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Low High-Density Lipoprotein Cholesterol

Physiological Background, Clinical Importance and Drug Treatment

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Abstract

Low high-density lipoprotein (HDL) cholesterol is an important risk factor for coronary heart disease (CHD). *In vitro*, HDL exerts several potentially anti-atherogenic activities. HDLs mediate the reverse cholesterol transport (RCT) from peripheral cells to the liver, inhibit oxidation of low-density lipoprotein (LDL), adhesion of monocytes to the endothelium, apoptosis of vascular endothelial and smooth muscle cells and platelet activation, and stimulate the endothelial secretion of vasoactive substances as well as smooth muscle cell proliferation. Hence, raising HDL-cholesterol levels has become an interesting target for anti-atherosclerotic drug therapy. Levels of HDL cholesterol and the composition of HDL subclasses in plasma are regulated by apolipoproteins, lipolytic enzymes, lipid transfer proteins, receptors and cellular transporters. The interplay of these factors leads to RCT and determines the composition and, thereby, the anti-atherogenic properties of HDL. Several inborn errors of metabolism, as well as genetic animal models, are characterised by both elevated HDL cholesterol and increased rather than decreased cardiovascular risk. These findings suggest that the mechanism of HDL modification rather than simply increasing HDL cholesterol determine the efficacy of anti-atherosclerotic drug therapy.

In several controlled and prospective intervention studies, patients with low HDL cholesterol and additional risk factors benefited from treatment with fibric acid derivatives (fibrates) or HMG-CoA reductase inhibitors (statins). However, only in some trials was prevention of coronary events in patients with low HDL cholesterol and hypertriglyceridaemia related to an increase in HDL cholesterol. We discuss the clinical and metabolic effects of fibrates, statins, nicotinic acid and sex steroids, and present novel therapeutic strategies that show promise in modifying HDL metabolism.

In conclusion, HDL-cholesterol levels increase only moderately after treatment with currently available drugs and do not necessarily correlate with the functionality of HDL. Therefore, the anti-atherosclerotic therapy of high-risk cardiovascular patients should currently be focused on the correction of other risk factors present besides low HDL cholesterol. However, modification of HDL

metabolism and improvement of RCT remain an attractive target for the development of new regimens of anti-atherogenic drug therapy.

Coronary heart disease (CHD) is a leading cause of death and morbidity in both industrialised and developing countries. Coronary atherosclerosis is multifactorial in origin. The initiation and progression of atherosclerosis and the final precipitation of cardiovascular events results from a series of parallel and sequential events which involves the occurrence of pathogens ('risk factors'), endothelial dysfunction, retention of atherogenic lipoproteins in the arterial wall, inflammation, foam cell and fibrous plaque formation, plaque rupture and thrombosis.[1,2]

Low-density lipoproteins (LDL) have been shown to interact with several of these pathogenetic pathways. Many epidemiological studies have demonstrated that elevated LDL cholesterol is an important risk factor for CHD. Moreover, several inborn errors of LDL metabolism (e.g. familial hypercholesterolaemia due to LDL receptor gene defects) and genetic animal models of hypercholesterolaemia provided consistent evidence for the atherogenicity of LDL. Finally, six controlled and prospective landmark studies which together followed up more than 50 000 individuals for at least 5 years, clearly demonstrated that lowering of LDL cholesterol with HMG-CoA reductase inhibitor (statin) or fibric acid derivative (fibrate) therapy reduces coronary event rates by an average of 30% (fatal and non-fatal myocardial infarction [MI] as well as the need for coronary intervention). This outcome was reached without any increases of non-cardiovascular morbidity and mortality.[3-8]

Therefore, although statins were shown to exert a broad scope of pleiotropic (i.e. non-LDL-related effects) on the vascular wall, LDL is currently considered as a causal factor in atherosclerosis. As a consequence, the control of LDL cholesterol has become a cornerstone in both the primary and secondary prevention of CHD. Nevertheless, about 60–70% of coronary events are not prevented by statin therapy.[3-8]

Low high-density lipoprotein (HDL) cholesterol is another important risk factor of CHD,[9] which has been found in more than 40% of patients experiencing an MI.[10] This prevalence may be even higher in some ethnic populations, for example those from Turkey, Israel or Arab countries, who for unknown reasons have 0.26–0.4 mmol/L lower mean HDL-cholesterol levels than Caucasians and who face a dramatic increase in the incidence of cardiovascular disease.[11,12] Moreover, some controlled intervention studies have demonstrated a significant benefit of lipid lowering drug therapy to patients with isolated low HDL cholesterol.[13-15] Hence, HDL cholesterol has attracted a lot of interest from clinicians and the pharmaceutical industry, not only as a marker of increased coronary risk but also as a therapeutic target.

While current international guidelines consider low HDL cholesterol an indication to start treatment of risk factors,[16,17] a plasma HDL-cholesterol level >1 mmol/L is increasingly advocated as a therapeutic goal.[18] However, to date the majority of the presently available drugs do not increase HDL-cholesterol levels effectively enough to reach this goal in many patients with manifest CHD or increased cardiovascular risk. Moreover, experience from patients with inborn errors of HDL metabolism and from genetic animal models of HDL metabolism indicate that it is not the increase of HDL cholesterol *per se* but the mechanism of altered HDL metabolism and function that is relevant for protection from atherosclerosis.

In this atmosphere of hope, promise and caveats, clinical and basic HDL research has become very active in recent years, and has identified novel regulators of HDL metabolism and potential targets for anti-atherosclerotic drug therapy.

1. Structure of High-Density Lipoprotein (HDL)

HDLs are heterogenous but share a high density (>1.063 g/mL), a small size (Stoke's diameter: 5–17nm) and the absence of apolipoprotein (apo) B. More than 20 proteins are associated with HDL particles (table I). Most of them are apolipoproteins that serve as structural components, cofactors or inhibitors of enzymes and ligands of receptors. Several HDL-associated enzymes contribute to lipid metabolism and transfer. In addition, proteins with diverse functions are carried by HDL. By definition, HDL transports various lipids, which are traditionally considered as a passive cargo. Most recently, however, some quantitatively minor HDL lipids have been recognised as bioactive mediators of potentially anti-atherogenic functions.[19-22]

1.1 Apolipoproteins

The majority of HDL-associated apolipoproteins (A-I, A-II, A-IV, C-I, C-II, C-III and E) have a secondary structure which is characterised by the presence of single or tandem 11-amino acid repeats that form amphipathic α-helices.[23] The amphipathic structure allows apolipoproteins to complex lipids and is, therefore, crucial for the formation of HDL particles.[24] ApoA-I is the quantitatively predominant apolipoprotein of HDL and the absence of apoA-I virtually causes HDL deficiency. Heterozygotes for apoA-I deficiency or certain apoA-I variants have half-normal HDL-cholesterol levels indicating that the availability of apoA-I is an important rate-limiting factor for the determination of HDL-cholesterol levels.

In the nascent discoidal HDL particle, two apoA-I molecules form belts[25] on the surface of particles,[26] which allow the hydrophobic surfaces of the α-helices to interact with the hydrophobic side chains of phospholipids and cholesterol, whereas the hydrophilic surfaces mediate solution of the lipoprotein in the aqueous plasma environment. This spatial arrangement is also thought to support the conversion of discoidal HDL precursors into spherical mature HDL for which no structural information is yet available. In addition to their function as structural proteins of HDL, several apolipoproteins fulfil specific functions as cofactors or inhibitors of enzymes and receptor ligands (see table I for details).

