospective studies have revealed that CHD risk is elevated by 3% in women and 2% in men for each decrement of 1 mg/dl in HDL-C (Wilson, 1990). Conversely, a decreased risk of CV events is frequently observed in subjects with elevated HDL-C levels (Maron, 2000; Doggen et al., 2004; Gotto and Brinton, 2004); in addition, high concentrations of HDL-C (>60 mg/dl) are typically associated with longevity (Barzilai et al., 2003; Barter, 2004). The prevalence of low HDL-C levels can vary from 20% in a general population to up to 60% in patients with established CHD (Franceschini, 2001). Not only are low HDL-C levels associated with an increased incidence of CHD but also with a greater risk for carotid atherosclerosis and ischemic stroke mortality and with a more aggressive progression of angiographically defined coronary artery disease (CAD) (Maron, 2000; Gotto and Brinton, 2004). Finally, it is noteworthy that in the recent Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) trial in patients with acute coronary syndromes treated with atorvastatin, baseline HDL-C levels, rather than those of LDL-C, predicted the occurrence of CV events (Olsson et al., 2005).

A. Inflammation and Oxidative Stress in the Progression of Atherosclerosis

The imbalance between circulating levels of cholesterol transported in HDL relative to that in apoB-containing particles is intimately associated with induction of both endothelial dysfunction and oxidative stress in the arterial wall, which are in turn closely related to inflammation (Chisolm and Steinberg, 2000; Lusis, 2000); as a result, dyslipidemia, oxidative stress, and inflammation are closely interrelated in the development of atherosclerosis.

Oxidative stress is defined as an imbalance between prooxidant and antioxidant factors in favor of prooxidants and is central to the pathophysiology of atherosclerosis and CV disease (Fig. 2). Analysis of plaque composition has revealed products of protein and lipid oxidation, such as oxidized, chlorinated, and nitrated amino acids, lipid hydroperoxides (LOOH), short-chain aldehydes, oxidized phospholipids (PL), F2α-isoprostanes, and oxysterols, thereby suggesting the presence of local oxidative stress (Heinecke, 1998). The preferential retention of LDL in the arterial wall makes this lipoprotein a major substrate for oxidation by prooxidants produced by arterial wall cells. Various oxidative systems potentially contribute to LDL oxidation in vivo, and these include NAD(P)H oxidases, xanthine oxidase, myeloperoxidase, uncoupled nitric oxide synthase (NOS), lipoxygenases, and the mitochondrial electron transport chain (Madamanchi et al., 2005; Mueller et al., 2005). Accordingly, reactive oxygen, chlorine and nitrogen species, and lipid-derived free radicals are major prooxidants involved in the formation of oxidized LDL

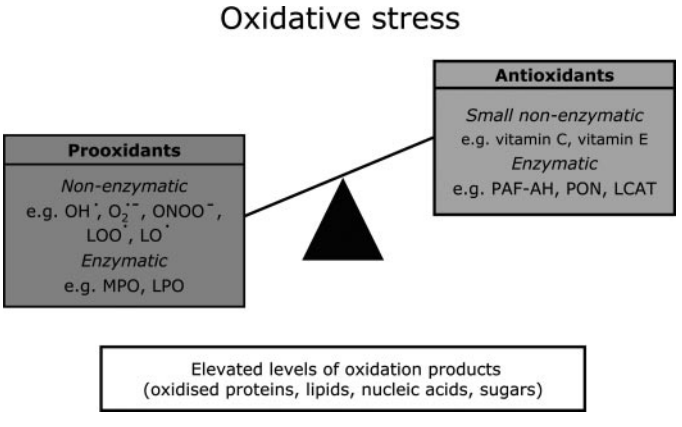


FIG. 2. Oxidative stress as an imbalance between prooxidant and antioxidant factors in favor of prooxidants. LPO, lipoxygenase; MPO, myeloperoxidase.

(oxLDL) in vivo. Significantly, production of both chlorine- and nitrogen-containing prooxidants is increased at sites of inflammation (Heinecke, 1998), suggesting that focal inflammation significantly contributes to the initiation of LDL oxidation at early stages of plaque formation. Consistent with the role of oxidative stress and oxidative modification of LDL in atherosclerosis, both urinary levels of F2α-isoprostanes, currently the most robust and integrative marker of oxidative stress in vivo in humans (Morrow, 2005) and plasma levels of oxLDL constitute strong and independent risk factors for CHD (Schwedhelm et al., 2004; Meisinger et al., 2005).

Inflammation is a systemic body response aimed to decrease the toxicity of harmful agents and repair damaged tissue. Chronic inflammation, which may be measured as circulating levels of an acute-phase protein, such as C-reactive protein (CRP), represents a major CV risk factor (Ridker et al., 2004b; Willerson and Ridker, 2004; Verma et al., 2005). A key feature of the inflammatory response involves activation of phagocytic cells involved in host defense, which produce an oxidative burst of reactive oxygen, chlorine, and nitrogen species, with subsequent creation of a highly prooxidative environment to combat invading pathogens. Local and systemic infections, arterial wall injury, and excessive retention of LDL may all potentiate activation of macrophages in the arterial wall, thereby triggering excessive production of prooxidant species (Hansson, 2005). As a result, oxidation of proteoglycan-bound LDL may occur in the extracellular space of the arterial intima (Memon et al., 2000).

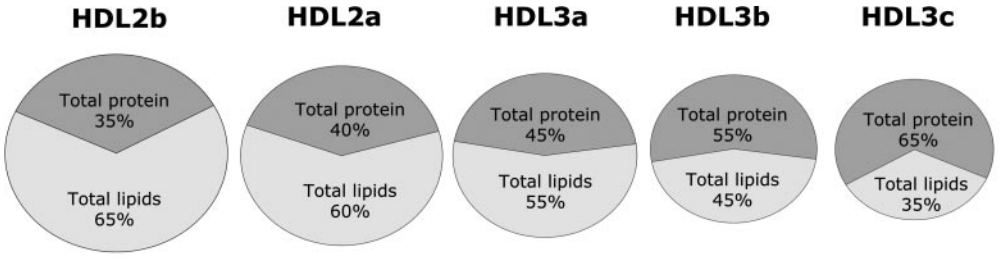
OxLDL particles exhibit multiple atherogenic properties, which include uptake and accumulation in macrophages, as well as proinflammatory, immunogenic, apoptotic, and cytotoxic activities (Chisolm and Steinberg, 2000). In contrast to unmodified LDL, oxLDL is taken up through macrophage scavenger receptor pathways that are not down-regulated by excess ligand and lead to the formation of cholesterol-loaded foam cells, characteristic components of atherosclerotic plaques. The proinflammatory activities of oxLDL include chemoattractant action on circulating monocytes, induction of the expression of adhesion molecules on endothelial cells, promotion of monocyte differentiation into macrophages, induction of the production and release of proinflammatory cytokines and chemokines from macrophages, and inhibition of macrophage motility (Chisolm and Steinberg, 2000; Lusis, 2000). Most of the proinflammatory properties of oxLDL arise from products of LDL lipid peroxidation, such as 1-palmitoyl-2(5-oxovaleroyl)-*sn*-glycero-3-phosphorylcholine, 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine, cholesteryl linoleate hydroperoxide, 7β-hydroperoxycholesterol, hydroxyoctadecadienoic acid, and 4-hydroxynonenal (Chisolm and Steinberg, 2000; Lusis, 2000; Van Lenten et al., 2001a). As a result, LDL oxidation further propagates the inflammatory process in the arterial wall, thereby accelerating atherogenesis (Lusis, 2000). Atherosclerosis can therefore be regarded as a chronic inflammatory disease of the arterial wall mediated by oxLDL in concert with a spectrum of additional proinflammatory agents.

HDL particles are distinguished from atherogenic apoB-containing lipoproteins by their capacity to exert a wide spectrum of antiatherogenic biological activities, including 1) their capacity to mediate cellular cholesterol efflux by acting as primary acceptors, thereby facilitating reverse cholesterol transport (RCT) from the arterial wall and peripheral tissues to the liver, 2) the protection of LDL against oxidative stress, 3) anti-inflammatory actions on arterial wall cells, and 4) antiapoptotic, 5) vasodilatory, 6) antithrombotic, and 7) antiinfectious activities. In this review, we will consider recent evidence for the heterogeneity of the atheroprotective properties of HDL particle subpopulations with emphasis on their ability both to protect against accumulation of lipids and to attenuate oxidative stress and inflammation in the arterial wall. Furthermore, new findings on functionally defective HDL will be discussed in the context of metabolic diseases associated with elevated CV risk; these data indicate that the potent antiatherogenic activities of small, dense HDL particles are impaired in the dyslipidemic and inflammatory state associated with type 2 diabetes and MetS. Finally, we will critically appraise innovative therapeutic strategies to normalize defective functionality of small, dense HDL particles; these exciting developments open new horizons for the treatment of atherogenic dyslipidemia in metabolic disease.

II. Functional High-Density Lipoprotein

A. Structure, Composition, and Heterogeneity

Functional plasma HDL are spherical or discoidal particles of high hydrated density (1.063–1.21 g/ml) due to elevated protein content (>30% by weight) compared with other lipoproteins (Fig. 3) (Asztalos and Schaefer, 2003; Barter et al., 2003b). Discoidal HDL are small particles consisting primarily of apoA-I embedded in a lipid monolayer constituted of PL and free cholesterol (Segrest et al., 1999, 2000). Spherical HDL are larger and additionally contain a hydrophobic core formed by cholesteryl esters (CE) and small amounts of TG. ApoA-I (molecular mass 28 kDa) is the major structural HDL apolipoprotein and accounts for ~70% of total HDL protein, whereas the second major HDL apolipoprotein, apoA-II, represents ~20%. Minor HDL protein components (typically <10% of the HDL protein moiety) include apoE, apoA-IV, apoA-V, apoJ, apoC-I, apoC-II, and apoC-III (Asztalos and Schaefer, 2003; Barter et al., 2003b; Karlsson et al., 2005). In small discoidal HDL, two molecules of apoA-I adopt a "double belt" orientation with their helixes oriented parallel to the plane of the disc and perpendicular to the lipid acyl chains in such a way that they wrap around the lipid bilayer disc forming



	HDL2b	HDL2a	HDL3a	HDL3b	HDL3c
Diameter (nm)	10.4	10.3	9.9	8.0	7.3
Density (g/ml)	1.099	1.107	1.123	1.155	1.186
M_r (kDa)	410	400	360	200	160
Plasma level (μM)	1.7	1.8	1.9	1.3	1.0
			mol/mol HDL		
Apolipoproteins					
ApoA-I	4-5	4	3-4	3	2-3
Others	≤2	≤2	≤2	1	≤1
Surface lipids					
Phospholipid	130	140	120	45	25
Free cholesterol	70	40	25	15	10
Core lipids					
Cholesteryl ester	180	160	140	70	40
Triglyceride	30	20	15	10	5

FIG. 3. Heterogeneity in the physicochemical properties of normal functional HDL in healthy normolipidemic subjects (Blanche et al., 1981; Barter et al., 1999; Kontush et al., 2003).

two stacked rings in an antiparallel orientation (Segrest et al., 1999, 2000; Silva et al., 2005); furthermore, apoA-I molecules appear to slide in relation to each other between two stable conformations (Silva et al., 2005). Plasma HDL particles also carry enzymes involved in lipid metabolism, including lecithin/cholesterol acyltransferase (LCAT), enzymes with plausible antioxidative activities, such as platelet-activating factor-acetyl hydrolase (PAF-AH, also called lipoprotein-associated phospholipase A_2), paraoxonase 1 (PON1) and glutathione selenoperoxidase (GSPx) (Navab et al., 2004b), and other proteins and peptides, such as serum amyloid A (SAA), a major positive acute-phase reactant (Uhlar and Whitehead, 1999), α-1-antitrypsin, a potent inhibitor of serine proteinases (Karlsson et al., 2005), or amyloid-β, the principal constituent of senile plaques in Alzheimer's disease (Kontush, 2004).

Plasma HDL particles are highly heterogeneous in their physicochemical properties, metabolism, and biological activity (Fig. 3) (Asztalos and Schaefer, 2003; Barter et al., 2003b). Such heterogeneity results from differences in relative contents of apolipoproteins and lipids in HDL and is intimately related to the amphipathic helical structure of human apoA-I (Reschly et al., 2002; Maiorano et al., 2004); these helixes possess a hinge domain that allows apoA-I to switch between two conformations corresponding to HDL particles of different size. When fractionated by ultracentrifugation, human HDL is typically separated into two major subfractions, HDL2 (*d* 1.063–1.125 g/ml) and HDL3 (*d* 1.125–1.21 g/ml) (Chapman et al., 1981). Nondenaturing polyacrylamide gradient gel electrophoresis has been used to separate HDL into five distinct subpopulations of decreasing size, HDL2b, 2a, 3a, 3b, and 3c (Anderson et al., 1977); equivalent subpopulations can be quantitatively isolated using isopycnic density gradient ultracentrifugation (Fig. 3) (Tall et al., 1982; Goulinet and Chapman, 1997; Guerin et al., 2000a). Other separation methods, such as two-dimensional electrophoresis, allow identification of more than 10 HDL subspecies in which spherical α-HDL predominate (Asztalos et al., 1993; Asztalos and Schaefer, 2003); each subspecies may, however, be heterogeneous in physicochemical properties, as in the case of ultracentrifugally isolated subfractions. HDL can also be immunoseparated on the basis of apolipoprotein composition into particles containing only apoA-I (LpA-I) and both apoA-I and apoA-II (LpA-I/A-II) (Duriez and Fruchart, 1999). In most human subjects, apoA-I is distributed approximately equally between LpA-I and LpA-I/A-II, whereas virtually all apoA-II is in LpA-I/A-II. Finally, ultrafiltration (Atmeh, 1990; Atmeh and Abd Elrazeq, 2005) and size-exclusion chromatography (Nanjee and Brinton, 2000) allow isolation of small, protein-rich HDL particles of low molecular mass (40–70 kDa). Given the complexity of HDL particle heterogeneity, small, dense HDL will be defined for present purposes as lipid-poor and protein-rich discoidal and spherical HDL particles of small size (≤9 nm), low molecular mass (≤200 kDa), and high density (1.125–1.24 g/ml). Depending on the fractionation method, small, dense HDL may include HDL3a, 3b, and 3c and very high-density lipoprotein separated by ultracentrifugation and pre-β-HDL separated by gradient gel electrophoresis.

On a particle basis, HDL are the most numerous per unit volume of plasma and are present at the highest (micromolar) levels compared with other lipoproteins. Concentrations of major HDL2 and HDL3 subfractions typically are in the range of 2 to 6 μM corresponding to 50 to 200 mg total mass/dl (Kontush et al., 2003, 2004, 2005; Hansel et al., 2004; Nobecourt et al., 2005).

The clinical relevance of circulating levels of individual HDL subfractions to atherosclerosis and CV disease is, however, unclear. Concentrations of HDL2-C and HDL3-C as estimates for plasma levels of the two major HDL subfractions were measured in several studies, which differed in separation methods (polyanion precipitation versus ultracentrifugation). Conflicting results were obtained, with evidence that either HDL2-C or HDL3-C constitutes a strong predictor of CHD or CV risk factors (Johansson et al., 1991; Drexel et al., 1992, 1994, 1996; Skinner, 1994; Robins et al., 2001; Alagona et al., 2002; Barter et al., 2003a; Yu et al., 2003; Desai et al., 2005). Furthermore, plasma levels of either large (Rosenson et al., 2002) or small (Mackey et al., 2002) HDL were reported to be associated with the progression of coronary atherosclerosis. Similarly controversial is the clinical significance of pre-α-HDL, pre-β-HDL, and LpA-I/A-II levels. By contrast, plasma levels of α1-HDL and LpA-I are typically associated with protection from atherosclerosis (Duriez and Fruchart, 1999; Asztalos et al., 2003; Asztalos and Schaefer, 2003).