1.2 Enzymes

The majority of HDL-associated enzymes mediate metabolism or transport of HDL-associated lipids (see table I for details). Lecithin-cholesterol-acyltransferase (LCAT) catalyses the transfer of the sn-2 fatty acid from phosphatidylcholine to cholesterol and, thereby, produces lysolecithin and cholesterol esters. ApoA-I acts as a cofactor of LCAT which provides the required lipid-water interface. LCAT deficiency causes virtual HDL deficiency in homozygotes and reduces HDL cholesterol by 30% to 50% in heterozygotes, indicating LCAT activity is another important rate limiting factor of HDL cholesterol.[27]

Active phospholipid transfer protein (PLTP) transfers phospholipids between HDL and LDL or triglyceride-rich lipoproteins, and thereby contributes to the conversion of small HDL particles into large HDL as well as to the release of HDL precursors from HDL.[28] However, the majority of PLTP in plasma is inactive.[29] Both the inactivation mechanism and the role of inactive PLTP are as yet unknown. Genetic PLTP deficiency is not known in humans. In genetically modified mice, both knockout and overexpression of PLTP causes decreased

Table I. Characteristics of pivotal proteins in high-density lipoprotein (HDL) metabolism

Gene/protein	Function in RCT	Major sites of synthesis	Regulation	
			up	down
Apolipoproteins (apo)				
apoA-I	Major protein component of HDL, activation of LCAT, stimulation of cholesterol efflux, ligand of HDL binding sites (e.g. SR-BI, ABCA1)	Liver, small intestine	Estradiol, thyroxin, glucocorticoids, IL-6, retinoids, fibrates, statins, hunger	Testosterone, TGFβ, cholic acid
apoA-II	Major protein component of HDL, ligand of HDL binding sites, inhibition of HL	Liver	Retinoids, fibrates	Thyroxin, TNFα
apoA-IV	Activation of LCAT, modulation of LPL, stimulation of cholesterol efflux	Small intestine	Unsaturated fatty acids, bile acids, peptide YY	Leptin
apoC-I	Activation of LCAT, inhibition of hepatic TGRL uptake	Liver		
apoC-II	Activation of LPL, inhibition of hepatic TGRL uptake	Liver		
apoC-III	Inhibition of LPL, inhibition of hepatic TGRL uptake	Liver	Retinoids, cytokines	Insulin, TNFα, fibrates, fish oil
apoD	Formation of preβ3-LpA-I	Liver, spleen, CNS, small intestine, adrenals, placenta	Testosterone, glucocorticoids	Estradiol
apoE	Ligand of apoE-receptors, mobilisation of cellular cholesterol in macrophages and cholesterol efflux	Liver, macrophages, CNS	Cholesterol, estradiol, oxysterols	Cytokines
apoF	Inhibition of CETP			
Enzymes and lipid transfer proteins				
LPL	Hydrolysis of triglycerides in TGRL, generation of HDL precursors and surface remnants	Muscle, adipose tissue	Vitamin D_3, cAMP, insulin, glucose, α1-adrenergic, β3-adrenergic, free fatty acids, fibrates, thiazolidinediones, fish oil	Endothelin-1, IL-1, IL-11, TNFα, IFNγ, LPS, PTH, PGE_2
HL	Hydrolysis of triglycerides and phospholipids in HDL; cofactor of cellular HDL-binding; generation of lipid-free apoA-I	Liver	Testosterone	Estradiol, growth hormone, dexamethasone, fibrates
EL	Hydrolysis of phospholipids in HDL	Endothelium, liver, macrophages	TNFα, IL-1β, shear stress	
LCAT	Cholesterol esterification, maturation of HDL	Liver, testes, CNS	IL-6, dexamethasone, free fatty acids, corticotrophin	TGFβ
CETP	Exchange of cholesteryl esters and triglycerides between HDL and LpB, conversion of HDL, generation of lipid-free apoA-I	Liver, small intestine, spleen, macrophages	Cholesterol, oxysterols, retinoids	Statins
PLTP	Transfer of phospholipids (surface remnants) from TGRL onto HDL, fusion of small HDL, generation of lipid-free apoA-I, hepatic assembly of apoB-containing lipoproteins	Liver, endothelium	Fibrates, bile acids, hypoxia	

Continued next page

Table I. Contd

Gene/protein	Function in RCT	Major sites of synthesis	Regulation	
			up	down
Cellular receptors and transporters				
ABCA1	Mediation of cholesterol and phospholipid efflux, maturation of HDL	Liver, macrophages, intestine, fetal tissues (ubiquitous)	Cholesterol, oxysterols, retinoids, cAMP, fibrates	IFNγ, statins, unsaturated fatty acids, acetoacetate, lipopolysaccharides
SR-BI	Mediation of selective uptake and cholesterol efflux	Liver, adrenals, testes, ovary, macrophages	Estradiol (Kupffer cells), gonadotropins, corticotrophin, cAMP, fibrates, statins, $PGF_{2\alpha}$, testosterone	Estradiol (hepatocytes), cholesterol, glucocorticoids
Cubilin	Tubular resorption of HDL and apoA-I	Renal tubulus cells, intestine, yolk sac, placenta	Shear stress	

ABCA1 = ATP binding cassette transporter A1; **cAMP** = cyclic adenosine monophosphate; **CETP** = cholesteryl ester transfer protein; **EL** = endothelial lipase; **HL** = hepatic lipase; **IFNγ** = interferon gamma; **IL** = interleukin; **LCAT** = lecithin: cholesterol-acyltransferase; **LpB** = lipoproteins containing apoB; **LPL** = lipoprotein lipase; **LPS** = lipopolysaccharide; **PGE_2** = prostaglandin E2; **$PGF_{2\alpha}$** = prostaglandin F2α; **PLTP** = phospholipid transfer protein; **preβ$_3$-LpA-I** = lipoproteins containing apoA1 with electrophoretic preβ$_3$ mobility; **PTH** = parathyroid hormone; **RCT** = reverse cholesterol transport; **SR-BI** = scavenger receptor B1; **TGFβ** = tissue growth factor-β; **TGRL** = triglyceride rich lipoproteins; **TNFα** = tumour necrosis factor-α.

HDL-cholesterol levels.[28] PLTP has also been shown to contribute to the intracellular lipidation of apoB containing lipoproteins.[30]

Cholesterol ester transfer protein (CETP) catalyses the exchange of HDL-associated cholesterol esters against triglycerides and phospholipids predominantly from very low-density lipoprotein (VLDL) and, thereby, generates small HDL at the expense of large HDL. CETP also exchanges cholesteryl esters between LDL and VLDL; however, this transfer is modulated by apoF which inhibits the transfer between LDL and VLDL.[31] In human plasma, apoF is associated with LDL particles[32] and limited studies suggest that apoF disrupts the association of CETP with lipoproteins, so that CETP mediated transfer from HDL to VLDL is favoured.[31,32]

Paraoxonase hydrolyses arylesters including lipid hydroperoxides and oxidised phospholipids, and organophosphates used as insecticides. Similar antioxidative functions are exerted by platelet activating factor acylhydrolase (PAF-AH) and glutathione peroxidase, which are also present in HDL.[33,34]

1.3 Other Proteins

Several other proteins have been found in HDL (table I). ApoJ (clusterin) exerts multiple functions including the inhibition of the terminal complement complex C5b-9 and the mediation of receptor-mediated uptake of phospholipid complexes.[35]

Serum amyloid A displaces apo A-I and apo A-II from HDL during acute phase inflammation and, thereby, modifies HDL function. It has been speculated that serum amyloid A could direct HDL to sites of inflammation where it could play a role in the protection and regeneration of cell membranes.[36] However, during acute phase inflammation HDL looses its anti-inflammatory properties as well as the ability to protect LDL from oxidation.[37] The loss of the anti-inflammatory properties of HDL may even turn HDL into a pro-atherogenic particle.

1.4 Lipids

On average, lipids constitute 50% of total HDL mass, namely 30% phospholipids, 10–20% cholesterol and cholesterol esters, and 5% triglycerides. The quantity and quality of lipids vary considerably among HDL subclasses. In particular, the phospho-

lipid class is very heterogenous. Phosphatidylcholine (about 80% of phospholipids) and sphingomyeline (about 20% of HDL phospholipids) are indispensable structural components of HDL and are also needed to dissolve unesterified cholesterol.[34]

In addition, specific phospholipids are present at low concentrations in HDL which were shown to have important functions previously thought to be mediated by protein components of HDL. Such lipids, such as arachidonic acid or linoleic acid containing phosphatidylcholine, promote the inhibitory effects of HDL on the adhesion of leucocytes to the endothelium. Other lipids, such as sphingosylphosphorylcholine and lysosulfatide, suppress apoptosis of endothelial cells, and stimulate proliferation of fibroblasts and vascular smooth muscle cells.[19-21,34] Hence, some of the atheroprotective function of HDL is mediated by lipids.

1.5 HDL Subclasses

Differences in the quantitative and qualitative content of lipids and proteins result in the formation of distinct HDL subclasses which are characterised by shape, density, size, charge and antigenicity.[38] Following agarose gel electrophoresis of plasma and anti-apoA-I-immunoblotting, the majority of apoA-I is present in a fraction which migrates with α-electrophoretic mobility and is designated α-LpA-I. This fraction eventually contains all of the cholesterol, which is quantified in the routine laboratory as HDL cholesterol, and can be further differentiated according to density and size into HDL_2 and HDL_3 or according to apolipoprotein composition into LpA-I (lipoproteins containing apoA-I) and LpA-I/A-II (lipoproteins containing both apoA-I and apoAII).[38]

Approximately 5–15% of apoA-I in human plasma is associated with particles which have electrophoretic preβ-mobility and which can be further distinguished by size into small $preβ_1$-LpA-I, intermediate $preβ_2$-LpA-I and large $preβ_3$-LpA-I. $Preβ_1$-LpA-I contains apoA-I either as a lipid-free apolipoprotein or in association with a few molecules of sphingomyelin and phosphatidylcholine.[39,40] Similar lipid-poor particles contain only apoE (γ-LpE) or apoA-IV (LpA-IV) as their apolipoproteins.[41-43] It is also important to note that, relative to the concentration of lipid-rich α-HDL, the concentration of these lipid-poor particles is increased in extravascular compartments.[40,44,45]

2. Functions of HDL

HDL-associated proteins and lipids exert a broad scope of potentially anti-atherogenic effects that have previously been reviewed by us in detail.[22] Therefore, only essential hallmarks are summarised below.[34]

2.1 Cholesterol Efflux

In contrast to most other cells of the body, which regulate their cholesterol content by a finely tuned interplay of LDL-receptor mediated lipoprotein uptake and endogenous cholesterol synthesis, macrophages (including those of the arterial wall) can accumulate large amounts of cholesterol by uncontrolled scavenger receptor (SR)-A–mediated uptake of modified lipoproteins and phagocytosis (figure 1). This process turns macrophages into activated foam cells which produce various growth factors, cytokines and proteases and, thereby, influence the course of atherosclerosis.[1,2]

Efflux is the only mechanism by which macrophages can limit or reverse the cellular cholesterol accumulation. The importance of this pathway is highlighted by a broad spectrum of cholesterol efflux pathways. Two of them are independent of extracellular cholesterol acceptors, namely the secretion of lipid-rich apoE-containing particles and the oxidation of cholesterol into 27-hydroxycholesterol together with the subsequent secretion of this oxysterol. The appearance of foam cells in genetically modified mice lacking macrophage apoE, as

well as in cholesterol-27-hydroxylase (CYP27) deficient patients with cerebrotendinous xanthomatosis, highlight the relevance of these pathways for the regulation of macrophage cholesterol homeostasis.[46-49]

However, the HDL-dependent cholesterol efflux pathways are generally considered as more important. HDL and lipid-free apolipoproteins induce at least four kinds of cholesterol efflux, two of which are independent of cellular proteins and slow, and two of which depend on cellular proteins and are fast[50] (figure 1).

2.1.1 Receptor-Independent Cholesterol Efflux Pathways: Aqueous Diffusion and Microsolubilisation

Unesterified cholesterol desorbs from the plasma membrane and diffuses across a concentration gradient to extracellular lipoproteins including HDL. This passive process of diffusional efflux is enhanced by esterification of cholesterol by LCAT which functions to maintain the gradient of cholesterol between the cell surface and the HDL particle.[50] This passive process is not very efficient in mediating cholesterol removal from macrophages.[50]

Lipid-free apolipoproteins A-I, A-II, A-IV, C and E, and also amphipathic synthetic peptides, via the abundance of amphipathic helices rather than a specific domain, can associate spontaneously with phospholipids of the cell membrane and sequester them into the extracellular compartment.[50-53] This so-called microsolubilisation is followed or paralleled with some cholesterol efflux, but does not lead to the formation of distinct HDL precursors that maturate to HDL. This efflux involves amino-terminal domains, and the interaction of amino-terminal and carboxyterminal domains of apoA-I.[54]

2.1.2 Scavenger Receptor B1

Expression of SR-BI in macrophages enhances HDL-mediated cholesterol efflux.[55-57] This is in contrast to the effect of SR-BI in liver or steroidogenic cells, where SR-BI mediates influx of cholesterol.[58] The cellular and extracellular metabolism of cholesterol may dictate the inward or outward direction of cholesterol flux. According to this model, the synthesis of bile acids, lipoproteins or steroid hormones favours influx of cholesterol into liver and steroidogenic organs, respectively, whereas cellular cholesterol ester hydrolysis and extracellular LCAT-mediated cholesterol esterification favours cholesterol efflux from macrophages.

Another open question relates to the mechanism by which SR-BI facilitates cholesterol efflux. Since another HDL binding protein of the SR-B family, i.e. CD36, does not mediate cholesterol efflux, it has been suggested that binding of HDL to SR-BI facilitates cholesterol efflux by reorganisation of lipids within the plasma membrane.[56] Both SR-BI– mediated cholesterol efflux and SR-BI–mediated HDL lipid-uptake into the liver are potentially anti-atherogenic pathways. In agreement with this, inactivation of SR-BI increases atherosclerosis in mice despite increasing HDL cholesterol[59,60] and overexpression of SR-BI decreases atherosclerosis in mice despite decreasing HDL cholesterol.[61,62]

2.1.3 ATP Binding Cassette Transporter A1

Lipid-free apoA-I induces phospholipid and cholesterol efflux from various cells, including macrophages, via interaction with the ATP binding cassette transporter A1 (ABCA1).[63] This pathway depends on a distinct domain within the carboxyterminus of apoA-I, whereas 'microsolubilisation' is also mediated by amino-terminal domains and by the interaction of amino-terminal and carboxyterminal domains of apoA-I.[54,64] Moreover, ABCA1-mediated lipid efflux, but not microsolubilisation, results in the formation of HDL precursors which subsequently mature to HDL.[54]

The mechanism by which ABCA1 mediates lipid efflux is not resolved. Originally it has been suggested that the transmembrane domains of ABCA1 form a pore within the plasma membrane into which

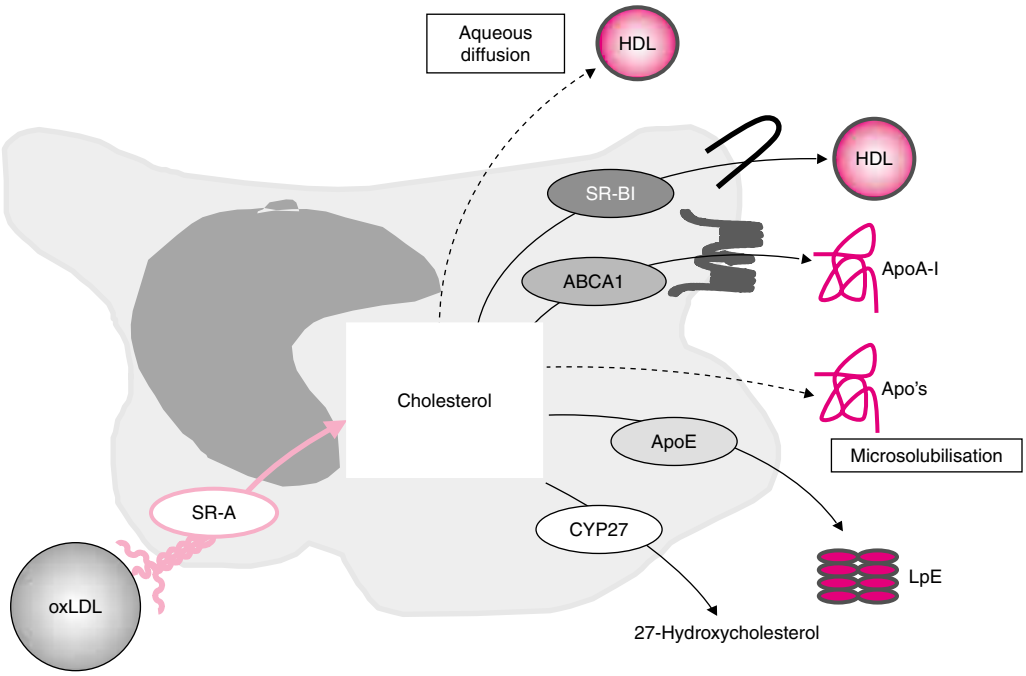

Fig. 1. Pathways of cholesterol efflux from macrophages. Macrophages can accumulate cholesterol by phagocytic or pinocytic uptake of cell debris, and by uptake of modified lipoproteins through scavenger receptors. Aqueous diffusion of cholesterol and SR-BI-mediated cholesterol efflux from cells is induced by HDL. Lipid-free apolipoproteins stimulate efflux of phospholipids and cholesterol by microsolubilisation and by interaction with ABCA1. In addition, macrophages can release cholesterol independently of exogenous cholesterol acceptors, either by secretion of apoE/lipid-particles or by secretion of 27-hydroxycholesterol after oxidation of cholesterol by the cytochrome P450 oxygenase CYP27. **ABCA1** = ATP binding cassette transporter A1; **Apo** = apolipoprotein; **CYP27** = cholesterol-27-hydroxylase; **HDL** = high-density lipoprotein; **LpE** = particles containing only apoE; **oxLDL** = oxidised low-density lipoprotein; **SR-A** = scavenger receptor A; **SR-BI** = scavenger receptor B1.