Discordance in these data reflects complex relationships between HDL subfractions separated by different methods. For example, immunoisolated LpA-I/A-II is found predominantly in the HDL3 density range, whereas LpA-I is a prominent component of both HDL2 and HDL3 (Duriez and Fruchart, 1999). On the other hand, α-migrating HDL predominate in both HDL2 and HDL3 subfractions, whereas pre-β-HDL coisolates with small, dense HDL particles (Asztalos and Schaefer, 2003). Another important example concerns ultracentrifugally isolated small, dense HDL3c, which does not precisely correspond to small HDL subpopulations as determined by other methods. Human HDL3c represents a minor subfraction accounting for approximately 6% of total HDL mass and 10% of apoA-I (Kontush et al., 2003, 2004, 2005; Hansel et al., 2004; Nobecourt et al., 2005). In two-dimensional electrophoresis, HDL3c reveals further heterogeneity and produces multiple signals corresponding to small α3-, preβ-3- and preβ-1-HDL (S. Chantepie, A. Kontush, and M. J. Chapman, unpublished data). By contrast, small HDL α3, preβ-3, and preβ-1 subfractions measured by two-dimensional electrophoresis in whole plasma account for approximately 37, 4, and 12% of apoA-I, respectively (Asztalos et al., 2004a); moreover, α3 together with α2 represent two major HDL subfractions in normolipidemic subjects. It is essential to emphasize that routine clinical measurement of plasma HDL-C primarily reflects levels of large, cholesterol-rich HDL particles and frequently lacks sensitivity to detect small cholesterol-poor HDL.

B. Metabolism

Spherical plasma HDL are mature particles generated by intravascular processes from lipid-free apoA-I or lipid-poor pre-β-HDL (Fig. 4) (Rye and Barter, 2004). These small HDL precursors are produced as nascent HDL by the liver or intestine, are also released as surface fragments from lipolysed TG-rich lipoproteins (VLDL and chylomicrons), and finally may be generated during the interconversion of HDL3 and HDL2 (von Eckardstein et al., 2001). Small nascent HDL are unstable and readily acquire lipids (Atmeh and Abd Elrazeq, 2005); their initial lipidation occurs at cellular membranes via the ATP-binding cassette transporter (ABC) A1-mediated efflux of cholesterol and PL from cells (Fig. 4) (Oram, 2002). ABCA1 is a major player in HDL metabolism; indeed, genetic defects in ABCA1 as occur in Tangier disease may result in low HDL-C levels, with cholesterol accumulation in peripheral tissues and premature atherosclerosis (Oram, 2002).

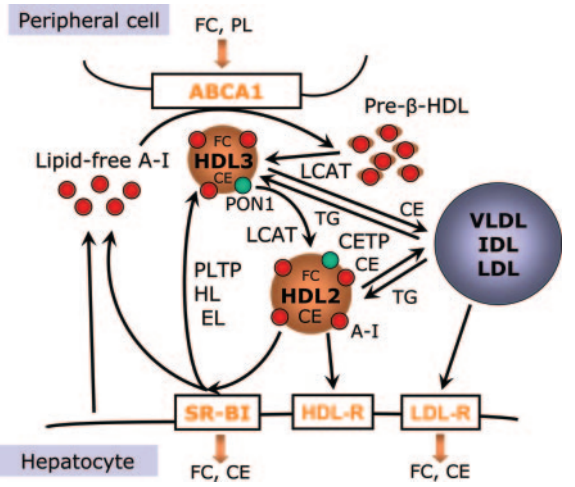


FIG. 4. Intravascular HDL particle remodeling and metabolism in normolipidemia. Spherical plasma HDL are generated from lipid-free apoA-I or lipid-poor pre-β-HDL, which are produced as nascent HDL by the liver or intestine but can also be released as surface fragments from lipolysed TG-rich lipoproteins and/or during the interconversion of HDL3 and HDL2. Initial lipidation of small nascent HDL occurs at cellular membranes via the ABCA1-mediated efflux of cholesterol and PL from cells. Subsequent LCAT-mediated cholesterol esterification generates large spherical HDL2 particles, which undergo further remodeling via particle fusion and surface remnant transfer mediated by PLTP. Large HDL2 can be converted in turn to small HDL3 upon CETP-mediated transfer of CE from HDL to apoB-containing lipoproteins, upon SR-BI-mediated selective uptake of CE by the liver and steroidogenic organs, and HL- and endothelial lipase-mediated hydrolysis of TG. When CETP-mediated transfer of CE occurs between HDL and TG-rich lipoproteins, TG-rich HDL are generated, which can be further hydrolyzed by HL to small, TG-rich HDL particles. The concerted action of CETP and HL promotes reduction in HDL size, formation of lipid-poor HDL particles, and shedding from HDL of lipid-free apoA-I, which can interact with ABCA1 in the next lipidation cycle. HDL lipids are catabolized either separately from HDL proteins by selective uptake or via CETP transfer or as holoparticles together with HDL proteins primarily in the liver via uptake through LDL receptors for apoE-containing HDL and through hitherto unidentified receptors for HDL holoparticles. EL, endothelial lipase; FC, free cholesterol; HDL-R, HDL holoparticle receptor; LDL-R, LDL receptor.

Subsequent LCAT-mediated esterification of cell-derived cholesterol generates large spherical HDL2 particles with a neutral lipid core of CE and TG (Jonas, 2000); such particles undergo further remodeling via particle fusion and surface remnant transfer mediated by phospholipid transfer protein (PLTP) (van Tol, 2002). Large HDL2 can be converted in turn to small HDL3 upon cholesteryl ester transfer protein (CETP)-mediated transfer of CE from HDL to apoB-containing lipoproteins, upon scavenger receptor type BI (SR-BI)-mediated selective uptake of CE by the liver and steroidogenic organs and hepatic lipase (HL) and upon endothelial lipase-mediated hydrolysis of core TG (Fig. 4) (von Eckardstein et al., 2001). When CETP-mediated transfer of CE occurs between HDL and TG-rich lipoproteins, TG-rich HDL are generated (Le Goff et al., 2004), which can be further hydrolyzed by HL to small, TG-rich HDL particles (Santamarina-Fojo et al., 2004). The concerted action of CETP and HL promotes reduction in HDL size, formation of lipid-poor HDL particles and shedding from HDL of lipid-free apoA-I, which can interact with ABCA1 in the next lipidation cycle (Clay et al., 1992). HDL lipids are catabolized either separately from HDL proteins by selective uptake or via CETP transfer or as holoparticles primarily in the liver, via uptake through LDL receptors for apoE-containing HDL and through hitherto unidentified receptors for HDL holoparticles.

C. Biological Activities

HDL particles possess multiple antiatherogenic activities (Stein and Stein, 1999; Nofer et al., 2002; Assmann and Nofer, 2003; Assmann and Gotto, 2004; Navab et al., 2004b). The central role of HDL in cellular cholesterol efflux and RCT is considered to form a basis for the capacity of HDL to attenuate atherogenesis (von Eckardstein et al., 2001; Nissen et al., 2003). However, compelling evidence has emerged that additional dimensions of the antiatherogenic action of HDL may be of major physiological and pathological relevance (Nofer et al., 2002; Assmann and Nofer, 2003; Assmann and Gotto, 2004; Navab et al., 2004b).

1. Cholesterol Efflux Capacity. The cholesterol efflux capacity of HDL particles is related to their ability to remove cholesterol from membranes of peripheral cells and particularly macrophages and foam cells via interaction with the ABCA1 and ABCG1 transporters and/or SR-BI receptor. Lipid-free apoA-I, apoA-II, apoE, and other HDL apolipoproteins induce fast, saturable, unidirectional and LCAT-independent efflux of cellular cholesterol and PL (von Eckardstein et al., 2001; Lewis and Rader, 2005); as a result, HDL particles efficiently acquire cholesterol in the extravascular compartment (Nanjee et al., 2001). ApoA-I is thought to play a central role in cholesterol transport from macrophages to the liver, consistent with the demonstration of accelerated RCT in mice overexpressing human apoA-I (Zhang et al., 2003); apoA-II is also able to act as as a primary acceptor and to efficiently remove cholesterol from macrophages in vivo (Rotllan et al., 2005).

Apolipoprotein-mediated lipid efflux involves specific interactions with membrane proteins, desorption of membrane lipids from caveolae, lipidation of lipid-free apolipoproteins and production of small, lipid-poor HDL (Rothblat et al., 1999; von Eckardstein et al., 2001). Lipid-free apolipoproteins remove cholesterol and PL from macrophages, aortic smooth muscle cells, and normal human skin fibroblasts but not from fibroblasts of patients with Tangier disease (Brousseau et al., 2000; Oram, 2000). Defective ABCA1 transporter function in Tangier disease has provided clear evidence that ABCA1 has a central role in lipid efflux mediated by lipid-poor apolipoproteins. In support of this mechanism, apoA-I-mediated cholesterol efflux is severely decreased by inhibition of ABCA1 with either antisense oligonucleotides or pharmacological compounds but is increased by the overexpression of ABCA1 (Oram, 2002). Thus, ABCA1 is a pivotal regulator of cellular cholesterol efflux and of the lipidation of apoA-I, a key step in formation of mature, spherical HDL particles.

ABCA1 has two highly conserved cytoplasmic ATP binding cassettes and two transmembrane domains, each of which consists of six membrane-spanning segments (Langmann et al., 1999; Santamarina-Fojo et al., 2000). It has been suggested that ABCA1 forms a channel within the plasma membrane through which cholesterol and PL are transferred ("flopped") from the inner to the outer leaflet of the plasma bilayer membrane (Hamon et al., 1997, 2000). There the lipids may be picked up by lipid-free apolipoproteins or lipid-poor particles, which bind to ABCA1 (Oram et al., 2000; Wang et al., 2000).

In addition to ABCA1, there are several other sterol-regulated ABC transporters, including ABCG1 and ABCG4, which are involved in cholesterol efflux from macrophages to mature HDL2 and HDL3 particles (Nakamura et al., 2004; Wang et al., 2004; Kennedy et al., 2005). Within the plasma membrane, ABCG1 redistributes cell cholesterol to domains that interact preferentially with mature HDL particles but not with lipid-poor apolipoproteins (Vaughan and Oram, 2005). The relative quantitative importance of cholesterol efflux mediated by ABCA1 compared with ABCG1 in macrophages remains unclear.

In contrast to lipid-free apolipoproteins, lipid-containing HDL particles induce both specific and nonspecific forms of cholesterol efflux (von Eckardstein et al., 2001). Nonspecific cholesterol efflux can be also mediated by PL vesicles, synthetic cyclodextrins, albumin or partially proteolysed HDL; it is slow, unsaturable, and bidirectional and thus appears to occur by aqueous diffusion (Rothblat et al., 1999; von Eckardstein et al., 2001). It has been suggested that SR-BI mediates the bidirectional flux between mature HDL and plasma membranes through the binding of HDL particles and subse-

quent reorganization of lipids within cholesterol- and caveolae-rich domains in the plasma membrane (de la Llera-Moya et al., 1999; Yancey et al., 2004). The PL content of HDL is an important determinant of such SR-BI-mediated cholesterol efflux (Yancey et al., 2000).

Another mechanism implicated in HDL-mediated cholesterol efflux is retroendocytosis, i.e., the uptake of HDL into clathrin-coated endosomes followed by intracellular enrichment with lipids and resecretion (Heeren et al., 1999; Takahashi and Smith, 1999). Finally, HDL-mediated cholesterol efflux from macrophages may be facilitated by apoE secretion (Mazzone, 1996). Indeed, macrophage-derived apoE can associate with HDL and improve its cholesterol acceptor properties.

Distinct cholesterol efflux properties of lipid-free and lipid-containing HDL are indicative of functional heterogeneity of HDL particles. Indeed, a decrease in the lipid content of HDL is generally thought to increase its capacity to remove cellular cholesterol (Ohta et al., 1995; Sasahara et al., 1998); small, dense, lipid-poor, protein-rich HDL particles are therefore considered to represent more efficient cholesterol acceptors compared with their large, light, lipid-rich, protein-poor counterparts (Asztalos et al., 1997). For example, small, lipid-poor HDL predominate in rabbits expressing human apoA-I; in parallel, the cholesterol efflux capacity of rabbit serum increases (Duverger et al., 1996a,b).

Interestingly, pre-β1-HDL, the initial product of apoA-I lipidation, is not essential for cellular cholesterol efflux (Sviridov et al., 2002), thereby suggesting that lipid-free, rather than lipid-poor, apolipoproteins function as primary cholesterol acceptors (Asztalos et al., 1997). Lipid-free and/or lipid-poor HDL apolipoproteins induce cholesterol uptake via interaction with ABCA1; consistent with this observation, plasma levels of small pre-β1-HDL particles correlate with serum capacity to induce ABCA1-mediated cholesterol efflux from J774 macrophages (Asztalos et al., 2005). Conversely, large, lipid-rich HDL particles appear to represent a better ligand for cellular uptake of CE mediated by SR-BI compared with small, lipid-poor HDL (de Beer et al., 2001; Thuahnai et al., 2004), consistent with the role of these particles in RCT from peripheral cells to the liver (von Eckardstein et al., 2001; Asztalos et al., 2005).

2. Antioxidative Activity. HDL antioxidative activity is typically observed as inhibition of LDL oxidation by HDL; LDL is thought to represent the major physiological target of HDL antioxidative action in vivo (Van Lenten et al., 2001a; Navab et al., 2004b). HDL is also able to inhibit generation of reactive oxygen species (ROS) in vitro under conditions of cell culture (Robbesyn et al., 2003; Lee et al., 2005) and in vivo in a rabbit model of acute arterial inflammation (Nicholls et al., 2005b). In addition, inhibitory actions of HDL on LDL oxidation have been reported in vitro upon their coincubation (Parthasarathy et al., 1990) and in vivo upon HDL injection (Klimov et al., 1993). HDL potently protects both lipid and protein moieties of LDL and inhibits accumulation of various oxidation products in LDL, including oxidized PL and short-chain aldehydes (Van Lenten et al., 2001a; Navab et al., 2004b).

The antioxidative activity of HDL is related to the presence of several apolipoproteins and enzymes with antioxidative properties in HDL particles. Apolipoproteins that possess antioxidative activity include apoA-I, apoE, apoJ, apoA-II, and apoA-IV. It appears that a major component of the antioxidative activity of HDL can be ascribed to apoA-I which can prevent and/or delay LDL oxidation by removing oxidized PL, including 1-palmitoyl-2(5-oxovaleroyl)-*sn*-glycero-3-phosphorylcholine and 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine, from LDL and from arterial wall cells (Navab et al., 2000a,b). The capacity of apoA-I to remove oxidized lipids is not specific for arterial wall cells, because similar effects have been reported for erythrocytes and astrocytes (Klimov et al., 2001; Ferretti et al., 2003, 2004). Circulating HDL accumulate LOOH and have been proposed to function as a "sink" for oxidized lipids (Bowry et al., 1992), ensuring their efficient elimination from the circulation through the liver.

ApoE possesses established antiatherosclerotic activity, which is normally ascribed to its lipid transport properties (Davignon, 2005). However, the action of apoE goes beyond such activity. Indeed, apoE possesses distinct antioxidative properties (Miyata and Smith, 1996) and can promote regression of atherosclerosis independently of lowering plasma cholesterol levels (Thorngate et al., 2000; Tangirala et al., 2001; Raffai et al., 2005). HDL-associated apoJ can inhibit oxidation of LDL by artery wall cells (Navab et al., 1997); in addition, apoJ is cytoprotective at low physiological levels (Trougakos et al., 2005). The beneficial actions of apoJ may be related to its ability to maintain integrity of membrane and lipoprotein lipids via its hydrophobic-binding domains (Jordan-Starck et al., 1992). Antioxidative properties have also been reported for apoA-II (Boisfer et al., 2002) and apoA-IV (Ostos et al., 2001). The capacity of apoA-II to protect LDL from oxidation is, however, questionable, given the fact that overexpression of human apoA-II in dyslipidemic mice accelerates atherosclerosis, increases aortic accumulation of oxLDL, and reduces antioxidative activity of HDL (Ribas et al., 2004; Rotllan et al., 2005). Such proatherogenic actions of apoA-II may be related to the displacement of antiatherogenic apoA-I and PON1 by apoA-II from HDL particles (Ribas et al., 2004). Finally, HDL is able to function as a preventive antioxidant through its capacity to bind transition metal ions (Kunitake et al., 1992), which in free form are potent catalyzers of LDL oxidation. Intriguingly, plasma HDL carry amyloid-β peptide, a major component of senile neuritic plaques and a strong chelator of transition metals (Kontush, 2004).

Major HDL enzymes possessing antioxidative activity are PON1, PAF-AH, LCAT, and GSPx (Van Lenten et

al., 2001a; Navab et al., 2004b). PON1 is a component of HDL that is thought to hydrolyze LDL-derived short-chain oxidized PL once they are formed (Aviram et al., 1998). PON1 is anchored to lipids via its hydrophobic N terminus (Josse et al., 2002; Harel et al., 2004); the association of PON1 with HDL is a prerequisite for maintaining normal serum activity of the enzyme. HDL provides the optimal physiological acceptor complex for PON1, in terms of both stimulating enzyme secretion and stabilizing the secreted peptide (James and Deakin, 2004); PON1 interaction with apoA-I is critical for enzyme stability (Gaidukov and Tawfik, 2005). HDL and, less efficiently, VLDL but not LDL promote PON1 secretion from cells; the differences between these lipoproteins are related to differences in their lipid composition (Deakin et al., 2005).

PAF-AH and LCAT can also hydrolyze LDL-derived short-chain oxidized PL; the relationship between the hydrolyzing activities of PON1, PAF-AH, and LCAT toward oxidized PL remains unclear. Recent data question the ability of PON1 to hydrolyze oxidized PL and suggest that PAF-AH, rather than PON-1, is the oxidized PL hydrolase in HDL (Marathe et al., 2003; Connelly et al., 2005). Consistent with this conclusion, HDL-associated PAF-AH is thought to play an antiatherogenic role, in contrast to the LDL-associated enzyme (Quarck et al., 2001; Tsimihodimos et al., 2003; Zalewski and Macphee, 2005). Indeed, local arterial expression of PAF-AH reduces accumulation of oxLDL and inhibits inflammation, shear stress-induced thrombosis, and neointima formation in balloon-injured carotid arteries of nonhyperlipidemic rabbits (Arakawa et al., 2005).

The antioxidative activity of PON1 purified from human serum has recently been ascribed to the presence of detergents or some other unidentified proteins (Teiber et al., 2004). Interestingly, PON1 has been reported to catalyze the hydrolysis of a variety of lactones, including homocysteine thiolactone, suggesting that its native activity is as a lactonase (Jakubowski, 2000; Draganov et al., 2005; Khersonsky and Tawfik, 2005). Plasma levels of homocysteine are a strong CV risk factor (Duell and Malinow, 1997); by detoxifying homocysteine thiolactone, PON1 could protect against homocysteinylation, a post-translational modification of proteins associated with attenuated biological activity and a potential contributing factor to atherosclerosis.

In addition, HDL-associated PON1 enhances cholesterol efflux from macrophages via increased HDL binding mediated by ABCA1 (Rosenblat et al., 2005). PON1-induced cellular accumulation of lysophosphatidylcholine, which stimulates cholesterol efflux via the ABCA1 pathway, may account for this effect (Hara et al., 1997). One can hypothesize that both lactonase activity and an RCT-related mechanism may contribute to the antiatherosclerotic effects of PON1 observed in vivo (Shih et al., 1998; Tward et al., 2002).

Another HDL enzyme, GSPx, can reduce LOOH to corresponding hydroxides and thereby detoxify them (Maddipati and Marnett, 1987; Arthur, 2000; Chen et al., 2000). LOOH-reducing activity mediated by Met residues of apoA-I and apoA-II has also been reported (Sattler et al., 1994; Garner et al., 1998). Finally, upon HDL oxidation with peroxynitrite, apoA-I increases generation of PL core aldehydes that are subsequently hydrolyzed by HDL-associated enzymes, such as PAF-AH and/or PON1, with formation of lysophospholipids (Ahmed et al., 2001). Such a PAF-AH/PON1-coupled protective function of apoA-I can effectively divert proatherogenic LOOH to less harmful products (Van Lenten et al., 2001a; Tselepis and Chapman, 2002; Navab et al., 2004b).

Apolipoproteins and enzymes with antioxidative activities are nonuniformly distributed across HDL subfractions. In vivo PON1 is preferentially associated with large HDL but can be displaced to small, dense particles upon ultracentrifugation (Cabana et al., 2003; Kontush et al., 2003; Bergmeier et al., 2004). The size and shape of HDL seem to be critical for PON1 binding (Josse et al., 2002). By contrast, apoJ is associated with a subset of small HDL, which also contains PON1 (Kelso et al., 1994). Similarly, LCAT activity (Kontush et al., 2003), PAF-AH activity (Kontush et al., 2003), and apoA-IV (Bisgaier et al., 1985) are enriched in small, dense HDL isolated by ultracentrifugation. As a consequence, HDL particles are heterogeneous in their antioxidative activity. Under mild oxidative stress induced by an azo initiator 2,2′-azobis-(2-amidinopropane) hydrochloride or Cu^{2+}, the antioxidative activity of HDL subfractions isolated by density gradient ultracentrifugation against LDL oxidation increases with increment in density in the order: HDL2b < HDL2a < HDL3a < HDL3b < HDL3c, thereby establishing that small, dense HDL act as potent protectors of LDL from oxidative stress (Kontush et al., 2003). Similarly, HDL3 is a more potent protector of LDL from in vitro oxidation compared with HDL2 (Yoshikawa et al., 1997; Huang et al., 1998). The antioxidative activity of small, dense HDL is related to the inactivation of proatherogenic products of LDL lipid peroxidation, primarily LOOH (Kontush et al., 2003). Mechanistically, this activity may arise from synergy in inactivation of oxidized lipids by enzymatic (hydrolysis) and nonenzymatic (physical removal) mechanisms, in part reflecting distinct intrinsic physicochemical properties of the small, dense HDL3c subfraction (Kontush et al., 2003).

The relative importance of HDL antioxidative activity in the overall cardioprotective effect of HDL compared with other biological actions remains indeterminate. A recent study proposed that the antioxidative activity of HDL is less important than cholesterol efflux capacity, as suggested by the absence of antioxidative effects of human apoA-I expression in $apoE^{-/-}$ mice accompanied by delayed atherosclerosis (Choudhury et al., 2004). The

increase in non-HDL-C levels observed in this animal model, however, renders interpretation of these results complex. By contrast, both cholesterol efflux capacity and antioxidative activity of HDL were impaired in parallel to a similar extent in apoA-I$^{-/-}$ mice in another recent study (Moore et al., 2005).

3. Anti-Inflammatory Activity. The anti-inflammatory activity of HDL is illustrated by the ability of HDL to decrease cytokine-induced expression of adhesion molecules on endothelial cells and to inhibit monocyte adhesion to these cells. HDL efficiently inhibit expression of the vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and E-selectin in vitro induced by tumor necrosis factor-α (TNF-α), interleukin (IL)-1, or endotoxin (Cockerill et al., 1995; Calabresi et al., 1997; Baker et al., 1999). Moreover, this potent anti-inflammatory activity observed in vitro can be translated into inhibition of adhesion molecule expression and a decrease in neutrophil infiltration in the arterial wall by reconstituted HDL (rHDL) in a rabbit model of acute arterial inflammation (Nicholls et al., 2005b). The ability of HDL to inhibit adhesion molecule expression may be related to the presence of apoA-I, apoA-II, apoA-IV, and/or distinct molecular species of PL, including sphingosine-1-phosphate (S1P) and sphingosylphosphorylcholine (Baker et al., 1999; Recalde et al., 2004; Nofer and Assmann, 2005). The anti-inflammatory action of HDL involves inhibition of TNF-α-stimulated activation of sphingosine kinase and production of S1P, which induces adhesion molecule expression in endothelial cells (Xia et al., 1999); transforming growth factor β may function as an important mediator of the anti-inflammatory activity (Norata et al., 2005). In addition, HDL attenuate IL-6 production in endothelial cells exposed to proinflammatory stimuli, such as TNF-α or endotoxin (Gomaraschi et al., 2005).

The anti-inflammatory action of HDL also involves hydrolysis of oxidized lipids by HDL-associated enzymes (PAF-AH and PON1) and is mechanistically similar to the antioxidative activity of HDL (Van Lenten et al., 2001a; Navab et al., 2004b; Recalde et al., 2004). Oxidized PL possess potent proinflammatory activities and can trigger arterial inflammation (Furnkranz et al., 2005). Inactivation of oxidized lipids by HDL may be associated with decreased expression of adhesion molecules in and decreased macrophage adhesion to endothelial cells (Theilmeier et al., 2000; Navab et al., 2004b).

Direct interaction of apoA-I with T lymphocytes, which can block subsequent activation of monocytes by lymphocytes, represents another plausible mechanism of HDL anti-inflammatory action (Burger and Dayer, 2002). In addition, apoA-I has been reported to diminish neutrophil activation in vitro (Liao et al., 2005). The anti-inflammatory activity of HDL in vivo is consistent with elevated levels of CRP in subjects with hypoalphalipoproteinemia (Sampietro et al., 2002), with negative correlation between plasma levels of CRP and HDL-C (Pirro et al., 2003) but also between plasma levels of intercellular adhesion molecule-1 and HDL-C and particularly small, dense HDL3-C (Kent et al., 2004).

The potential heterogeneity of HDL anti-inflammatory activity remains poorly characterized. HDL3 has been reported to be superior to HDL2 in terms of its capacity to inhibit vascular cell adhesion molecule-1 expression in endothelial cells (Ashby et al., 1998), a finding that is consistent with the potent antioxidative activity of small, dense HDL3 particles (Yoshikawa et al., 1997; Huang et al., 1998; Kontush et al., 2003).

4. Antiapoptotic, Vasodilatory, Antithrombotic, and Anti-Infectious Activities. Other antiatherogenic activities of HDL include antiapoptotic and vasodilatory actions, mitogenic activity in endothelial cells, attenuated platelet activation, and anticoagulant and anti-infectious activities (Calabresi et al., 2003).

HDL potently inhibit apoptosis in endothelial cells induced by oxLDL (Suc et al., 1997; Robbesyn et al., 2003) or TNF-α (Sugano et al., 2000); this effect is paralleled by decreased intracellular generation of ROS and diminished levels of apoptotic markers, suggesting that it can be related to the intracellular antioxidative actions of HDL or HDL components (Suc et al., 1997; Sugano et al., 2000; Robbesyn et al., 2003). Indeed, HDL contain bioactive lysophospholipids, including S1P (Nofer and Assmann, 2005; Zhang et al., 2005), a potent antiapoptotic agent, which may mediate the antiapoptotic effect of HDL via increased NO production (Kwon et al., 2001).

Similarly, HDL vasodilatory activity may be related to the stimulation of NO release by endothelial cells mediated by intracellular Ca^{2+} mobilization and phosphorylation of NOS upon association with apoA-I (Drew et al., 2004; Nofer et al., 2004). Such activation of NO production involves HDL binding to SR-BI with a subsequent increase in intracellular ceramide levels (Yuhanna et al., 2001; Li et al., 2002). Furthermore, HDL can stimulate production of prostacyclin, which possesses potent vasorelaxing activity (Beitz and Forster, 1980; Norata et al., 2004). Again, the vasoactive effects of HDL can be mediated by S1P acting via the lysophospholipid receptor S1P3 (Nofer et al., 2004). S1P may be equally important for mitogenic effects of HDL in endothelial cells and for the inhibitory action of HDL on the migration of vascular smooth muscle cells (Kimura et al., 2003; Nofer and Assmann, 2005; Tamama et al., 2005).

Similarly, increased production of NO may form a basis for the inhibitory action of HDL on platelet aggregation (Chen and Mehta, 1994). The antithrombotic activity of HDL is observed as inhibitory actions on factors that promote blood coagulation, including tissue factor, factors X, Va, and VIIIa (Nofer et al., 2002; Calabresi et al., 2003). Mechanistically, this effect may be related to the presence of cardiolipin and phosphatidylethanolamine, two minor anionic PL with potent anticoagulant properties that are enriched in the HDL fraction (Degu-

chi et al., 2000). In addition, HDL acts via its protein moiety to enhance the anticoagulant activity of protein S and activated protein C (Griffin et al., 1999).

Finally, HDL play a major role in the binding and clearance of circulating endotoxin to the bile and thereby inhibit endotoxin-induced cellular activation, resulting in potent anti-infectious activity (Pajkrt et al., 1996; Levels et al., 2001; Stoll et al., 2004). The inactivation of endotoxin by HDL is mediated by direct interaction with apoA-I (Ma et al., 2004) and involves reduced CD14 expression on monocytes as a key step (Pajkrt et al., 1996). In addition, human HDL possess specific trypanosome-lytic activity, which selectively protects humans from *Trypanosome brucei brucei* (Hajduk et al., 1989).

The potential heterogeneity of these antiatherogenic activities among HDL particles is indeterminate. The anticoagulant activity of tissue factor pathway inhibitor in human plasma has been reported to be preferentially associated with dense subspecies of HDL and LDL (Lesnik et al., 1993). Similarly, the trypanosome-lytic activity is associated with a minor large and dense HDL subfraction with a molecular mass of 490 kDa (Hajduk et al., 1989). Finally, our recent data suggest that small, dense HDL potently inhibit apoptosis induced in endothelial cells by oxLDL (Suc et al., 1997; Robbesyn et al., 2003; J. de Souza, M. J. Chapman, and A. Kontush, unpublished data).

III. Functionally Defective High-Density Lipoprotein in Dyslipidemic and Inflammatory States

HDL is known to undergo dramatic modification in structure and composition as a result of the concerted actions of the acute-phase response and inflammation (Khovidhunkit et al., 2004b; Esteve et al., 2005). The close association between inflammation, oxidative stress, dyslipidemia, and atherosclerosis suggests that such HDL alterations play a significant role in disease progression. As a result, HDL particles progressively lose normal biological activities and acquire altered properties. Such altered HDL particles have been termed "dysfunctional HDL" (Navab et al., 2001b), and HDL has been proposed to possess "chameleon-like properties" (Navab et al., 1996; Van Lenten et al., 2001a). It is essential to emphasize that the degree of loss of normal HDL function compared with the absence of this function depends on the assay used to characterize HDL functionality. Indeed, HDL can be dysfunctional (with total loss of function) in cell-based or cell-free assays aimed at measuring anti-inflammatory activity (Navab et al., 2001b; Ansell et al., 2003), whereas measurements of antioxidative activity (Kontush et al., 2003, 2004, 2005; Hansel et al., 2004; Nobecourt et al., 2005) or cholesterol efflux capacity (Banka et al., 1995; Cavallero et al., 1995; Brites et al., 2000; Khovidhunkit et al., 2001) reveal a deficiency in normal HDL function rather than a complete dysfunction.