lipids are translocated ('flopped') from the inner leaflet of the plasma membrane. From there they are then picked up by lipid-free apolipoproteins which may even bind to ABCA1.[65,66] More recent data do not support this model[67] but rather suggest that ABCA1 organises the intracellular trafficking of lipids and proteins.[68,69] HDL and apoA-I were previously found to be internalised by macrophages into an endosomal compartment from where they are resecreted together with lipids.[70,71] Macrophages from patients with Tangier disease appear to have a defect in resecretion and aberrantly target internalised HDL to lysosomes for degradation. For this reason, and because of the presence of hyperplastic Golgi structures within lipid laden macrophages of patients with Tangier disease or ABCA1 deficient mice, it has been suggested that ABCA1 serves as a protein component of vesicles which shuttles lipids (and proteins including interleukin [IL]-1β and apoE) between lipid-rich intracellular organelles such as the trans-Golgi network, lysosomes and the plasma membrane.[70,72-74] Actually, the transfection of cells with fluorescent ABCA1 showed intensive cycling of fluorescent vesicles between the trans-Golgi network and the plasma membrane.[68]

Whatever may be the mechanism, HDL deficiency and the abundance of macrophage foam cells in patients with Tangier disease highlight the importance of ABCA1 for the regulation of both plasma HDL-cholesterol level and the regulation of cellular cholesterol homeostasis. However, the site of foam

cell formation is variable and involves arteries, liver, spleen, tonsils and peripheral nerves. Some, but not all, patients with Tangier disease have premature atherosclerosis, others have hepatosplenomegaly and peripheral neuropathy.[75]

These biochemical, pathological and clinical findings make ABCA1 an interesting target for anti-atherosclerotic drug therapy. To reach this aim it is important to understand the regulation of ABCA1. The promotor of the ABCA1 gene contains binding motifs for several transcription factors including the sterol regulatory binding protein (SREBP) and the liver-X-receptor/retinoid-X-receptor (LXRα/RXRα).[76] In agreement with a regulatory role of these transcription factors, ABCA1 expression and lipid efflux are up-regulated by cholesterol, oxysterols, retinoids and cAMP analogues.[77-80]

On the other hand, ABCA1 expression is downregulated in mice with diabetes mellitus, possibly as a result of the inhibitory effects of free unsaturated fatty acids and acetoacetate on oxysterol- and retinoid-induced ABCA1 expression.[81] This observation and the stimulatory effect of unsaturated fatty acids on ABCA1 degradation[82] provide a direct link between defective cholesterol efflux and the accelerated development of atherosclerosis in patients with diabetes mellitus. ABCA1 expression is also repressed by the inflammatory cytokine interferon-γ and by lipopolysaccharides, but upregulated by tumour growth factor (TGF)-β.[83-85] Inflammation may hence represent another pathological state in which disturbed cholesterol efflux favours the development of atherosclerosis.

2.2 Anti-Oxidative Effects

The oxidation of LDL is an important pathogenic step in the initiation and development of atherosclerosis. Oxidised LDL is a chemoattractant for monocytes, transforms macrophages into foam cells, exerts cytotoxic effects on endothelial cells, increases platelet activation, stimulates migration and proliferation of smooth muscle cells, and antagonises the vasodilative effect of nitric oxide (NO).[1,2,86] Several cell culture experiments support evidence that HDL counteracts these pro-atherogenic properties of oxidised LDL.

Discoidal and spherical HDL inhibit the oxidation of LDL by transition metal ions as well as the 12/15-lipooxygenase-mediated formation of lipid hydroperoxides.[34] HDL exerts these anti-oxidative effects by various mechanisms. Lipophilic antioxidants carried by HDL can scavenge oxygen-derived free radicals and, thereby, interrupt the cascade of LDL oxidation. Furthermore, HDL depletes LDL from lipid peroxides, and either transports these oxidised lipids to the liver for elimination or metabolises them enzymatically. The most effective mechanism by which HDL diminishes lipid peroxide accumulation is the enzymatic hydrolysis of phospholipid hydroxyperoxides by the HDL-bound enzyme paraoxonase.[33] This was shown to reduce the oxidised ox-LDL stimulated cytokine production and adhesion of monocytes to the endothelial surface.[33] In addition, paraoxonase decreases the lipid peroxide content in human coronary and carotid lesions.[87,88]

The importance of paraoxonase in the prevention of atherosclerosis development became evident in mice lacking this enzyme. Upon a fat-rich diet, paraoxonase knock-out mice were significantly more susceptible to the development of atherosclerosis than control animals and HDL isolated from paraoxonase-deficient mice failed to prevent LDL from oxidation.[89,90] In contrast, overexpression of paraoxonase was shown to decrease the development of atherosclerotic lesions in cholesterol-fed and apoE-deficient mice without altering plasma lipid levels.[91]

Platelet activating factor acetylhydrolase and glutathione peroxidase are two further enzymes in HDL which prevent the formation or degrade bioactive LDL oxidation products. And there is *in vitro*

indication that apoA-I and apoA-II reduce lipid peroxides through sulfoxidation of specific methionine residues.[92] Interestingly, such sulfoxidised apo A-I is not impaired in its ability to bind lipids, to induce cholesterol efflux or to activate LCAT.[93]

2.3 Effects on Endothelial Function and Viability

Atherosclerosis is thought to be initiated by injuries to the endothelium via physical (shear) stress, and exposure to atherogenic lipids and toxins. The dysfunctional endothelium is impaired in its ability to serve as a barrier against atherogenic lipoproteins, to regulate vascular tone by the production of NO and other vasoactive molecules, and to prevent thrombosis. Activated endothelial cells also express selectins and adhesion molecules to which circulating monocytes and T lymphocytes bind before they transmigrate into the subendothelial space.[94]

In vivo studies revealed an inverse correlation between serum HDL-cholesterol level and endothelium-dependent vasodilatation of coronary arteries,[95-99] whereas infusion of reconstituted HDL improved endothelium-dependent vasorelaxation in hypercholesterolaemic patients.[100] In organ bath experiments, HDL prevented the inhibition of acetylcholine-induced vasorelaxation by oxidised LDL or lysophosphatidylcholine – one of the major products of LDL oxidation.[101,102]

One mechanism accounting for the protective effect of HDL on endothelial-dependent vasoreactivity is the direct stimulation of NO formation by HDL, which appears to depend on the binding of HDL to SR-BI.[103] Another important vasorelaxing molecule produced by endothelial cells is prostacyclin (PGI_2) which, in addition, inhibits platelet activation and diminishes the release of growth factors. Physiological concentrations of HDL stimulate PGI_2 production, probably by supplying endothelial cells with arachidonic acid, i.e. the essential fatty acid precursor of prostanoids, and by enhancing the expression of cyclooxygenase-2, i.e. the rate-limiting enzyme of prostanoid synthesis.[104-109]

Transmigration of leucocytes, including monocytes, through endothelial cells involves reversible adhesion to E-selectin ('rolling') and irreversible adhesion to vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1. Expression of VCAM-1 is induced by cytokines including tumour necrosis factor (TNF)-α or IL-1β but also by the lipid lysophosphatidylcholine present in oxidised LDL and in products of lipolysis. HDL inhibits the adhesion of monocytes to endothelial cells induced by oxidised LDL as well as the cytokine-induced expression of VCAM-1, ICAM-1 and E-selectin.[110-112] The inhibitory effect of HDL is related to the inhibition of TNFα-stimulated activation of sphingosine kinase, thereby decreasing the production of sphingosine-1-phosphate.[106,113] The inhibitory effect is mediated by phosphatidylcholine complexed to apoA-I. Phosphatidylcholine species containing polyunsaturated fatty acids appear to be most effective.[114,115]

HDL sustains endothelial cell proliferation under serum-free conditions and prevents endothelial cell death induced by oxidised LDL, TNFα, remnants of triglyceride-rich lipoproteins or growth factor deprivation.[21,116-119] The latter effect is mediated by the two phospholipids sphingosylphosphorylcholine and lysosulfatide, which bind to specific receptors of the EDG (endothelial differentiation genes) family and activate Akt – an ubiquitous serine/threonine kinase and a principal mediator of anti-apoptotic activity in mammalian cells.[21]

In addition to preventing apoptosis, HDL inhibits endothelial cell damage and necrosis resulting from the activation of the complement system. HDL and apoA-I inhibit complement-mediated cell lysis by inhibiting the formation of the C5a-C9 complex.[120-123] Furthermore, HDL is a carrier of protectin (CD59), a glycoprotein that inhibits the cytolytic activity of complement activation.[124] The inhibito-

ry effects of HDL on the complement activation are of particular interest because of reduced atherosclerosis in animals lacking the C6 complement factor.[125]

2.4 Inhibition of Platelet Activation and Coagulation

Low HDL cholesterol is an independent predictor of acute platelet-dependent thrombus formation.[126] *In vitro*, HDL inhibits thrombin-, collagen-, ADP- and adrenalin-induced platelet aggregation, and thrombin-induced binding of fibrinogen to platelets.[127-130] HDL interacts by at least two pathways with platelets.