A. Altered High-Density Lipoprotein Composition and Enzymatic Activities in Dyslipidemic and Inflammatory States

1. Apolipoproteins. Both the plasma levels and apolipoprotein content of HDL can be significantly altered during the acute phase as well as during acute and chronic inflammation. Levels of apoA-I and apoA-II decrease, whereas those of apoA-IV, apoA-V, apoJ, and apoE increase (Khovidhunkit et al., 2004a,b). The decrease in HDL apoA-I levels in inflammatory states is related to both decreased apoA-I synthesis in the liver and apoA-I replacement in HDL particles by SAA (Fig. 5) (Khovidhunkit et al., 2004b; Esteve et al., 2005). SAA is a 12-kDa acute-phase protein whose circulating levels can be induced up to 1000-fold (Malle et al., 1993). HDL is a major carrier of SAA in human, rabbit, and murine plasma (Hoffman and Benditt, 1982a; Marhaug et al., 1982; Cabana et al., 1996). In the circulation, SAA does not exist in a free form and associates with non-HDL lipoproteins in the absence of HDL (Cabana et al., 2004). In the presence of HDL, SAA is specifically associated with small, dense HDL3 subspecies (Benditt and Eriksen, 1977; Hoffman and Benditt, 1982a; Coetzee et al., 1986; Cabana et al., 1996) via its N-terminal domain (Liang et al., 1996), but it is also present in large and intermediate HDL (Coetzee et al., 1986; Cabana et al., 1996).

SAA is able to replace apoA-I in small, dense HDL upon induction of the acute phase (Parks and Rudel, 1985; Coetzee et al., 1986); as a result, plasma levels of apoA-I decrease (Cabana et al., 1996). In dense HDL, SAA can account for up to 80% of total protein; such enrichment can further increase HDL protein content and density (Cabana et al., 1989). Elevated plasma levels of SAA are accompanied by elevated levels of lipid-free apoA-I, probably due to the dissociation of apoA-I from HDL (Cabana et al., 1996). In rabbits and mice, SAA can completely replace apoA-I in a subset of small, dense HDL particles, thereby functioning as a structural apolipoprotein (Cabana et al., 1996, 1999). In such SAA-only HDL, 20 molecules of SAA have been estimated to replace all 3 molecules of apoA-I in each HDL particle. SAA is mainly produced by the liver but also by arterial wall cells and adipocytes (Hoffman and Benditt, 1982b; Malle et al., 1993). Primary murine hepatocytes secrete SAA as a monomer, which subsequently associates with small, lipid-poor, apoA-I-containing HDL secreted via a separate pathway (Hoffman and Benditt, 1982b).

Similar to CRP, elevated plasma levels of SAA have been reported to represent a CV risk factor. In a prospective case-control study, plasma level of hs-CRP was the strongest univariate predictor of CV risk in apparently healthy postmenopausal women; the relative risk of events for women in the highest compared with the

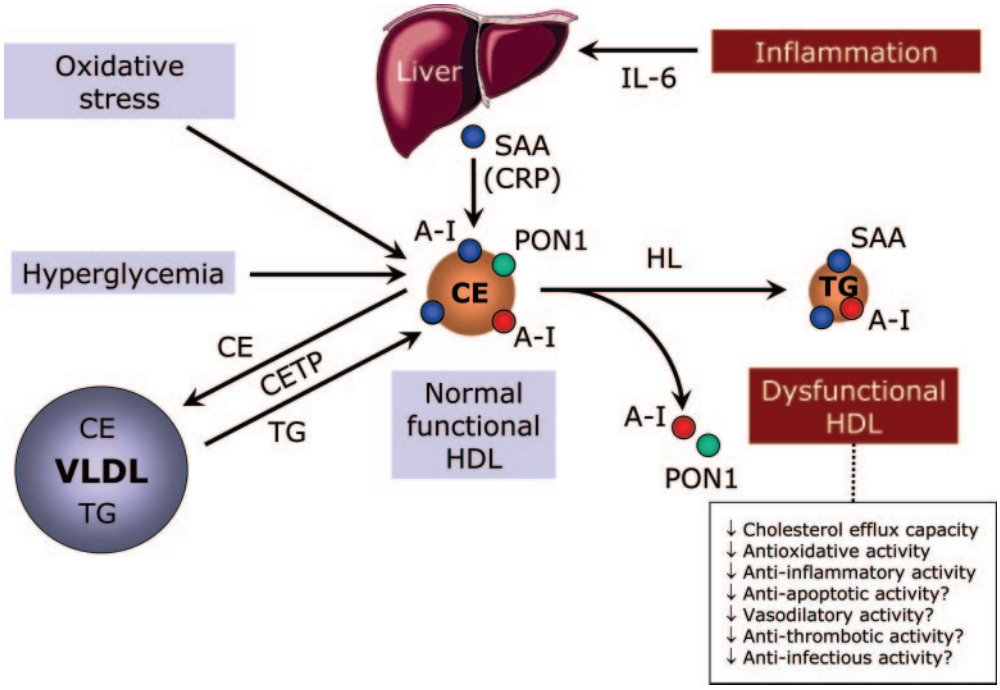


FIG. 5. Abnormal metabolism and deficient biological activities of HDL in atherogenic dyslipidemias of metabolic disease. Chronic inflammation characteristic of metabolic disease, such as MetS and type 2 diabetes, is associated with elevated plasma levels of IL-6. As a result, the liver produces SAA, which replaces apoA-I and PON1 in HDL. Oxidative stress, hyperglycemia, and elevated activity of CETP are other important modulators of HDL function. Oxidative stress modifies specific amino acids in apoA-I, whereas hyperglycemia results in apoA-I glycation. CETP exchanges CE and TG between HDL and TG-rich lipoproteins, such as VLDL; as a result, HDL become enriched in TG. Such enrichment in TG induces conformational changes in apoA-I, which becomes less accessible for the interaction with other lipoproteins, including LDL, and cannot eliminate oxidized lipids from LDL. Subsequent HDL hydrolysis by HL produces small, dense HDL that are enriched in TG and in SAA and contain apoA-I in an incorrect conformation; such HDL possess deficient functionality compared with normal HDL particles.

lowest quartile was 4.4 (95% confidence interval, 2.2–8.9) (Ridker et al., 2000). However, SAA levels also revealed a significant, albeit weaker, association with CV events (relative risk for the highest versus lowest quartile 3.0). Remarkably, the levels of hs-CRP and SAA were significant predictors of CV risk even in the subgroup of women with low LDL-C levels (Ridker et al., 2000).

In addition, baseline SAA levels were independently associated with angiographic CAD (Liuzzo et al., 1994) and were highly predictive of 3-year CV events in women referred for coronary angiography for suspected myocardial ischemia (Liuzzo et al., 1994) as well as of the progression of carotid atherosclerosis in patients undergoing ultrasound investigations (Schillinger et al., 2005). By comparison, hs-CRP was not associated with angiographic CAD but, like SAA, was strongly and independently predictive of adverse CV outcome. Elevation of hs-CRP and SAA levels at the time of hospital admission predicted poor outcome in patients with unstable angina (Liuzzo et al., 1994). Elevated SAA levels were associated with increased mortality in transplant patients with CAD (Fyfe et al., 1997). Furthermore, circulating SAA is elevated in CHD patients (Fyfe et al., 1997; Delanghe et al., 2002), subjects with CAD (Fyfe et al., 1997; Winkler et al., 2005), and patients with type 2 diabetes (Choudhury and Leyva, 1999) compared with healthy control subjects. Local concentrations of SAA are elevated in the coronary artery at sites of plaque rupture compared with concentrations in the aorta in patients with acute myocardial infarction (Maier et al., 2005). Finally, plasma levels of SAA correlate with the development of atherosclerosis in mouse models of this disease (Herrington and Parks, 2004; Lewis et al., 2004).

The proatherogenic properties of SAA are intimately related to its biological activities. SAA is present in human and murine atherosclerotic lesions and colocalises with apoA-I, apoB, and the proteoglycan perlecan (Yamada et al., 1996; O'Brien et al., 2005). HDL enrichment in SAA enhances in vitro HDL binding to proteoglycans due to the presence of a proteoglycan-binding domain in the SAA molecule (Lewis et al., 2004; O'Brien et al., 2005); thus, SAA might immobilize HDL particles in the arterial wall, which would otherwise transport cholesterol from the plaque to the liver. In addition, SAA-enriched HDL are rapidly cleared from the circulation (Hoffman and Benditt, 1983); SAA may then play a role in the lipoprotein redistribution to the arterial wall. Finally, enrichment in SAA may impair the normal atheroprotective activities of HDL (see below).

Human LDL contain low levels of SAA; LDL-associated SAA has been recently proposed to represent a risk factor for cardiac events in stable CAD (Ogasawara et al., 2004). SAA-carrying LDL represent 5 to 6% of total LDL, contain increased levels of products of lipid and protein oxidation and may represent circulating oxLDL

(Ogasawara et al., 2004). The increased SAA content of such LDL may enhance its retention in atherosclerotic lesions. Such SAA-mediated accumulation of LDL lipids in the lesions may lead to plaque instability and result in plaque rupture (Johnson et al., 2004).

Apart from its replacement by SAA, apoA-I can undergo other modifications in the circulation. Amino acid residues in apoA-I, such as methionine, cysteine, tyrosine, and lysine residues, can be selectively modified under the action of prooxidants secreted by arterial wall cells (Bergt et al., 2004; Zheng et al., 2004; Nicholls et al., 2005d) and nonenzymatically glycosylated in the presence of high levels of glucose (Fievet et al., 1995). In human atherosclerotic lesions, proteins oxidized by HOCl or acrolein colocalise with extracellular apoA-I (Bergt et al., 2004; Shao et al., 2005c). Oxidized amino acid residues, including chlorotyrosines, nitrotyrosines and oxidized lysine and methionine residues, are present in apoA-I isolated from plasma and from human atherosclerotic lesions (Bergt et al., 2004; Zheng et al., 2004; Panzenbock and Stocker, 2005; Shao et al., 2005c); furthermore, the apoA-I content of chloro- and nitrotyrosines is increased in plasma of patients with CV disease. Myeloperoxidase, a major source of chlorinated ROS in the arterial wall, binds to apoA-I in vitro and in vivo and produces similar patterns of oxidized amino acids. Residue Tyr-192 represents the major target for myeloperoxidase-catalyzed oxidation in the apoA-I molecule both in vitro and in vivo, whereas three other tyrosine residues at positions 29, 166, and 236 are modified to a lesser extent (Zheng et al., 2005). Thus, apoA-I represents a selective target for chlorination and nitration in human atheromatous tissue catalyzed by myeloperoxidase. In vivo oxidation of apoA-I is equally consistent with the observation that HDL from hypercholesterolemic chickens contain higher amounts of oligomeric apoA-I and are more susceptible to in vitro oxidation than HDL from control animals (Artola et al., 1997).

2. Enzymes with Antioxidative and Anti-Inflammatory Properties. HDL-associated enzymes, including PAF-AH, PON1, and LCAT, can become dysfunctional and/or depleted under inflammatory conditions (Navab et al., 1997; Van Lenten et al., 2001b), in metabolic diseases involving low HDL levels (type 2 diabetes, MetS) (Hansel et al., 2004; Nobecourt et al., 2005), and in premature CHD (Ansell et al., 2003). Induction of the acute-phase response is associated with decreased PON1 activity, probably due to the replacement of PON1 by SAA (Fig. 5) (Navab et al., 1997; Van Lenten et al., 2001b). Furthermore, decreased PON1 activity may be caused by enzyme inactivation as a result of oxidation (Jaouad et al., 2003) and/or glycation (Hedrick et al., 2000; Ferretti et al., 2001). Consistent with these observations, serum concentrations of PON1 are decreased in subjects with MetS (Blatter Garin et al., 2005) and in patients with type 1 and type 2 diabetes (Boemi et al., 2001; Costa et al., 2005), who feature elevated levels of inflammation and oxidative stress (Ridker et al., 2004a). Serum PON1 activity decreases with age (Costa et al., 2005) and is lower in subjects with MetS (Blatter Garin et al., 2005) and low HDL-C (Brites et al., 2004) and patients with type 2 diabetes (Boemi et al., 2001; Costa et al., 2005) and familial hypercholesterolemia (FH) (Mackness et al., 1991) compared with age-matched healthy control subjects. Moreover, low PON1 activity toward paraoxon has been reported to represent an independent risk factor for coronary events in men at high CV risk (Mackness et al., 2003).

HDL-associated PAF-AH activity, expressed as a percentage of total serum PAF-AH activity, is lower in FH patients than in control subjects (Karabina et al., 1997; Tsimihodimos et al., 2002). By contrast, LDL-associated PAF-AH activity is elevated in homozygous FH subjects (Tsimihodimos et al., 2002), indicating a major redistribution of PAF-AH activity in plasma of FH individuals from apoA-I- to apoB-containing lipoproteins (Tsimihodimos et al., 2002). Finally, LCAT activity is diminished under inflammatory conditions (Jonas, 2000).

3. Lipid Components. Although apolipoproteins and enzymes are major determinants of altered HDL function, it is considerably influenced by changes in lipid content. HDL core enrichment in TG with CE depletion is the most frequent abnormality of HDL lipid composition (Fig. 5) and occurs in hypertriglyceridemic states associated with decreased activity of LPL, decreased activity of HL, and/or decreased activity of LCAT; all these metabolic alterations are frequently observed in the acute phase and during inflammation (Cabana et al., 1996). In addition, HDL-TG content can be raised as a consequence of elevated CETP-mediated TG transfer from VLDL to HDL (de Grooth et al., 2004a; Le Goff et al., 2004).

Under such conditions, TG typically replace CE in the HDL core, resulting in a low CE/TG ratio and in a decrease in plasma HDL-C levels, another feature of the acute phase response (Khovidhunkit et al., 2004b). Interestingly, a similar elevation in HDL-TG, decrease in HDL-C, and increase in inflammatory markers are observed in the postprandial phase (Schaefer et al., 2005). Human acute-phase HDL obtained from patients undergoing bypass surgery are enriched in TG and depleted of CE (Pruzanski et al., 2000). Acute-phase HDL also contain elevated levels of nonesterified fatty acids (NEFA), lysophosphatidylcholines and isoprostanes compared with normal HDL; in addition, CE levels are decreased (Pruzanski et al., 2000). Similarly, HDL3 from subjects with myocardial infarction are enriched in TG and depleted of PL (Clifton et al., 1985). Induction of the acute-phase response in monkeys increases plasma TG levels and TG content in total HDL and in all HDL subfractions and also decreases HDL-C and HDL-CE; these effects are paralleled by cytokine-induced decreases in the activities of HL, LPL, and LCAT and increases in the activity of CETP (Auerbach and Parks, 1989; Ettinger et

al., 1990; Cabana et al., 1996). In addition, acute-phase HDL obtained from hamsters display reduced CE content and elevated PL and FC contents compared with normal HDL (Khovidhunkit et al., 2001). As a consequence of decreased LCAT activity, increased HDL concentrations of free cholesterol are frequently observed in inflammatory states; in addition, HDL free cholesterol is elevated in genetic LCAT deficiency (Jonas, 2000). By contrast, reduced PL content of HDL is a less consistent finding (Khovidhunkit et al., 2004b). The reduction of HDL-PL reported in some studies (Clifton et al., 1985; Cabana et al., 1989) may reflect elevated activity of secretory phospholipase A2 frequently observed in the acute phase (Crowl et al., 1991; Pruzanski et al., 1993; Tietge et al., 2002).

Equally, HDL composition can be abnormal in other forms of dyslipidemia. In FH, HDL levels of TG and the TG/CE ratio are increased (Bagdade et al., 1991; Frenais et al., 1999). HDL enrichment in TG is associated with accelerated CE transfer from LDL and HDL to TG-rich lipoproteins in plasma of FH patients, an observation that has been linked to abnormal properties of plasma VLDL1 (Bagdade et al., 1991; Guerin et al., 1995a, 2000a). By contrast, decreased CE transfer from LDL and HDL to TG-rich lipoproteins, such as those observed in plasma of subjects with heterozygous CETP deficiency, results in reduced HDL content of TG, elevated content of CE, and increased HDL size (Koizumi et al., 1991).