One pathway involves NO, since the inhibitory effect of (apoE containing) HDL on platelet activation is diminished by NO synthase inhibitors and increased in the presence of NO precursors.[131-134] The alternative pathway involves the activation of the sodium-proton antiport and the alkalisation of the cytoplasmic compartment. This in turn inhibits the release of calcium ions from intracellular storage sites and ultimately inhibits platelet activation.[135] Furthermore, activation of protein kinase C inhibits the activity of phosphatidylinositol specific phospholipase C (PI-PLC), which mediates agonistic effects of thrombin and collagen.[130]

Activation of blood coagulation is accompanied by the production of extrinsic tenase (a complex consisting of tissue factor, factor VIIa, phospholipids and calcium ions) and prothrombinase (a complex consisting of blood coagulation factors Va, Xa, II, phospholipids and calcium ions). Whereas LDL and VLDL stimulate both the secretion of tissue factor and the activation of extrinsic tenase, HDL inhibits tissue factor synthesis.[136,137] In addition, HDL antagonises the activation of factor X by extrinsic tenase.[138] The inhibitory effect of HDL may be related to the presence of tissue pathway factor inhibitor. In addition, both HDL and apoA-I inhibit the calcium ionophore-induced production of the prothrombinase complex on the surface of platelets.[139] Finally, by virtue of cardiolipin and phosphatidylethanolamine on the HDL surface, HDL enhances the anticoagulatory effect of activated protein C, which inactivates factors Va and VIIIa.[140]

2.5 Modulation of Smooth Muscle Cell Proliferation

HDL was reported to exert both stimulatory and inhibitory effects on cell proliferation.[34] However, the most recent studies clearly demonstrated the mitogenic effects of HDL on vascular smooth muscle cells. A direct effect on cell cycle regulation was evident through induction of cyclins and oncogens.[20] The phospholipids sphingosylphosphorylcholine and lysosulfatide have previously been identified to exert the mitogenic activities of HDL by activation of PI-PLC and liberation of intracellular calcium.[20]

In contrast to the lipid mediated mitogenic effect, lipid-free apolipoproteins A-I and E were found to inhibit growth factor-induced proliferation and cell cycle progression of VSMC and lymphocytes through inhibition of cell-cycle related proteins such as cyclin D1.[141,142] At first sight the mitogenic effects of HDL-lipids appear proatherogenic. However, the strong growth-promoting effects of HDL were observed at high concentrations usually not encountered in the interstitial fluid under physiological conditions. Thus, vascular smooth muscle cells are probably exposed to mitogenic concentrations of HDL only after loss of the endothelial barrier, so that HDL-induced proliferation may help to replenish cell loss in the fibrous cap and, thereby, contribute to plaque stability.

3. HDL Metabolism and Reverse Cholesterol Transport

Reverse cholesterol transport (RCT) describes both the metabolism and an important anti-ather-

ogenic function of HDL; namely, the HDL-mediated efflux of cholesterol from non-hepatic cells and its subsequent delivery to the liver and steroidogenic organs where it is used for the synthesis of lipoproteins, bile acids, vitamin D and steroid hormones.

Approximately 9mg cholesterol per kilogram bodyweight is synthesised by peripheral tissues every day and has to be delivered to the liver for effective catabolism.[22] Distortion of RCT contributes to the deposition of cholesterol within the arterial wall and, thereby, to the development of atherosclerosis.

Lipid-rich α-HDLs arise from lipid-poor particles (i.e. preβ1-LpA-I) or even lipid-free apolipoproteins.[38-40,143] These lipid-poor HDL-precursors are produced either as nascent HDL by hepatocytes[144] and the intestinal mucosa,[145] or dissociate from chylomicrons and VLDL during lipoprotein lipase (LPL)-mediated hydrolysis of triglycerides,[146,147] or are generated by the conversion of HDL_2 and HDL_3 by CETP,[148,149] PLTP[150,151] hepatic lipase, SR-BI[152] or endothelial lipase[153] (figure 2).

Lipid-free apolipoproteins or lipid-poor particles acquire phospholipids and unesterified cholesterol from hepatic and non-hepatic cells.[154] This initial step of HDL formation is interrupted in patients with Tangier Disease[71] and in mice lacking ABCA1. In

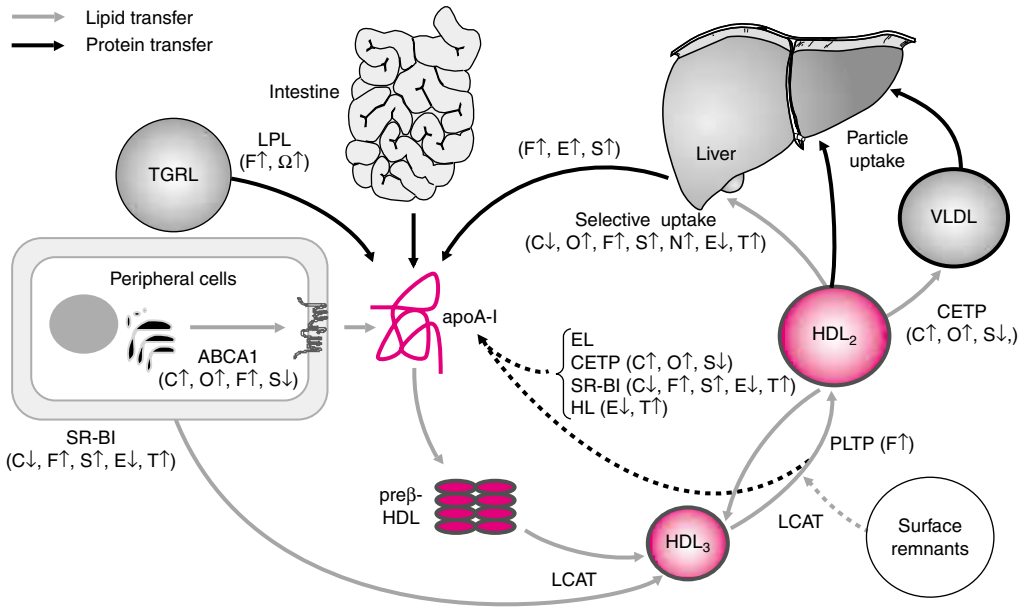

Fig. 2. Pathways of HDL metabolism and regulation by metabolites, drugs and hormones. Mature HDL_3 and HDL_2 are generated from lipid-free apoA-I or lipid-poor preβ1-HDL as the precursors. These precursors are produced as nascent HDL by the liver or intestine, or are released from lipolysed VLDL and chylomicrons, or by interconversion of HDL_3 and HDL_2. ABCA1-mediated lipid efflux from cells is important for initial lipidation; LCAT-mediated esterification of cholesterol generates spherical particles that continue to grow upon under ongoing cholesterol esterification, and PLTP-mediated particle fusion and surface remnant transfer. Larger HDL_2 are converted into smaller HDL_3 upon CETP-mediated export of cholesteryl esters from HDL onto apoB-containing lipoproteins, SR-B1-mediated selective uptake of cholesterol esters into liver and steroidogenic organs, and HL- and endothelial lipase-mediated hydrolysis of phospholipids. HDL lipids are catabolised either separately from HDL proteins, i.e. by selective uptake or via CETP-transfer, or together with HDL proteins, i.e. via uptake through as yet unknown HDL receptors or apoE receptors. Both the conversion of HDL_2 to HDL_3 and the PLTP-mediated conversion of HDL_3 to HDL_2 liberate lipid-free or poorly lipidated apoA-I. For further details see text. **ABCA1** = ATP binding cassette transporter A1; **apo** = apolipoprotein; **C** = cholesterol; **CETP** = cholesteryl ester transfer protein; **E** = estradiol; **EL** = endothelial lipase; **F** = fibrates; **HDL** = high-density lipoproteins; **HL** = hepatic lipase; **LCAT** = lecithin: cholesterol-acyltransferase; **LPL** = lipoprotein lipase; **N** = nicotinic acid; **O** = oxysterols; **PLTP** = phospholipid transfer protein; **S** = statins; **SR-BI** = scavenger receptor B1; **T** = testosterone; **TGRL** = triglyceride-rich lipoproteins; **VLDL** = very low-density lipoproteins; Ω = fish oil; ↑ indicates stimulatory effects; ↓ indicates inhibitory effects.

patients with Tangier disease and in gene-targeted mice, cellular lipid efflux is drastically reduced, which causes absence of lipid-rich α-HDL in plasma and macrophage foam cell formation.[63] Since ABCA1 is expressed in many cells including hepatocytes and enterocytes,[78,155] this cellular lipid transporter probably plays an important role not only in lipid efflux from peripheral cells, but also in the hepatic and intestinal generation of HDL.

HDL precursors generated by ABCA1-mediated lipid efflux become mature, lipid-rich and spherical α-HDL_3 by acquisition of additional phospholipids and unesterified cholesterol either from cells or apoB-containing lipoproteins (at least partially PLTP-mediated), by the LCAT-mediated esterification of cholesterol and by the association of additional apolipoproteins.[143,156-158] Ongoing LCAT-mediated cholesterol esterification and PLTP-mediated fusion with other HDL_3 further increases the size of these initially small HDL_3.[143,159-161] In addition, lipolysis of VLDL and chylomicrons by LPL generates surface remnants of triglyceride-rich lipoproteins which, again with the help of PLTP, are transferred onto HDL (figure 2).

Lipids or proteins of α-HDL are removed from the circulation by at least two direct pathways that involve the selective uptake of lipids, and the holoparticle-uptake by the apoE-receptors and possibly apoA-I-receptors. Furthermore, two indirect pathways that involve the actions of CETP, hepatic lipase and endothelial lipase were shown to play a role in HDL catabolism (figure 2).[49,162-167] The selective uptake of cholesterol esters from HDL into hepatocytes and steroidogenic cells is mediated by the binding of HDL to SR-BI.[58] SR-BI appears to internalise HDL into a cellular compartment of hepatocytes from where apolipoproteins are directed to the basolateral site for resecretion and lipids to apical membranes for secretion into the bile.[152] Selective uptake by SR-BI may depend on the presence of cofactors such as hepatic lipase, which hydrolyses phospholipids on the surface of both HDL and plasma membranes, and thus enables the flux of cholesteryl esters from the lipoprotein core into the plasma membrane.[165,166]

Whereas SR-BI internalises HDL lipids but not HDL-apolipoproteins, the apoE-receptors and the putative apoA-I receptors, like for example the previously identified β-chain of ATP synthase,[168] mediate holoparticle uptake of HDL. CETP exchanges cholesteryl esters of α-HDL with triglycerides of VLDL, intermediate-density lipoprotein (IDL) and LDL which are then removed via the LDL-receptor pathway.[164] Endothelial lipase hydrolyses phospholipids and generates free fatty acids taken up by endothelial cells.[167]

The removal of lipids from HDL_2 by SR-BI, CETP and hepatic lipase, and the subsequent conversion of HDL_2 to HDL_3 as well as the conversion of HDL_3 to HDL_2 by PLTP, regenerates preβ_1-LpA-I or lipid-free apoA-I.[39,143,148-152] These small apolipoproteins or particles can leave the plasma into the extravascular space[38-40] where they serve as acceptors of cellular lipids and, thus, again initiate the generation of HDL. In the kidney, these small particles are filtered and removed from the plasma. Again, apoA-I is recycled in the proximal tubulus lumen by a cubilin-mediated re-uptake.[169,170]

4. HDL and Atherosclerosis

4.1 Evidence from Clinical Studies

4.1.1 Observational Population Studies

Since 1977, numerous clinical and epidemiological studies have demonstrated the inverse and independent association between HDL cholesterol and the risk of fatal and non-fatal CHD events.[9] From population study data, it has been calculated that every 0.026 mmol/L (1 mg/dL) increase in HDL cholesterol lowers coronary risk by 1%. In patients with angiographically assessed CHD, this associa-

tion may even be stronger since the prospective and multicentric European Concerted Action on Thrombosis and Disabilities (ECAT) study as well as the Baltimore Longitudinal Heart Study identified low HDL cholesterol as the most important biochemical risk factor for coronary events.[171,172]

However, it is not clear whether this rule can be extrapolated to the whole range of HDL cholesterol. Whereas a low HDL-cholesterol level (e.g. <20th percentile) has been consistently found to increase cardiovascular risk, it has not been consistently shown that a high HDL-cholesterol level is protective. At least in some studies including the Münster Heart Study (Prospective Cardiovascular Münster [PROCAM])[173] and the ECAT angina pectoris study,[171] individuals with the highest levels of HDL cholesterol (e.g. >80th percentile) did not experience fewer coronary events than individuals with HDL cholesterol in the high-normal range (e.g. 60–80th percentile).

In patients with certain metabolic conditions a high level of HDL cholesterol is associated with excess cardiovascular risk. Hypertriglyceridaemic participants of the Copenhagen City Heart Study and the PROCAM study with high levels of HDL cholesterol were at higher coronary risk than hypertriglyceridaemic probands with intermediate HDL levels.[174,175] Interestingly, although low HDL cholesterol is also significantly associated with reduced life expectancy, HDL-cholesterol levels in the fifth quintile were also associated with excess mortality compared with intermediate HDL levels in the PROCAM and also in a Belgian study population.[173,176]

By convention, the risk threshold values of HDL cholesterol have been defined as 0.9 or 1.05 mmol/L (35 or 40 mg/dL) in men and 1.15 or 1.3 mmol/L (45 or 50 mg/dL) in women.[17,177,178] However, the strength of the association between HDL cholesterol and cardiovascular risk depends on the presence of additional risk factors.[9] For example, in the PROCAM subgroup of men aged 35–65 years, the incidence of MI was 67.5 per 1000 in 10 years. An HDL cholesterol threshold value of 0.9 mmol/L distinguishes between men at higher or lower average risk. However, this threshold value is much higher in men with diabetes mellitus, hypercholesterolaemia or high global risk (i.e. in the presence of multiple risk factors) and much lower in the absence of other risk factors, i.e. in men at low global risk.[179]

Therefore, elimination of additional risk factors is of equal or greater importance than raising HDL-cholesterol levels in an effort to reduce overall risk for the individual. This also explains why in some of the statin trials baseline levels of HDL were strongly associated with the extent of benefit from LDL cholesterol lowering treatment.[3,7]

The potentiating effect of coinciding risk factors is a very well known phenomenon. However, from the metabolic point of view, the coincidence of elevated LDL cholesterol or smoking with other risk factors occurs by chance in many individuals. In contrast, low HDL cholesterol is frequently confounded with hypertriglyceridaemia, the presence of small dense LDL, impaired glucose tolerance or overt type 2 diabetes mellitus, hypertension and overweight. Actually, in many populations a low HDL cholesterol is a typical component of the metabolic syndrome or insulin resistance syndrome which precedes the manifestation of the other components including diabetes.[180,181] Thus, although the association of HDL cholesterol with CHD is statistically independent of other risk factors, a low HDL-cholesterol level is frequently not the sole risk factor in a given individual.

Therefore, it is important to correct the metabolic basis of this disorder, i.e. by reduction of bodyweight as well as by increasing physical activity.[11,12,176] Since metformin and glitazones increase both insulin sensitivity and HDL cholesterol,[182] it is an important question whether these

drugs can help to prevent coronary events, especially in patients with diabetes.