Finally, HDL lipids can be oxidized in vivo with formation of biologically active compounds. For instance, HDL oxidation by HOCl produces 2-chlorohexadecanal, a chlorinated fatty aldehyde formed upon oxidative cleavage of plasmalogen, which exerts inhibitory actions on endothelial NOS (Marsche et al., 2004).

B. Abnormal High-Density Lipoprotein Metabolism in Dyslipidemic and Inflammatory States

HDL metabolism is substantially altered in dyslipidemic states, including hypertriglyceridemia, hypercholesterolemia, mixed dyslipidemia and hypo- and hyperalphalipoproteinemia and also during infection and inflammation. As discussed above, hypertriglyceridemia is characterized by decreased levels of HDL-C and increased HDL-TG content due to the action of CETP. Such low HDL-C dyslipidemias associated with hypertriglyceridemia are characteristic of metabolic diseases associated with elevated CV risk, such as type 2 diabetes and MetS. Mechanisms leading to reduced plasma HDL-C levels and HDL particle numbers in hypertriglyceridemic states are as follows: 1) small HDL particles, which result from the intravascular lipolysis of TG-enriched HDL, are cleared more rapidly from the circulation; 2) TG-enriched HDL are intrinsically more unstable in the circulation, with apoA-I loosely bound; 3) lipolysis of TG-enriched HDL lower HDL particle numbers by causing apoA-I to be shed from HDL particles and cleared from the circulation; 4) dysfunctional LPL or reduced LPL activity contributes to the lowering of HDL levels by reducing the availability of surface constituents of TG-rich lipoproteins that sequester to the plasma pool of nascent HDL particles (Lamarche et al., 1999).

The CE/TG ratio therefore represents a critical factor in determining HDL particle stability and plasma residence time; HDL possessing decreased CE/TG ratios are less stable than normal particles (Sparks et al., 1995; Rashid et al., 2002; Borggreve et al., 2003; Rashid et al., 2003). Importantly, a decrease in circulating HDL-C levels and an increase in TG levels are typical components of the acute-phase reaction (Khovidhunkit et al., 2004b; Esteve et al., 2005). In humans, influenza infection is associated with decreased levels of HDL-C (Marchesi et al., 2005); in rodents, endotoxin injection increases plasma TG levels through increased hepatic secretion and/or delayed clearance (Feingold et al., 1992).

HDL metabolism critically depends on the activity of CETP. In metabolic diseases such as type 2 diabetes and MetS, elevated CETP activity results in increased CE transfer from HDL to TG-rich lipoproteins and in reciprocal TG transfer, producing TG-enriched HDL and decreasing HDL-C levels (Fig. 5) (Le Goff et al., 2004). Conversely, CETP deficiency reduces the exchange of TG and CE between HDL and TG-rich lipoproteins and elevates HDL-C due to CE retention. As a consequence, increased CETP activity is thought to be proatherogenic in humans (Barter et al., 2003b). Consistent with this hypothesis, the low-active CETP *Taq*IB variant B2B2 is associated with higher HDL-C plasma levels and a lower risk of CAD than the high-active variant B1B1 (Boekholdt et al., 2005). In addition, baseline CETP levels positively correlated with carotid intima-media thickness (IMT) in 2 years in FH patients treated with statins (de Grooth et al., 2004b). Finally, baseline CETP levels were associated with future CAD in a subset of hypertriglyceridemic subjects from an apparently healthy population (Boekholdt et al., 2004).

Elevated activity of CETP may therefore form a basis for low HDL-C phenotypes; alternatively, they may result from a deficiency of apoA-I (Ng et al., 1995), elevated activities of HL (Tato et al., 1995), reduced activities of LCAT (Kuivenhoven et al., 1997; Hovingh et al., 2005) or LPL (Blades et al., 1993), or a combination of these. Furthermore, HDL metabolism is altered in hyperalphalipoproteinemia, which can arise from a genetic deficiency of CETP and/or HL, as well as from increased production of apoA-I (Yamashita et al., 2000). Familial CETP deficiency is associated with accumulation of large CE-rich HDL2 particles (Yamashita et al., 2000); in addition, large HDL predominate in familial HL deficiency (Cohen et al., 1999).

In hypercholesterolemia, abnormalities of HDL metabolism include moderate decreases in plasma apoA-I and HDL-C levels (Schaefer et al., 1992; Frenais et al.,

1999). Subnormal plasma levels of apoA-I in FH are probably related to an increased fractional catabolic rate of apoA-I observed both in homozygous (Schaefer et al., 1992) and heterozygous (Frenais et al., 1999) forms of FH. In homozygous FH, the deleterious influence of altered apoA-I metabolism on HDL-C levels is further aggravated by decreased rates of apoA-I production (Schaefer et al., 1992). Moreover, elevated CETP activity due to the increased number of apoB-containing lipoproteins—mainly LDL—also contributes to depletion of CE from the plasma HDL pool in FH (Guerin et al., 1995, 2000b).

HDL heterogeneity and particle profile largely reflect abnormalities in HDL metabolism. In the atherogenic dyslipidemias of MetS and type 2 diabetes, circulating levels of large, cholesterol-rich HDL decrease in parallel with decrease in HDL-C (Syvanne et al., 1995; Blatter Garin et al., 2005). By contrast, levels of small, dense, cholesterol-poor HDL particles and their content of apoA-I are rarely reduced in low HDL-C dyslipidemia (Hansel et al., 2004; Kontush et al., 2005; Nobecourt et al., 2005). Consistent with this observation, the relative apoA-I contents of HDL2b and HDL2a decrease, whereas those of pre-β1, pre-β2, HDL3a, HDL3b, and HDL3c increase in hypercholesterolemia, hypertriglyceridemia, and mixed hyperlipidemia (Ishida et al., 1987; Xu and Fu, 2003; Yang et al., 2005). Furthermore, plasma concentrations of small pre-β-HDL are increased in hypercholesterolemia, hypertriglyceridemia, LCAT deficiency, and CHD but not in CETP deficiency (Ishida et al., 1987; Miida et al., 1997). Finally, plasma levels of small 70-kDa HDL are elevated in mixed hyperlipidemia (Atmeh and Robenek, 1996). Familial low HDL-C dyslipidemia appears to represent the only low HDL-C phenotype that is characterized by the presence of reduced concentrations of small pre-β-HDL particles (Soderlund et al., 2005).

In obesity and insulin resistance, frequent features of both MetS and type 2 diabetes, plasma levels of large HDL decrease in parallel with those of HDL-C, whereas levels of small HDL do not (Garvey et al., 2003; Festa et al., 2005; Goff et al., 2005; Okazaki et al., 2005). Levels of α1-HDL are lower and levels of α2-, α3-, and pre-β1-HDL are higher in obese subjects compared with lean control subjects (Sasahara et al., 1997). As a result, MetS, type 2 diabetes, obesity, and insulin resistance are all characterized by the prevalence of small, dense HDL in the HDL particle profile, indicating either impaired conversion from small to large HDL or accelerated turnover and remodeling of large HDL2-like particles.

Small, dense HDL also prevail in CHD patients. In male participants in the Framingham Offspring Study, subjects with CHD displayed higher levels of small pre-β1- and α3-particles and lower levels of large α1-, pre-α1-, and pre-α3-particles than subjects without CHD (Asztalos et al., 2004a). Similarly, subjects with new CV events possessed higher levels of small pre-β1- and α3-HDL and lower levels of large α1-, α2-, pre-α1-, and pre-α2-HDL than subjects without such events in the Veterans Affairs HDL Intervention Trial (VA-HIT) study (Asztalos et al., 2005). CAD patients also display elevated levels of lipid-poor apoA-I (Suzuki et al., 2005). The increase in small HDL and decrease in HDL of intermediate size as measured by nuclear magnetic resonance are associated with CAD severity in men admitted for diagnostic coronary arteriography (Freedman et al., 1998). Small HDL also prevail in peripheral arterial disease (Mowat et al., 1997). By contrast, CETP deficiency elevates plasma levels of large HDL particles, particularly buoyant, apoE-containing HDL1 but in also the bulk of HDL2; levels of small particles are affected to a minor degree (Asztalos et al., 2004b). Similarly, large HDL prevail in patients with type 1 diabetes (Colhoun et al., 2002).

Abnormalities in HDL subfraction distribution in hypercholesterolemia include reduced levels of apoA-I in large HDL2b and HDL2a and elevated levels of apoA-I in small pre-β1, pre-β2, HDL3b, and HDL3c subfractions (Ishida et al., 1987; Xu and Fu, 2003). FH HDL are further characterized by elevated content of apoE in association with increased plasma levels of apoE-enriched large HDL1, which represents only a minor fraction of total HDL in normolipidemic individuals (Keidar et al., 1990).

C. Impaired High-Density Lipoprotein Biological Activities in Dyslipidemic and Inflammatory States

1. Cholesterol Efflux Capacity. Alterations in HDL composition and metabolism as occur in dyslipidemia and inflammation are intimately associated with impaired biological activities (Fig. 5). However, data on HDL cholesterol efflux capacity in atherogenic dyslipidemia are conflicting. In primary hypertriglyceridemia associated with low HDL-C levels, TG-enriched HDL particles, whose intrinsic cholesterol efflux capacity from hepatoma Fu5AH cells is impaired, accumulate; the cholesterol efflux capacity of serum is also reduced (Brites et al., 2000). Similarly, LpA-I from hypertriglyceridemic patients with well-controlled type 2 diabetes exhibits decreased capacity to induce cholesterol efflux from adipose cells (Cavallero et al., 1995). TG-enriched HDL produced in vitro by coincubation of normal HDL with CETP are weak activators of cholesterol esterification by LCAT and poor donors of CE to HepG2 cells (Skeggs and Morton, 2002). Consistent with these findings, HDL capacity to deliver CE to hepatic cells through interaction with SR-BI diminishes as a result of HDL enrichment in TG (Greene et al., 2001). Furthermore, the presence of TG-rich lipoproteins may have deleterious consequences for HDL-mediated cellular cholesterol efflux as demonstrated by preincubation of lipid-loaded macrophages with TG-rich lipoproteins (Palmer et al., 2004).

By contrast, others have reported normal cholesterol efflux capacity of serum from hypertriglyceridemic subjects in Fu5AH cells, an observation that can be related to normal contents of HDL-PL, a key determinant of HDL-mediated efflux (Fournier et al., 2001). Furthermore, HDL from hypertriglyceridemic CAD patients with low HDL-C levels possess a normal capacity to extract cholesterol from smooth muscle cells (Uint et al., 2003). Consistent with these results, TG-enriched HDL are not deficient in cholesterol efflux properties from cholesterol-loaded J774 macrophages (Skeggs and Morton, 2002).

The intrinsic cholesterol efflux capacity of HDL is considerably impaired during inflammation. Cellular cholesterol efflux is largely mediated by apoA-I-containing HDL particles (Ohta et al., 1992); apoA-I replacement by SAA can therefore have a significant impact on efflux. Enrichment of HDL with SAA (up to high SAA contents of 86% of total HDL protein) results in increased HDL binding to, decreased cholesterol efflux capacity from, and increased selective CE uptake by macrophages (Banka et al., 1995; Artl et al., 2000). Importantly, SAA selectively impairs cholesterol efflux properties of small, dense HDL3 particles. Less pronounced enrichment of HDL with SAA in vivo (up to 27% of total HDL protein) does not influence cholesterol efflux but enhances HDL binding to macrophages (Banka et al., 1995).

The presence of SAA increases both HDL affinity to and selective CE uptake by macrophages but reduces affinity to and CE uptake by hepatocytes (Kisilevsky and Subrahmanyan, 1992; Artl et al., 2002; Cai et al., 2005); furthermore, SAA efficiently promotes cholesterol efflux from hepatoma cells (van der Westhuyzen et al., 2005). During inflammation, the number of binding sites for HDL-bound SAA increases on macrophages and decreases on hepatocytes; in addition, macrophage expression of ABCA1 is diminished (Baranova et al., 2002). Decreased PL contents in inflammatory HDL constitute another factor that contributes to deficient HDL cholesterol efflux properties as suggested by studies in patients with periodontitis (Pussinen et al., 2004). Together, these changes lead to a significant shift in the HDL-mediated cholesterol transport from hepatocytes toward macrophages under acute-phase conditions (Kisilevsky and Subrahmanyan, 1992). Biologically, such alterations may serve to redirect cholesterol to immune cells and to sites of injury and inflammation.

Similarly, acute-phase HDL from hamsters display diminished cholesterol efflux capacity from J774 macrophages, elevated cholesterol influx capacity, and decreased LCAT activity (Khovidhunkit et al., 2001). In vitro inactivation of LCAT in control HDL results in similar effects on cholesterol transport, identifying LCAT as another key player in the abnormal cholesterol transport properties of HDL particles in inflammatory states (Khovidhunkit et al., 2001).

Abnormal lipid composition may also impair cholesterol efflux properties of HDL particles, as demonstrated by the diminished capacity of large, CE-enriched HDL2 isolated from subjects with homozygous CETP deficiency to accept cholesterol from lipid-loaded mouse peritoneal macrophages (Ishigami et al., 1994). Normalization of the lipid composition of such HDL, as a result of the transfer of excess CE to SR-BI-overexpressing cells, improves HDL cholesterol efflux capacity (Kinoshita et al., 2004).

Oxidative modification represents another factor involved in the impairment of HDL cholesterol efflux capacity. In vitro oxidation of apoA-I by myeloperoxidase results in selective inhibition of ABCA1-dependent cholesterol efflux from macrophages (Bergt et al., 2004; Zheng et al., 2004); oxidation of Tyr-192 and Tyr-166 residues appears to specifically account for this effect (Shao et al., 2005a; Zheng et al., 2005). In parallel, the lipid-binding capacity of apoA-I is progressively impaired (Zheng et al., 2005). Similarly, both in vitro HDL oxidation by Cu^{2+} and HDL modification by acrolein decrease HDL-mediated cholesterol efflux from cultured cells (Rifici and Khachadurian, 1996; Shao et al., 2005b). Oxidized forms of cholesterol, including 7-ketocholesterol, may account for impaired cholesterol efflux from macrophage-derived foam cells mediated by apoA-I, such oxysterols act through alterations in cell membrane properties (Gelissen et al., 1999; Gaus et al., 2001). Finally, the cholesterol efflux capacity of apoA-I may be impaired as a consequence of nonenzymatic glycosylation (Fievet et al., 1995; Ferretti et al., 2005).

The central role of apoA-I in HDL-mediated cholesterol efflux is consistent with the deleterious role of apoA-I mutations. ApoA-I Oslo carrying the R160L substitution and apoA-I mutant carrying the P165R substitution, two naturally occurring apoA-I variants associated with low HDL-C levels, are less effective in promoting cholesterol efflux from smooth muscle cells compared with normal HDL (Daum et al., 1999). Both mutants display a reduced ability to activate LCAT. Furthermore, cholesterol efflux from human fibroblasts and murine peritoneal macrophages mediated by apoA-I Nichinan, a naturally occurring human apoA-I variant with a deletion of glutamic acid at codon 235, is reduced relative to normal apoA-I (Han et al., 1999; Huang et al., 2000). In addition, familial low HDL-C deficiency is characterized by reduced HDL3- and apoA-I-mediated cellular cholesterol efflux in the absence of abnormalities in cellular HDL3 binding (Marcil et al., 1999).

However, not all mutations in apoA-I lead to decreased cholesterol efflux capacity. ApoA-I Milano, a molecular variant of apoA-I characterized by the Arg173Cys substitution, displays potent capacity for cholesterol efflux, a unique feature that is related to the formation of apoA-I Milano homodimers with prolonged plasma residence time (Franceschini et al., 1999; Chiesa and Sirtori, 2003). Carriers of apoA-I Milano exhibit

severe hypoalphalipoproteinemia but are not at increased risk for premature CHD (Sirtori et al., 2001) (see below).

Finally, the capacity of HDL particles to extract cholesterol from peripheral cells may be impaired as a result of alterations in cellular HDL receptors, primarily ABCA1. Thus, individuals from families with ABCA1 mutations display lower levels of HDL-C, higher IMT, and lower cholesterol efflux from fibroblasts compared with matched control subjects (van Dam et al., 2002).

2. Antioxidative Activity. Recent evidence indicates that HDL particles are deficient in antioxidative activity in atherogenic dyslipidemias involving low HDL-C levels (Hansel et al., 2004; Kontush et al., 2005; Nobecourt et al., 2005). Thus, the antioxidative activity of small, dense HDL subfractions against LDL oxidation induced by 2,2′-azobis-(2-amidinopropane) hydrochloride is significantly impaired in patients with MetS (up to −23%) (Hansel et al., 2004) and well-controlled type 2 diabetes (up to −47%) (Nobecourt et al., 2005). HDL antioxidative activity is deficient both on a unit particle mass and on a particle number basis. In another study, large, light HDL2 show decreased protection of LDL against oxidation mediated by THP1 macrophages in poorly controlled type 2 diabetes (Gowri et al., 1999). The impaired antioxidative activity of small, dense HDL in MetS and type 2 diabetes is intimately related to the concomitant presentation of hypertriglyceridemia, hyperinsulinemia, and insulin resistance, thereby suggesting that abnormalities in both lipid and glucose metabolism underlie the antioxidative deficiency of HDL particles (Hansel et al., 2004; Nobecourt et al., 2005). Furthermore, all HDL subfractions from subjects with a normotriglyceridemic, normocholesterolemic, normoglycemic low HDL-C phenotype display lower antioxidative activity (up to −43%) than their counterparts from normolipidemic control subjects (Kontush et al., 2005). Interestingly, the intrinsic antioxidative activity of HDL particles is equally reduced in subjects with hyperalphalipoproteinemia associated with low HL activity and high HDL-TG content (Kontush et al., 2004).

The antioxidative HDL deficiency in low HDL-C dyslipidemias of MetS and type 2 diabetes and in a normotriglyceridemic low HDL-C phenotype is paralleled by decreased enzymatic activities and altered physicochemical properties of HDL (Hansel et al., 2004; Kontush et al., 2005; Nobecourt et al., 2005), thereby suggesting that the intrinsic properties of HDL particles, rather than low HDL-C levels per se, are determinants of antioxidative deficiency of HDL3 subfractions. In each study population (MetS, type 2 diabetes, and normotriglyceridemic low HDL-C phenotype), HDL3 subfractions were enriched in TG and CE depleted (Hansel et al., 2004; Kontush et al., 2005; Nobecourt et al., 2005), potentially reflecting elevated CETP activity and/or reduced HL activity; these alterations correlated with the diminished antioxidative activity of HDL3 subfractions.

Mechanistically, the relationship between TG enrichment of HDL particles and impairment of antioxidative activity can be explained by the fact that the replacement of CE by TG in the HDL lipid core considerably alters the conformation of the central and C-terminal domains of apoA-I, which are critical for HDL to act as an acceptor of oxidized lipids (Sparks et al., 1995; Curtiss et al., 2000). Moreover, replacement of CE by TG in spherical rHDL decreases the conformational stability of apoA-I (Sparks et al., 1995), resulting in TG-containing particles, which are unstable and which lose apoA-I upon storage.

Replacement of apoA-I by acute-phase proteins, primarily SAA, in small, dense HDL particles under conditions of chronic inflammation (Van Lenten et al., 2001a) may represent another mechanism contributing to the impairment of HDL antioxidative activity. As in the case of the replacement of CE by TG, the replacement of apoA-I by SAA may cause deficient activity of HDL as an acceptor of oxidized PL, resulting in their elevated accumulation in LDL.

Altered enzymatic activities also contribute to the antioxidative deficiency of small, dense HDL. PAF-AH and PON1 activities are consistently lower in all HDL subfractions from patients with type 2 diabetes compared with matched normolipidemic control subjects (Nobecourt et al., 2005). Moreover, PAF-AH and PON1 activities positively correlate with HDL3 antioxidative activity (Nobecourt et al., 2005), suggesting that these enzymes are implicated in the deficiency of HDL antioxidative function. In type 1 diabetes, serum concentrations of PON1 are reduced to such an extent that diminished oxidative protection of LDL by HDL in vitro results (Boemi et al., 2001). Consistent with this mechanism, inactivation of HDL-associated enzymes, such as PON1 or LCAT, by oxidation and/or glycation leads to decreased capacity of HDL to protect LDL from oxidative stress (Hedrick et al., 2000; Ferretti et al., 2001; Jaouad et al., 2003; Ferretti et al., 2005). The role of enzymes in HDL antioxidative deficiency is also consistent with data obtained in obese leptin-deficient (*ob*/*ob*), LDL-R$^{-/-}$ mice that possess dysfunctional HDL displaying not only decreased PON1 and LCAT activities, but also elevated levels of antibodies against oxLDL (Mertens et al., 2003).

An antioxidative deficiency of HDL may also be observed when antioxidative activity is measured in total HDL, rather than in individual HDL subfractions. Total HDL from humans and rabbits lose the ability to protect LDL against oxidation by artery wall cells in coculture during induction of the acute phase, concomitant with decreases in PON1 and PAF-AH activities (Watson et al., 1995a,b). Total HDL from mice that are genetically predisposed to diet-induced atherosclerosis do not protect LDL against oxidation in cocultures of artery wall cells when the mice are fed an atherogenic diet, injected with LDL-derived oxidized PL, or infected with influ-

enza A virus (Shih et al., 1996; Navab et al., 1997; Van Lenten et al., 2001b). Such loss of antioxidative activity of murine HDL is accompanied by decrease in PON1 activity. In addition, antioxidative deficiency of total HDL is observed in both apoE$^{-/-}$ (Navab et al., 1997) and apoA-II transgenic (Warden et al., 1993; Castellani et al., 1997) mice.

Furthermore, total HDL-mediated protection of LDL from oxidation by Cu^{2+} is compromised in postmenopausal compared with premenopausal women (Zago et al., 2004). This effect is paralleled by decreased plasma levels of HDL-C, elevated HDL levels of TG, and increased HDL oxidability in the postmenopausal group; the two latter parameters are significantly correlated (Zago et al., 2004). By contrast, serum PON1 activity did not differ between the groups, lending further support to our hypothesis that alterations of HDL core lipid composition are a key determinant of the antioxidative function of HDL particles. In addition, the antioxidative activity of total HDL toward LDL oxidation by γ-radiolysis of water is attenuated in elderly compared with young subjects (Jaouad et al., 2005). Finally, a diminished capacity of HDL to remove lipid hydroperoxides from erythrocyte membranes and attenuated HDL PON1 activity are features of poorly equilibrated type 1 diabetes (Ferretti et al., 2004).

In another study, no difference in HDL antioxidative activity, chemical composition, and serum PON activity was detected between type 2 diabetic patients with glycemic control and healthy control subjects (Sanguinetti et al., 2001). Similarly, patients with renal disease do not display antioxidatively deficient HDL despite low serum PON1 activity and low HDL levels of CE and α-tocopherol (Hasselwander et al., 1999). The high albumin content of HDL in this study is, however, noteworthy. These negative results suggest that measurement of the antioxidative activity of small, dense HDL may provide a more sensitive estimation of HDL antioxidative activity in some patient populations compared with measurements performed on total HDL.

Despite the fact that HDL antioxidative deficiency has been extensively documented in atherogenic dyslipidemias using in vitro assays, direct evidence for its presence in vivo is still lacking. Indirect evidence includes an association between elevated levels of plasma HDL and reduced levels of lipid peroxidation products after a single intravenous injection of a large dose of human HDL3 (200 mg of protein) in hypercholesterolemic rabbits (Klimov et al., 1993), suggesting compromised antioxidative activity of autologous rabbit HDL under conditions of hypercholesterolemia.

3. Anti-Inflammatory Activity. HDL particles possessing antioxidative activity within the normal range can prevent formation of or inactivate proinflammatory oxidized PL produced during LDL oxidation and are therefore anti-inflammatory (Navab et al., 2001a,b). Such potent anti-inflammatory activity becomes deficient and even transforms into in vitro pro-inflammatory action under conditions favoring development of atherosclerosis. In contrast with functional HDL, proinflammatory dysfunctional HDL is unable to protect LDL from oxidation by arterial wall cells and to prevent monocyte migration induced by oxLDL (Navab et al., 2001a,b). Total HDL from CHD patients with normal or elevated HDL-C levels are proinflammatory in both cell culture and cell-free fluorescent assays (Ansell et al., 2003). Similarly, HDL from mice that are genetically predisposed to diet-induced atherosclerosis become proinflammatory when the mice are fed an atherogenic diet, injected with LDL-derived oxidized PL or infected with influenza A virus (Shih et al., 1996; Navab et al., 1997; Van Lenten et al., 2001b). Proinflammatory HDL can also be detected in apoE$^{-/-}$ mice (Navab et al., 1997). In addition, transgenic mice overexpressing apoA-II possess proinflammatory HDL and develop atherosclerosis on a chow diet (Warden et al., 1993; Castellani et al., 1997). Finally, the anti-inflammatory activity of HDL is diminished in patients with obstructive sleep apnea subjected to repetitive cycles of hypoxia/reoxygenation (Tan et al., 2005); such patients frequently exhibit the MetS.

Formation of proinflammatory HDL correlates with decreases in the activities of various HDL-associated enzymes, such as PON1, PAF-AH, and LCAT, which are replaced by acute-phase proteins, such as SAA and ceruloplasmin; indeed, the content of these proteins in HDL increases during an inflammatory response (Navab et al., 2001a,b). Copper-containing ceruloplasmin may provide a source of transition metals for oxidative reactions, thereby accounting for the enhancement of LDL modification by acute-phase HDL (Navab et al., 2001a,b). Purified ceruloplasmin can induce LDL oxidation due to its content of a loosely bound copper atom (Ehrenwald et al., 1994; Ehrenwald and Fox, 1996); the in vitro enrichment of HDL with ceruloplasmin abrogates the ability of HDL to inhibit LDL modification in aortic wall cell cocultures (Van Lenten et al., 1995).

Interestingly, HDL PON1 activity is not decreased in CHD patients possessing proinflammatory HDL (Ansell et al., 2003), indicating that factors other than PON1 determine anti-inflammatory HDL dysfunction in vitro. In this study, plasma TG levels were markedly elevated (+76%) in CHD patients compared with control subjects (Ansell et al., 2003), suggesting that concomitant HDL enrichment in TG might have significantly contributed to the formation of dysfunctional HDL as proposed elsewhere (Hansel et al., 2004; Kontush et al., 2004, 2005; Nobecourt et al., 2005).

Intriguingly, all of the alterations in HDL composition that lead to attenuated anti-inflammatory and antioxidative activities (depletion in CE, apoA-I, PON1, and LCAT and increase in TG and SAA) are observed

during inflammation and in the acute-phase response (Khovidhunkit et al., 2004b; Esteve et al., 2005). The pro-inflammatory and prooxidative rearrangement of HDL particles and formation of LDL-derived oxidized PL have been hypothesized to form part of an evolutionary conserved mechanism of nonspecific innate immunity aimed to protect against infection (Navab et al., 2001a,b). Such an innate inflammatory response may include subnormal levels of HDL-C, increased HDL-TG content, and altered HDL apolipoprotein composition, all of which impair cholesterol efflux capacity as well as the antioxidant and anti-inflammatory activities of HDL particles. These modifications in HDL may be aimed to redirect cholesterol from the liver to immune cells, particularly macrophages, during infection (Kisilevsky and Subrahmanyan, 1992). Such a response to acute infection or injury can be advantageous in the short term but may become maladaptive in the long term. A sustained response that is not able to repair the injury, such as an emerging atherosclerotic plaque, which can be considered as a local inflammation (Libby, 2002), may lead to a chronic alteration in plasma lipid levels; such a response may become harmful, accelerating the formation of atherosclerotic lesions (Esteve et al., 2005). This mechanism is consistent with a recent hypothesis that accelerated development of atherosclerosis in old age is related to increased inflammation and concomitant endothelial dysfunction during early life (Finch and Crimmins, 2004; Charakida et al., 2005; Napoli et al., 2005). Within this concept, classic lipid changes associated with MetS (low HDL-C and elevated TG levels) are envisioned as a highly conserved evolutionary response aimed to repair tissue (Esteve et al., 2005).

It is indeterminate as to whether deficient anti-inflammatory activity of HDL is selectively associated with a subset of HDL particles as suggested by studies with rHDL (Nanjee et al., 1999; Nicholls et al., 2005b). The specific association of the deficient antioxidative activity with small, dense HDL (Hansel et al., 2004; Kontush et al., 2004, 2005; Nobecourt et al., 2005) together with the direct mechanistic link between anti-inflammatory and antioxidative activities (Navab et al., 2001a,b) suggests that small, dense HDL is a major subset of the total HDL particle population, which is responsible for both potent anti-inflammatory activity under normal conditions and for deficient activity under pro-atherogenic, pro-inflammatory conditions. This conclusion is consistent with the corrected anti-inflammatory properties of HDL from PLTP-deficient mice, which are characterized by the prevalence of small, lipid-poor HDL particles (Yan et al., 2004). Intriguingly, small, dense HDL3c represents the major HDL subfraction in newborns (Kherkeulidze et al., 1991), consistent with an elevated need for protection against infection in early life.

IV. Physiological Relevance of Defective High-Density Lipoprotein Function in Dyslipidemia and Metabolic Disease

The attenuated atheroprotective properties of HDL in metabolic disease raise the possibility of an indirect putative proatherogenic effect of these particles. Indeed, attenuated cholesterol efflux capacity of HDL can result in enhanced accumulation of cholesterol in the arterial wall and reduced RCT flux. Reduced efficiency of cholesterol flux through the RCT pathway is thought to account for the epidemiological link between subnormal HDL-C levels and increased incidence of CV disease (Nofer et al., 2002; Assmann and Nofer, 2003; Assmann and Gotto, 2004; Navab et al., 2004b). Impaired RCT has been shown to lead to accelerated atherosclerosis in subjects with Tangier disease (Oram, 2000) and in some cases of LCAT deficiency (Kuivenhoven et al., 1997). However, no data are available to our knowledge on the direct link between atherogenesis and the cholesterol efflux capacity of HDL particles, although infusion of apoA-I Milano/phospholipid complex has been shown to lead to a reduction in atheroma volume in patients with acute coronary syndromes, suggestive of plaque cholesterol efflux (Nissen et al., 2003) (see below).