Several studies have investigated the question on whether HDL subpopulations or apolipoproteins have a better prognostic value than HDL cholesterol. Data are conflicting and derive mostly from small case-control studies. Prospective data have been generated in the Physicians' Health Study and the Atherosclerosis Risk In Communities (ARIC) study,[183,184] which did not show any superiority of HDL_2, HDL_3 or apoA-I, and most recently in the PRIME study, which found apoA-I to be a better risk indicator than HDL cholesterol.[185]

4.1.2 Inborn Errors of HDL Metabolism

Several mutations in the genes for apoA-I, LCAT or ABCA1 lead to dose-dependent decreases in HDL cholesterol with a virtual absence of HDL in homozygotes and half normal levels of HDL cholesterol in heterozygotes. And several (but not all) homozygous patients with apoA-I, LCAT or ABCA1 deficiency have experienced premature CHD.[22,186]

It is not yet understood whether the specific mutation, or the presence of other genetic and exogenous (risk) factors, determine the manifestation of disease. Interestingly, heterozygous relatives of apoA-I deficient patients as well as heterozygous carriers of apoA-I mutants, who frequently present with HDL-cholesterol levels below the 10th percentile, did not show an increased risk of premature CHD.[22,186] In contrast, the apoA-I_{Milano} mutation was even claimed to convey protection from CHD, although the heterozygous carriers have very low concentrations of HDL cholesterol as a result of increased catabolism of both normal and variant allele products.[187] By contrast, heterozygosity for mutations in ABCA1 was associated with increased carotid intimal thickening and coronary event rates.[75,188,189] Some polymorphic mutations in the *ABCA1* gene were also associated with increased risk of CHD independent of HDL cholesterol.[190-192]

High levels of HDL cholesterol in individuals with CETP deficiency were originally found to be associated with reduced CHD risk but later on with increased CHD risk in hypertriglyceridaemic subpopulations.[31,193] Similarly, some genetic variants of CETP[193-195] were associated with increases of both HDL cholesterol and CHD risk, while an extensively investigated *Taq1B* polymorphism showed in some but not all populations the expected inverse association between HDL cholesterol and CHD risk.[196,197]

4.2 Evidence from Animal Studies

Knock-out of the *apoA-I* gene caused HDL deficiency but not atherosclerosis in wild-type mice.[198] However, in combined apoA-I knock-out and apoB overexpressing mice, apoA-I deficiency increased atherosclerosis compared with pure apoB transgenic mice. Thus, in the presence of an atherogenic lipoprotein profile, apoA-I appears to delay atherosclerosis.[199] In agreement with this, transgenic overexpression of the human *apoA-I* gene in atherosclerosis-susceptible mice or rabbits increased HDL-cholesterol levels and reduced atherosclerosis.[200-205] Somatic overexpression of human apoA-I even induced the regression of pre-existing lesions.[204,205] In line with improved HDL function and accelerated RCT, expression of a human *apoA-I* transgene in mice resulted in increased cholesterol efflux capacity of plasma.[206] Overexpression of a human *apoA-IV* transgene also reduced atherosclerosis in mice,[207,208] without changing HDL-cholesterol levels. These data suggest that stimulated production of HDL precursors enhances RCT and protects from atherosclerosis.

Like patients with Tangier disease, ABCA1 knock-out mice were characterised by HDL deficiency and reduced cellular cholesterol efflux activity.[72,209] Intriguingly, absence of ABCA1 had no effect on biliary sterol, bile acid and phospholipid secretion, which argues against a significant effect

of ABCA1 on total body cholesterol flux.[210] Bone marrow transplantation experiments provided evidence that the expression of ABCA1 in macrophages is important for protection from atherosclerosis but not for the determination of HDL cholesterol. Transplantation of ABCA1 deficient bone marrow into apoE or LDL receptor knockout mice increased atherosclerosis but did not decrease HDL cholesterol.[211,212]

Three mouse strains with overexpressed ABCA1 have been generated. Although macrophages of all three mouse strains showed increased cholesterol efflux, HDL cholesterol was only increased in two of them.[213,214] Data are also discrepant with respect to the effects of ABCA1 overexpression on biliary sterol secretion and atherosclerosis. In one but not in another ABCA1 mouse model, overexpression of ABCA1 led to increased bile acid secretion. Crossbreeding of ABCA1 overexpressors with apoE deficient mice led to reduced atherosclerosis when low level ABCA1 was overexpressed in several tissues including the liver and macrophages.[215] In contrast, liver specific expression of high levels of ABCA1 increased atherosclerosis in mice deficient in apoE.[216] The majority of the data from the ABCA1 animal models, taken together, provides strong evidence that *ABCA1* gene expression in macrophages is directly related to reduced atherosclerosis. Therefore, increasing ABCA1 expression in macrophages may be an important anti-atherogenic target.

Genetic inactivation of LCAT caused HDL deficiency in mice without affecting atherosclerosis. Overexpression of LCAT increased HDL cholesterol in both mice and rabbits, but was anti-atherogenic in rabbits and pro-atherogenic in mice.[217,218] However, when coexpressed with CETP, for which wild-type mice are deficient, LCAT also protected mice from atherosclerosis.[219] Likewise, CETP, apoA-II and HL exerted pro-atherogenic or anti-atherogenic effects in various mouse models depending on the activity of other genes.[220-228] However, it is important to note that despite the contradictory effects on atherosclerosis, all models showed the expected changes in HDL cholesterol, namely decreased HDL cholesterol in mice with overexpression of CETP or hepatic lipase and increased HDL cholesterol in hepatic lipase-deficient mice.[220-228] Thus, whether LCAT, CETP or hepatic lipase are pro- or anti-atherogenic appears to be strongly influenced by their relative ratios and by additional factors.

In addition, modulation of SR-BI activity did not show the expected inverse association between HDL cholesterol and atherosclerosis. Although SR-BI transgenic mice have reduced HDL-cholesterol levels, they are protected from atherosclerosis.[61,62,229] Conversely, SR-BI inactivation in mice causes reduced biliary cholesterol secretion[230] and increased atherosclerosis despite a doubling of HDL-cholesterol levels.[59,60] When crossed with apoE-deficient mice, these animals even developed (fatal) MI.[60] However, it is important to emphasise that SR-BI also mediates the removal of apoB-containing lipoproteins, so that overexpression of SR-BI causes not only a decrease in HDL cholesterol but also in LDL cholesterol. This effect makes upregulation of SR-BI an even more attractive therapeutic target. Interestingly, hepatic SR-BI expression is inhibited by estradiol and stimulated by testosterone,[231-233] so that it is questionable whether the estrogen-induced increase and androgen-induced decrease of HDL cholesterol translate into decreased and increased cardiovascular risk, respectively.

Taken together, the data indicate that changes in HDL cholesterol and atherosclerosis are not always inversely related. Only increases of HDL cholesterol by overexpression of human apoA-I or ABCA1 shows the expected beneficial effect on atherosclerosis. Modulation of SR-BI activity even shows the opposite relationship between HDL cholesterol and atherosclerosis. For many other genes, the relationships are variable as a result of interactions with

Table II. Effects of various hypolipidaemic drugs on plasma lipids and coronary heart disease (CHD) outcomes in controlled, interventional clinical endpoint trials

Trial (drug)	LDL cholesterol		Triglycerides		HDL cholesterol	
	mean change (%)	association to reduction of CHD events	mean change (%)	association to reduction of CHD events	mean change (%)	association to reduction of CHD events
Primary prevention trials						
HHS (gemfibrozil)[13]	−11	$p < 0.04$	−35	NS	+11	$p < 0.01$
AFCAPS/TexCAPS (lovastatin)[15]	−25	NS	−15	NS	+6[a]	NS[a]
WOSCOPS (pravastatin)[234]	−26	NS	−12	NS	+5	NS
Secondary prevention trials						
CARE (pravastatin)[235]	−32	NS	−14	NS	+5	NS
4S (simvastatin)[236]	−38	$p < 0.0001$	−15	NS	+8	NS[b]
VA-HIT (gemfibrozil)[237]	0	NS	−31	NS	+6	NS[c]
LIPID (pravastatin)[235]	−25	NA	−11	NA	+5	NA
BIP (bezafibrate)[238]	−6	NS	−21	NS	+18	NS
HERS (HRT)[239]	−14	NS	+10	NS	+8	NS

a Changes in apoA-I levels were significantly related to CHD events when data of men on placebo and lovastatin were pooled.

b Changes in HDL cholesterol were significantly related to CHD events when data of patients on placebo and simvastatin were pooled.

c Changes in HDL_3 cholesterol were significantly related to CHD events.

4S = Scandinavian Simvastatin Survival Study; **AFCAPS/TexCAPS** = AirForce/Texas Coronary Atherosclerosis Prevention Study; **apo** = apolipoproteins; **BIP** = Bezafibrate Infarction Prevention; **CARE** = Cholesterol and Recurrent Events; **HDL** = high-density lipoprotein; **HERS** = Heart and Estrogen/Progestin Replacement Study; **HHS** = Helsinki Heart Study; **HRT** = hormone replacement therapy; **LDL** = low-density lipoprotein; **LIPID** = Long-term Intervention with Pravastatin in Ischemic Disease; **NA** = not available; **NS** = not significant; **VA-HIT** = Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial; **WOSCOPS** = West of Scotland Coronary Prevention Study.

other genes. In addition, since most if not all HDL functions can be regarded as repair systems ('fire brigade'), atherogenic conditions ('fire') have to be generated to demonstrate the atheroprotective role of HDL. It may well be that specific atherogenic conditions such as LDL hypercholesterolaemia, remnant hypercholesterolaemia, hypertrigylceridaemia or inflammation are necessary to set the stage for HDL.