A deficiency in the antioxidative and anti-inflammatory properties of HDL may also result in accelerated atherosclerosis. The oxidation hypothesis of atherosclerosis postulates that oxidation of lipoproteins, primarily LDL, in the arterial wall is a key element in atherogenesis (Steinberg et al., 1989). The validity of this statement has been confirmed in innumerable studies (Chisolm and Steinberg, 2000; Steinberg and Witztum, 2002). Its important corollary is that deficient LDL protection from oxidation may accelerate atherogenesis. Our recent data indicate clearly that impairment of the antioxidative activity of small, dense HDL in dyslipidemias involving low HDL-C levels is intimately associated with elevated oxidative stress, a newly recognized CV risk factor (Schwedhelm et al., 2004; Meisinger et al., 2005), and may therefore contribute to enhanced atherogenesis (Hansel et al., 2004; Kontush et al., 2005; Nobecourt et al., 2005). Indeed, dyslipidemic subjects presenting with atherogenic low HDL-C levels (MetS, type 2 diabetes, and a normotriglyceridemic low HDL-C phenotype) are characterized by both deficient antioxidative activity of small, dense HDL (Hansel et al., 2004; Kontush et al., 2005; Nobecourt et al., 2005) and elevated systemic oxidative stress assessed as plasma levels of 8-isoprostanes, products of nonenzymatic oxidation of arachidonic acid (Davi et al., 1999; Devaraj et al., 2001; Hansel et al., 2004; Kontush et al., 2005; Nobecourt et al., 2005). Furthermore, HDL antioxidative activity and plasma 8-isoprostanes are negatively correlated (Hansel et al., 2004; Nobecourt et al., 2005). In addition, in subjects with controlled type 2 diabetes, plasma 8-isoprostanes negatively correlate with HDL-C levels (No-

becourt et al., 2005), whereas in subjects with a normotriglyceridemic low HDL-C phenotype, plasma 8-isoprostanes positively correlate with an elevated ratio of total cholesterol/HDL-C, thereby reflecting an excess of atherogenic nonHDL-C relative to antiatherogenic HDL-C levels (Kontush et al., 2005). The elevation of plasma 8-isoprostanes in subjects with low HDL-C dyslipidemias is consistent with the elevation of F2α-isoprostanes in apoA-I deficient mice, emphasizing the link between oxidative stress and HDL deficiency (Moore et al., 2003). Mechanistically, HDL enrichment in TG may play a role in both elevated oxidative stress and the deficiency in HDL antioxidative activity, as suggested by strong association between plasma levels of oxLDL and the TG/HDL-C molar ratio in elderly subjects (Holvoet et al., 2003).

The presence of antioxidatively deficient HDL can facilitate or even trigger accumulation of LDL-derived proinflammatory oxidized PL in vivo, resulting in compromised anti-inflammatory activity (Ansell et al., 2003). Functional small, dense HDL particles may in turn provide protection of LDL against oxidative stress in the subendothelial space of the arterial wall via removal of oxidized lipids from LDL, with inactivation and subsequent transfer to the liver mediated by SR-BI. This mechanism may account, at least in part, for the negative results of recent large-scale placebo-controlled trials that did not show any beneficial effect of low-molecular-weight antioxidants, primarily vitamin E, on the development of CV disease (Stocker and Keaney, 2004). The Nutrition Committee of the American Heart Association Council on Nutrition, Physical Activity and Metabolism has recently concluded that "the existing scientific database does not justify routine use of antioxidant supplements for the prevention and treatment of CV disease" (Kris-Etherton et al., 2004). Moreover, a meta-analysis of performed trials suggests that supplementation with vitamin E may even increase all-cause mortality (Miller et al., 2005). We interpret these data to indicate that low-molecular-weight antioxidants do not play a key role in the protection of LDL from oxidation in vivo; by contrast, small, dense HDL may constitute a central element of such protection.

The impaired antioxidative activity of small, dense HDL particles in atherogenic dyslipidemia is intimately linked to the presence of a constellation of CV risk factors, including hypertriglyceridemia, hyperglycemia, hyperinsulinemia, insulin resistance, and a disequilibrium between circulating levels of atherogenic apoB-containing lipoproteins and antiatherogenic HDL in favor of the former (Hansel et al., 2004; Kontush et al., 2005; Nobecourt et al., 2005). All of these factors are independently characterized by their significant association with elevated systemic oxidative stress (Morrow, 2005). Such correlational data (Hansel et al., 2004; Kontush et al., 2005; Nobecourt et al., 2005) strongly suggest then that small, dense HDL particles function as a biosensor of oxidative stress, integrating a wide spectrum of prooxidant signals; the integration of such signals is in turn expressed as attenuated HDL antioxidative activity. Diagnostic detection of small, dense HDL possessing deficient antioxidative activity may therefore serve as a novel biomarker to assess elevated CV risk.

V. Functionally Defective Small, Dense High-Density Lipoprotein as a Therapeutic Target

Subjects at elevated CV risk in primary prevention and patients in secondary prevention with symptomatic coronary atherosclerosis possess small HDL3 particles whose antiatherogenic properties are impaired. Such defective functionality of small, dense HDL is frequently paralleled by decreased levels of HDL-C, which can principally be accounted for on the one hand by subnormal levels of large, cholesterol-rich HDL2-like particles and on the other by altered particle structure and composition. The association between low HDL-C levels and functional deficiency of small, dense HDL particles led us to propose that the deficient antioxidative activity of HDL can be corrected and concomitantly that elevated oxidative stress and attenuated HDL anti-inflammatory activity can be normalized by therapeutic approaches targeted to raise HDL-C and apoA-I levels and to normalize HDL structure and composition (Ashen and Blumenthal, 2005; Nicholls et al., 2005c).

Approaches to raise HDL-C levels may involve upregulation of apoA-I synthesis in hepatocytes, increased lipidation of apoA-I, accelerated efflux of cholesterol and PL from peripheral cells mediated by ABCA1, decreased activity of CETP, which results in the diminished heteroexchange of CE and TG between HDL and TG-rich lipoproteins, and inhibition of HDL2 holoparticle uptake by the liver mediated by hitherto unidentified receptor(s) for HDL holoparticles (Fig. 6). Such approaches are focused on small molecules whose pharmacological action results in marked raising of HDL-C, such as a CETP inhibitor or niacin, either alone or in combination with a statin. In this way, the LDL-C/HDL-C ratio may be reduced in dyslipidemic subjects, together with normalization of HDL metabolism, composition, and antiatherogenic function. Such normalization can result from 1) a decrease in plasma TG levels and concomitant replacement of TG by CE in the HDL core and normalization of apoA-I conformation and function and/or 2) a decrease in the level of oxidative stress and inflammation potentially involving replacement of SAA by apoA-I with normalization of intravascular HDL particle remodeling. Therapeutic raising of HDL levels is intimately associated with slowed progression of atherosclerosis and reduced CV risk, as observed in large-scale clinical studies such as the Armed Forces Regression Study (AFREGS) (Personius et al., 1998), the Bezafibrate Infarction Prevention Trial (BIP) [The Bezafibrate

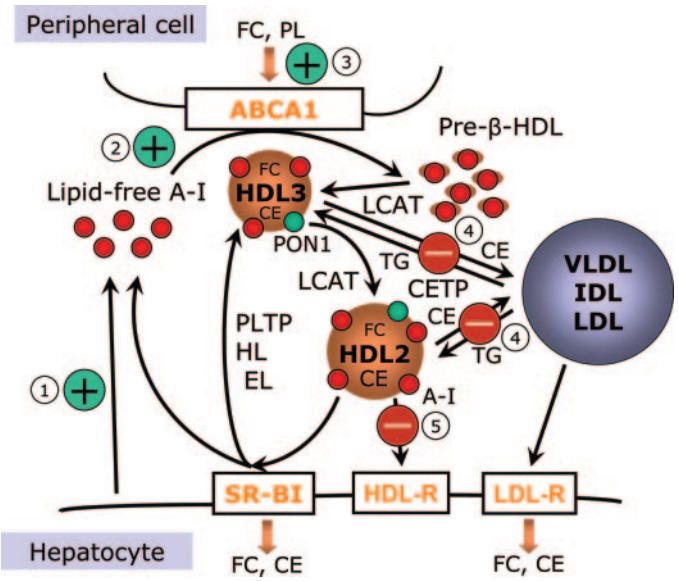


FIG. 6. Potential targets for therapeutic normalization of abnormal metabolism and deficient biological activities of HDL in atherogenic dyslipidemias of metabolic disease. ①, up-regulation of apoA-I synthesis in hepatocytes. ②, enhanced lipidation of apoA-I. ③, accelerated efflux of cholesterol and PL from peripheral cells mediated by ABCA1. ④, decreased activity of CETP, which results in the diminished heteroexchange of CE and TG between HDL and TG-rich lipoproteins. ⑤, inhibition of HDL2 holoparticle uptake by the liver mediated by hitherto unidentified receptor(s) for HDL holoparticles. EL, endothelial lipase; FC, free cholesterol; HDL-R, HDL holoparticle receptor; LDL-R, LDL receptor.

Infarction Prevention (BIP) Study, 2000], the VA-HIT (Robins et al., 2001), the HDL-Atherosclerosis Treatment Study (HATS) (Brown et al., 2001), and the Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2 trial (Taylor et al., 2004).

A. Cholesteryl Ester Transfer Protein Inhibitors

CETP inhibitors are promising therapeutic agents that markedly decrease the activity of plasma CETP (Le Goff et al., 2004; van der Steeg et al., 2004). The therapeutic strategy to inhibit CETP and thereby raise HDL-C derives from the fact that genetic deficiency of CETP is associated with increased HDL-C and decreased LDL-C levels, a profile that is typically antiatherogenic (Le Goff et al., 2004). Human subjects with heterozygous CETP deficiency and HDL-C levels >60 mg/dl exhibit a reduced risk of CHD (Curb et al., 2004). Such subjects display elevated levels of α1- and reduced levels of α3- and preβ-1-HDL subfractions, a profile consistent with corrected HDL antiatherogenicity (Asztalos et al., 2004b). Partial inhibition of CETP may therefore be atheroprotective; by contrast, the complete absence of CETP activity can create a potentially proatherogenic lipid profile (Brewer, 2004; Le Goff et al., 2004). The formation of large HDL particles with attenuated antiatherogenic activity and of heterogeneous LDL particles that are characteristic of lipoproteins in homozygous CETP-deficient patients can be avoided by partial inhibition of CETP. Studies in rabbits, a species with naturally high levels of CETP, support the therapeutic potential of partial CETP inhibition as an approach to retarding or even reversing atherogenesis (Barter et al., 2003b; Gaofu et al., 2005).

Small-molecule inhibitors of CETP have now been tested in human subjects and shown to increase the concentration of HDL-C while decreasing those of LDL-C and apoB. In healthy young normolipidemic subjects, torcetrapib dose-dependently inhibits CETP activity by 12 to 80% and increases HDL-C by 28 to 91% at 30 and 240 mg/day, respectively (Clark et al., 2004). With respect to modulation of apolipoprotein levels, apoA-I and apoE were elevated by 27 and 66%, respectively, whereas apoB was reduced by 26% at a dose of 240 mg/day in these studies. Significantly, CETP inhibition led to a reduction in the TG content of HDL particles and an increase in the CE content (Clark et al., 2004). In subjects with low levels of HDL-C, treatment with 120 and 240 mg of torcetrapib daily raised plasma HDL-C concentrations by 46 and 106%, respectively (Brousseau et al., 2004).

Torcetrapib treatment led to elevations in HDL2-C (+87%) to a greater extent than HDL3-C (+29%), increased plasma apoA-I and apoA-II and reduced plasma TG levels. In addition, torcetrapib altered the distribution of cholesterol among HDL and LDL subclasses, resulting in an increase in the mean particle size of both HDL and LDL particles in each cohort. Finally, torcetrapib increased the amount of apoA-I in α1-HDL and apoA-I pool size and decreased apoA-I fractional catabolic rate, thereby prolonging the residence time of apoA-I in the circulation (Brousseau et al., 2005). It is plausible that such a beneficial modulation of HDL metabolism results from normalization of apoA-I lipidation and thus conformation subsequent to a normalized CE/TG core lipid ratio (Brousseau et al., 2005).

Another small-molecule CETP inhibitor, JTT-705, at a dose of 900 mg/day, inhibits CETP by 37% and increases HDL-C by 34%, apoA-I by 15%, HDL2-C by 59%, and HDL3-C by 19% in subjects with mild hyperlipidemia (de Grooth et al., 2002); comparable effects were observed in patients with type II dyslipidemia (Kuivenhoven et al., 2005) and with familial hypoalphalipoproteinemia (Bisoendial et al., 2005). In rabbits, JTT-705 given at a high dietary dose of 0.75% inhibits CETP activity, increases both HDL-C concentrations and the ratio of HDL2-C/HDL3-C, and decreases the fractional esterification rate of HDL-C, again indicating a preferential increase in large HDL particles (Zhang et al., 2004). Levels of apoE in HDL, serum PON activity, and HDL-associated PAF-AH activity also increase, whereas plasma lysophosphatidylcholine concentration decreases; enhanced apoE content in HDL particles may be of special relevance in the potentiation of their catabolism in the liver and peripheral tissues through the LDL receptor pathway. Similarly, JTT-705 at a high dose of 300 mg/kg daily in rabbits increased plasma total cho-

lesterol, HDL-C, HDL2-C, and HDL3-C and reduced HDL-TG and CETP activity but did not influence the cellular cholesterol efflux capacity of HDL (Kobayashi et al., 2002). Interestingly, torcetrapib at 120 mg increased HDL-C substantially more than JTT-705 at 900 mg (Brousseau et al., 2004).

Despite minor impact of CETP inhibitors on circulating levels of small HDL particles, HDL functionality may be considerably improved, as suggested by elevated HDL content of CE and decreased TG (Clark et al., 2004). This conclusion may be particularly relevant for HDL antioxidative activity, which strongly depends on the CE/TG ratio in HDL particles (Hansel et al., 2004; Kontush et al., 2005; Nobecourt et al., 2005). Consistent with this hypothesis, JTT-705 decreases circulating levels of oxLDL in familial hypoalphalipoproteinemia (Bisoendial et al., 2005), whereas CETP inhibition in vitro by a monoclonal antibody renders LDL more resistant to oxidation (Sugano et al., 2000), observations that could translate into improved HDL-mediated protection of LDL from oxidation in vivo. The critical role of TG metabolism in the mechanism of action of CETP inhibitors is also supported by recent data suggesting that CETP inhibition may be especially effective in reducing CV risk in patients with elevated TG levels (Wolfe and Rader, 2004).

B. Niacin

Nicotinic acid (niacin), a vitamin of the B complex, has been used for almost 50 years as a lipid-modulating drug. The primary action of nicotinic acid is to suppress lipolysis of triacylglycerol in adipose tissue via inhibition of the hormone-sensitive TG lipase (Ganji et al., 2003; Rosenson, 2003; Karpe and Frayn, 2004; Meyers et al., 2004). The hormone-sensitive lipase is activated by reversible phosphorylation under the influence of protein kinase A. The antilipolytic action of nicotinic acid involves reduction of intracellular cyclic AMP levels in adipose tissue via a G-protein-coupled receptor that mediates inhibition of adenylyl cyclase. The recently discovered orphan G-protein-coupled receptor (HM74) in man has been identified as the nicotinic acid receptor in adipose tissue (Tunaru et al., 2003). HM74 appears to function as a low-affinity receptor for nicotinic acid, whereas the shorter homologous form (HM74A) represents a high-affinity receptor (Wise et al., 2003).

The key feature of the mechanism of action of nicotinic acid on lipid metabolism involves attenuated adipose tissue lipolysis, resulting in reduction of circulating levels of NEFA (Fig. 7). NEFA flux to the liver constitutes the main substrate for hepatic TG synthesis; this TG may either be integrated into nascent VLDL particles and secreted into the circulation or alternatively may be stored in the form of intracellular lipid droplets in the hepatocyte. Nicotinic acid is therefore distinguished as the sole pharmacological agent that markedly lowers NEFA and, as a direct consequence, plasma VLDL-TG levels.

TG levels are strongly inversely correlated with levels of HDL-C (Chapman et al., 2004). Thus, a nicotinic acid-mediated reduction in plasma TG levels predictably leads to marked raising of HDL-C. This effect is intimately linked to the action of CETP. By attenuating CETP-mediated depletion of HDL-CE in hypertriglyceridemic states such as MetS and type 2 diabetes, the TG-lowering action of nicotinic acid favors retention of CE in HDL with normalization of the HDL neutral lipid content, an increase in particle size, and a prolongation of plasma HDL-apoAI residence time in vivo, thus resulting in effective raising of HDL-C and apoA-I levels.

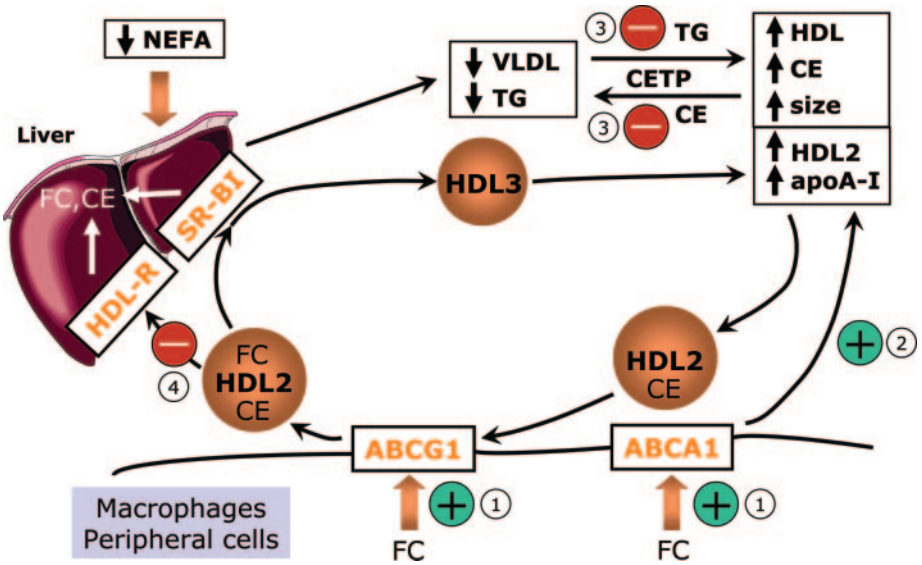


FIG. 7. Mechanisms involved in HDL raising by niacin. ① accelerated efflux of cholesterol from peripheral cells mediated by ABCA1 and ABCG1. ②, enhanced formation of mature HDL particles, primarily of HDL2. ③, diminished heteroexchange of CE and TG between HDL and TG-rich lipoproteins. ④, reduced uptake of HDL2 holoparticles by the liver mediated by receptor(s) for HDL holoparticles with maintained uptake of CE and free cholesterol from mature HDL by SR-BI. FC, free cholesterol; HDL-R, HDL holoparticle receptor.

A second mechanism that may contribute to nicotinic acid-induced raising of HDL-C involves the recent observation that this drug can stimulate cholesterol efflux from macrophages to primary HDL acceptors via the ABCA1 membrane transporter, thereby entering the RCT pathway (Fig. 7). Niacin activates ABCA1 via a nuclear peroxisome proliferator-activated receptor-γ-dependent pathway (Rubic et al., 2004). In addition, nicotinic acid decreases HDL uptake by the liver (Sakai et al., 2001) Whether such actions lead to local, intraplaque depletion of cholesterol is conjectural, although this mechanism is consistent with the capacity of nicotinic acid to facilitate regression of coronary artery stenoses as observed in the HATS trial (Brown et al., 2001).

Clearly then, reduction in lipolysis in adipose tissue and in the NEFA supply to the liver are essential features of the pharmacological action of nicotinic acid in modifying the atherogenic lipid profile. As a result of these effects, nicotinic acid effectively decreases plasma levels not only of TG-rich lipoproteins, but also of small, dense LDL and lipoprotein(a), and also raises levels of HDL-C by a preferential decrease in CE flux to VLDL driven by CETP (Fig. 7) (Chapman et al., 2004; McKenney, 2004).

Importantly, niacin is presently the most effective commercially available agent for increasing HDL-C; the HDL-C-raising effect of niacin may reach 35% (Chapman et al., 2004). Meta-analysis of 30 randomized controlled trials reveals that niacin increases HDL-C on average by 16%; in parallel, niacin decreases plasma TG typically by 20% (Birjmohun et al., 2005). The action of niacin results in elevated plasma levels of both large and small HDL (McKenney et al., 2001; Morgan et al., 2003). In addition, niacin favorably modifies HDL composition, preferentially increasing apoA-I in the form of large HDL2-like, CE-rich particles (Sakai et al., 2001; Morgan et al., 2003). Such increases in HDL levels of apoA-I and CE at the expense of TG are consistent with the view that niacin, by virtue of its action in normalizing HDL structure and chemical composition but also in increasing HDL particle numbers and concentrations, may normalize deficient antiatherogenic functions of HDL particles in atherogenic dyslipidemia.

C. Fibrates

Fibrates are peroxisome proliferator-activated receptor-α agonists that exert multiple effects on lipid and fatty acid metabolism and that also modulate the expression of genes of cellular cholesterol homeostasis, inflammation, and hemostasis (Steiner, 2005). Compared with CETP inhibitors and niacin, the average increase in HDL-C levels provided by fibrates is less pronounced and equaled +10% in 53 randomized, controlled trials (Birjmohun et al., 2005). The HDL-raising effect of fibrates is accompanied by a pronounced decrease in plasma levels of TG-rich lipoproteins of up to 48%. As a result, fibrates normalize HDL lipid composition, decreasing HDL-TG and increasing CE.

An alteration in the HDL subfraction profile is another central feature of fibrate therapy, which selectively increases circulating levels of small HDL particles, apoA-I and apoA-II. Fenofibrate (Sasaki et al., 2002; Ikewaki et al., 2004), bezafibrate (Miida et al., 2000; Kazama et al., 2003; Ikewaki et al., 2005), and gemfibrozil (Kahri et al., 1993) all selectively increase small and/or medium HDL particle numbers in patients with type 2 diabetes and in hypertriglyceridemic subjects. Remarkably, the increase in small HDL induced by fibrates may attain +168% in subjects with hypertriglyceridemia (Ikewaki et al., 2005). In addition, fenofibrate induces redistribution of PAF-AH from LDL to HDL in dyslipidemic patients, thereby lowering the proinflammatory potential of the enzyme (Tsimihodimos et al., 2003). Mechanistically, these effects can be accounted for by increased activities of both LPL, which provides release of surface fragments from TG-rich lipoproteins and their transfer to HDL during lipolysis, and of HL, which facilitates conversion of large to small HDL. Interestingly, plasma levels of small HDL3-C were a powerful predictor of CV risk in insulin-resistant subjects in the VA-HIT trial involving gemfibrozil treatment in insulin-resistant subjects (Robins et al., 2001). Fibrates may therefore be useful not only to induce an increase in circulating levels of HDL but also to enhance the functionality of small, dense HDL particles. The findings of the FIELD trial involving treatment of type 2 diabetes with fenofibrate and its effects on CV morbitity abd mortality are eagerly awaited (FIELD Study Investigators, 2004).

D. Statins

Statins are inhibitors of HMG-CoA reductase, whose major effect is to efficaciously decrease plasma levels of apoB-containing lipoproteins, primarily LDL, IDL, VLDL, and VLDL remnants. In addition, statins induce minor increases in HDL-C levels (by 5–10%) (Chong et al., 2002), consistent with a reduction in CETP activity (Guerin et al., 1995b, 2000b) and also with stimulation of apoA-I production (Schaefer et al., 1999). As a consequence, statins (atorvastatin and pravastatin) preferentially increase levels of HDL particles of large and medium size and α-mobility (Otvos et al., 2002; Schaefer et al., 2002; Kazama et al., 2003; Soedamah-Muthu et al., 2003).

Importantly, statins exert a number of pleiotropic effects, which include anti-inflammatory and antioxidative activities. Antioxidative actions of statins involve increases in the activity of HDL-associated enzymes, such as that demonstrated for PON1 in type IIa hyperlipidemic patients treated with atorvastatin (Harangi et al., 2004). Beneficial effects of statins on HDL functionality therefore appear to be mediated by a decrease in CETP activity (Guerin et al., 1995b, 2000b), a reduction

in LDL-C levels (i.e., the numbers of LDL particles to be protected by HDL) (Chong et al., 2002), and a decrease in systemic oxidative stress (Harangi et al., 2004; Cerielo et al., 2005). Consistent with this mechanism, treatment of CHD patients with simvastatin at 40 mg/day for 4 weeks potently enhanced HDL functionality, rendering HDL anti-inflammatory (Ansell et al., 2003).

E. Reconstituted High-Density Lipoprotein

rHDL typically consist of apoA-I and PL but may also include apoE and other lipids. rHDL may provide an innovative approach to the management of CV disease by its ability to rapidly raise circulating HDL levels upon intravenous injection and to act as primary cholesterol acceptors at the arterial wall and in peripheral tissues, thereby facilitating RCT (Nanjee et al., 1999). Infusion of rHDL leads to inhibition of adhesion molecule expression, attenuation of endotoxin-induced release of proinflammatory cytokines, reduced ROS generation, enhanced NO bioavailability, restored impaired flow-mediated dilatation, and stabilized vulnerable plaque in dyslipidemic subjects (Spieker et al., 2002; Bisoendial et al., 2003) and/or in animal models (Cockerill et al., 2001a,b; Cuzzocrea et al., 2004; Nicholls et al., 2005a,b). In addition, rHDL favorably affects the distribution of antioxidative enzymes, particularly PAF-AH, between HDL and other lipoproteins (Kujiraoka et al., 2004).

Because of a potent cholesterol efflux capacity, rHDL containing apoA-I Milano offer an especially promising approach to treat CV disease (Sirtori et al., 1999), particularly under acute conditions at the diseased site, namely the vulnerable, unstable atherosclerotic plaque (Newton and Krause, 2002). Consistent with this notion, 5-weekly infusions of apoA-I Milano induced a 4.2% reduction in plaque volume in patients with acute coronary syndromes (Nissen et al., 2003). These promising findings indicate outperformance of typical reductions in plaque volume established after statin therapy, the most efficient approach at present to delay progression of atherosclerosis, e.g., 0.9% reduction after 18 months of intensive therapy with 80 mg of atorvastatin in the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) study (Nissen et al., 2004; Birjmohun et al., 2005). In addition, apoA-I Milano-containing rHDL potently reduce atherosclerosis in atherosclerotic rabbits (Ameli et al., 1994; Soma et al., 1995) and mice (Shah et al., 1998, 2001). The regression of atherosclerosis induced by rHDL is most probably related to accelerated cholesterol efflux from the arterial wall with enhanced RCT to the liver (Nissen et al., 2003); the mechanistic relevance of other antiatherosclerotic activities of HDL to plaque regression remains unclear.

Importantly, injections of both apoA-I and apoA-I Milano result in the accumulation of small HDL particles (Nanjee et al., 1999), which also predominate in subjects with apoA-I Milano (Sirtori et al., 1999), thereby suggesting that small apoA-I-containing HDL are particularly cardioprotective. Selective elevation in circulating concentrations of small HDL particles forms a basis for another approach to raise plasma HDL levels which involves reinfusion of HDL after selective delipidation (Sacks et al., 2004).

F. Apolipoprotein-Mimetic Peptides

Oral or intravenous administration of small amphipathic helical peptides that mimic HDL apolipoproteins represents another promising strategy to raise circulating HDL levels and to attenuate atherosclerosis (Navab et al., 2005b). Apolipoprotein-mimetic peptides typically include those derived from apoA-I (Navab et al., 2005b) but also from apoE (Gupta et al., 2005b) and apoJ (Navab et al., 2005c). As a result of their beneficial impact on HDL metabolism, apoA-I-mimetic peptides improve HDL-mediated cholesterol efflux, activate cholesterol efflux from macrophages, increase PON1 activity, convert HDL from proinflammatory to anti-inflammatory particles, increase endothelial production of nitric oxide, decrease endothelial production of superoxide, improve vasodilation, induce vascular heme oxygenase and superoxide dismutase, inhibit endotoxin-induced inflammatory responses, and reduce atherosclerosis in mice and monkeys (Garber et al., 2001; Navab et al., 2002; J. Ou et al., 2003; Z. Ou et al., 2003; Li et al., 2004; Navab et al., 2004a; Gupta et al., 2005a; Kruger et al., 2005).

Whereas typical apoA-I mimetics and apoA-I itself consist of L-amino acids, which are rapidly degraded in the digestive system and need to by supplemented parenterally, one of these peptides, D-4F, consists of D-amino acids, is not digested by mammalian enzymes, and can be administrated orally (Navab et al., 2005b); the latter represents an important advantage in the development of apoA-I mimetics. Interestingly, covalent binding to an apoA-I mimetic of an apoE fragment critical for binding to the LDL receptor endows the resulting peptide with potent cholesterol-lowering capacity and further increases its antiatherogenic activity (Gupta et al., 2005b). Finally, orally administrated small zwitterionic tetrapeptides, which are too small to form an amphipathic helix associate with HDL and display potent antiatherosclerotic, antioxidative, and anti-inflammatory activities in apoE$^{-/-}$ mice (Navab et al., 2005).

Significantly, apoA-I mimetics cause rapid formation of small, lipid-poor pre-β-HDL, both in vivo when given to animal models and in vitro when added to human plasma (Navab et al., 2004a), consistent with a key antiatherosclerotic role for such particles. The anti-inflammatory properties of apoA-I mimetic peptides appear to depend on subtle differences in the configuration of the hydrophobic face of the peptides, which determines their ability to sequester pro-inflammatory oxidized lipids (Datta et al., 2004; Epand et al., 2004).

G. Combination Therapy

As distinct HDL-C raising agents function through complementary mechanisms, their effects may be additive; hence, association of such medications has been proposed. Another advantage of such combination therapy involves the potential for use of lower doses of each agent compared with their use in monotherapy to obtain additive elevations in HDL-C levels; such an approach may also lead to a reduction in adverse effects. Two trials evaluating niacin combined with either a statin or a bile acid sequestrant reported impressive HDL-C increases ranging from 25 to 41% with an unprecedented reduction in CV event rates ranging from 60 to 72% (Brown et al., 1995, 2001). Such effects can be accounted for by the additive benefit of concomitant reduction of LDL-C and raising of HDL-C. These impressive reduction rates also correspond well to the estimated reduction rates based on the increase in HDL-C obtained in these studies (Birjmohun et al., 2005) (i.e., 1% HDL-C increase being associated with a 1–3% reduction in CV events) (Robins et al., 2001). Furthermore, addition of extended-release niacin to statin therapy slows the progression of carotid atherosclerosis (measured as IMT), increases HDL-C (+21%), and decreases TG (−15%) and non-HDL-C (−7%) levels among individuals with established CHD and moderately low HDL-C (Taylor et al., 2004). Finally, one clinically important feature of the action of statins on HDL functionality involves their synergism with a CETP inhibitor (Brousseau et al., 2004, 2005) or apoA-I mimetic peptide (M. Navab et al., 2005a).

VI. Conclusions

HDL particles possess potent biological activities, including cellular cholesterol efflux capacity, antioxidative, anti-inflammatory, antiapoptotic, antithrombotic, anti-infectious, and vasodilatory activities, which provide protection from atherosclerosis or may even favor plaque regression. Small, dense HDL afford potent protection of LDL against oxidative stress, possess pronounced anti-inflammatory properties, and display high cholesterol efflux capacity. The atheroprotective properties of HDL can, however, be compromised under conditions associated with accelerated development of atherosclerosis, such as in atherogenic low HDL-C dyslipidemias typical of metabolic diseases, including MetS and type 2 diabetes. Such functional HDL deficiency is intimately associated with alterations in HDL metabolism and structure. Formation of small, dense HDL particles with attenuated antioxidative activity is mechanistically related to HDL enrichment in TG and SAA, depletion of CE and apoA-I, and covalent modification of key HDL apolipoproteins. Deficiency of HDL function may result in accelerated atherosclerosis; therapeutic normalization of HDL function in terms of both the quantity and quality of HDL particles, using CETP inhibitors, niacin, rHDL or other agents, may therefore represent a novel therapeutic approach to attenuate atherosclerosis in dyslipidemic subjects with metabolic disease. Induction of selective increases in the circulating concentrations of HDL particles possessing normal antiatherogenic activity is especially promising; more specifically, recent studies suggest that small, dense HDL3 particles represent a new therapeutic target in atherogenic dyslipidemia, particularly in view of its intimate association with a proinflammatory state.

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