5. Drug Effects on HDL Metabolism

Outcomes of several prospective intervention studies have been interpreted as proof for the beneficial effect of increasing HDL cholesterol on CHD prevention. However, it is important to emphasise that these studies used fibrates and statins that exert a broad spectrum of metabolic effects, only one of which is the moderate increase in HDL cholesterol (table II). Moreover, the conclusions on the beneficial effects of increased HDL cholesterol were drawn from results of *post-hoc* analyses of some big trials but not confirmed in *post-hoc* analyses of others.

In the Helsinki Heart Study (HHS) and Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT), both with gemfibrozil, increases in HDL cholesterol and HDL_3 cholesterol, respectively, were significantly associated with reduction in event rates.[13,237] However, no such associations were seen in the Bezafibrate Infarction Prevention (BIP) trial, the AirForce/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) with lovastatin, the Scandinavian Simvastatin Survival Study (4S), or the West of Scotland Coronary Prevention Study (WOSCOPS), Cholesterol and Recurrent Events (CARE) and Long-term Intervention with Pravastatin in Ischemic Disease

(LIPID) trials, all with pravastatin.[15,234-236,238] Likewise *post-hoc* and meta-analyses of some, e.g. Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT) and Lipoprotein and Coronary Atherosclerosis Study (LCAS, with fluvastatin),[240,241] but not all, e.g. Lopid Coronary Angiographic Trial (LOCAT) with gemfibrozil,[242] angiographic trials provided evidence for a moderate but significant association between changes in HDL cholesterol and regression of atherosclerotic lesions.

In view of the strong LDL-cholesterol lowering effects of statins and the strong triglyceride lowering effects of fibrates, together with the pleiotropic effects of both drug groups on vascular inflammation and insulin sensitivity, it is not justified to take these studies as proof for a general atheroprotective effect of increased HDL cholesterol. These studies do only show that patients with low HDL cholesterol and additional risk factors benefit from treatment with fibrates or statins. In this context, it is also important to reconcile that in the two controlled intervention studies on cardiovascular effects of hormone replacement therapy (HRT) in postmenopausal women, a combination of conjugated equine estrogens and medroxyprogesterone did not reduce (Heart and Estrogen/Progestin Replacement Study [HERS]) or even increased coronary event rates (Women's Health Initiative [WHI]), despite increased HDL cholesterol.[239,243,244]

5.1 Statins

In addition to decreasing LDL-cholesterol levels in a dose-dependent way,[245] statins also increase HDL-cholesterol levels by about 4–10%. However, there is no general dose-response or positive correlation between statin dosage and HDL-cholesterol levels.[246-248] In the Comparative Dose Efficacy of Atorvastatin, Simvastatin, Pravastatin, Lovastatin and Fluvastatin study (CURVES), pravastatin, lovastatin, fluvastatin and simvastatin produced HDL cholesterol increases of 4–10% independent of the applied dosage.[247] Atorvastatin elevated HDL cholesterol at low doses but this effect diminished markedly with increasing dosage.[249]

In large clinical trials for secondary and primary CHD prevention, statin therapy increased HDL cholesterol largely independent of baseline total, LDL- and HDL-cholesterol levels.[3-8] Most of these studies have systematically excluded patients with low HDL-cholesterol levels, except for the AFCAPS/TexCAPS primary prevention study which specifically included participants with an average total cholesterol of 5.7 mmol/L and LDL cholesterol of 3.9 mmol/L but low HDL cholesterol of 0.94 mmol/L for men and 1.03 mmol/L for women.[7] In this study, lovastatin treatment increased HDL cholesterol by 6% but the beneficial effect of this increase on coronary outcomes did not reach significance. Only the increase in apoA-I in the lovastatin treated group had a significant association with the decrease of CHD events.[15] Furthermore, HDL cholesterol elevation was not significantly associated with the reduction of CHD events in any of the large scale primary or secondary prevention studies. Nevertheless, in a recent *post-hoc* subgroup analysis of the 4S trial, patients with the lipid triad of elevated LDL cholesterol (mean 5.1 mmol/L), low HDL cholesterol (mean 0.86 mmol/L) and elevated triglycerides (mean 2.17 mmol/L) were shown to receive a greater benefit from statin treatment than patients with isolated LDL cholesterol elevation and high HDL cholesterol.[236] There was a trend towards more benefit with simvastatin for HDL cholesterol and triglyceride levels in the lipid triad subgroup but the differences were not statistically significant.

In a small turnover study using stable isotopes, pravastatin was found to increase the production and catabolism of apoA-I, while a more recent study found no effect of atorvastatin on apoA-I production and catabolism in patients with the metabolic syndrome.[250] There is evidence from *in vitro* experiments that some statins influence both the produc-

tion and catabolism of HDL. In cell culture experiments, statins directly up-regulate *apoA-I* gene transcription through the peroxisome proliferator-activated receptor (PPAR)-α.[251,252] This upregulation of PPARα is mediated through downregulation of cholesterol-derived geranylgeranyldiphosphate which is necessary for posttranslational prenylation of the GTP-binding protein Rho A. Such prenylation of Rho A leads to activation and membrane translocation, a process that is inhibited by statins.[253] It is not yet clear how suppression of Rho A prenylation finally up-regulates PPARα but it seems that the pathway is independent of PPARα activation by fibrates. Simultaneous treatment of statins and fibrates resulted in synergistic effects on PPARα transactivation.[251]

The hypercatabolic effect of statins on HDL metabolism appears to be mediated by up-regulation of SR-BI and down-regulation of ABCA1.[77,78,162,163] Statin-mediated down-regulation of cholesterol synthesis may enhance selective uptake of HDL cholesterol in the liver through up-regulation of SR-BI, which is expected to increase HDL catabolism and lower plasma HDL cholesterol.[61,62,162,163] Furthermore, down-regulation of ABCA1 by inhibition of cholesterol synthesis would reduce HDL maturation and as a secondary effect enhance the catabolism of apoA-I. SR-BI upregulation and ABCA1 downregulation are expected to lower HDL cholesterol in plasma and cannot explain the statin induced elevation of HDL cholesterol. However, statin-treated patients also had reduced CETP activity in plasma, which impairs cholesterol-ester transfer from HDL to apoB containing lipoproteins and, thereby, increases HDL cholesterol.[254,255]

Taken together, the *in vitro* data suggest that statins increase HDL cholesterol via enhanced apoA-I production that appears to override the HDL cholesterol lowering effect of increased HDL catabolism. However, for some statins or at high dosages, these relative effects may vary so that the HDL cholesterol increase is reduced. Because of the divergent effects of statins on the expression of ABCA1 (down) and SR-BI (up), and because of their inhibition of CETP, the effects of statins on RCT from peripheral cells to the liver are difficult to predict.

5.2 Fibric Acid Derivatives (Fibrates)

Fibrates were found to regulate the expression of several genes involved in lipoprotein metabolism and inflammation by activation of the nuclear receptor PPARα.[256] Activated PPARα binds to PPAR-response elements and increases the transcription of downstream genes. Several pivotal genes of HDL metabolism, including *apoA-I*, *apoA-II*, *SR-BI* and *ABCA1*, harbour such PPAR response elements and are upregulated in the presence of PPARα agonists.[257,258]

Enhanced hepatic secretion of apoA-I and apoA-II increase HDL production, whereas upregulation of ABCA1 helps to supply HDL precursors with phospholipid and cholesterol from peripheral cells. Furthermore, LPL is upregulated and apoC-III, an inhibitor of LPL, is suppressed by PPARα activation. Hence, fibrate treatment enhances lipolysis of triglyceride-rich lipoproteins and, thereby, increases the production of surface remnants and finally the formation of HDL. Stimulation of SR-BI expression will also increase HDL catabolism. The combination of these processes is thought to increase HDL-cholesterol levels and to enhance RCT. A recent turnover study of lipoprotein metabolism in patients with the metabolic syndrome showed that fenofirate stimulates both apoA-I production and catabolism.[250]

Fibrates primarily lower triglyceride levels and were shown to increase HDL cholesterol between 6% and 18% in three large clinical trials for primary prevention[259] and secondary prevention of CHD.[237,238] Effects of fibrates on serum levels of lipids and lipoproteins differ widely depending on

the baseline lipoprotein profile of treated patients. For fenofibrate, the increase in HDL cholesterol has been shown to be related to baseline HDL cholesterol. Individuals with low baseline levels of HDL cholesterol experience the strongest increase in HDL cholesterol.[260] In two large open-label trials, fenofibrate increased HDL cholesterol by 41% and 44% when baseline levels were <0.9 mmol/L.[260]

In a subgroup analysis of the HHS primary prevention trial, individuals with low HDL cholesterol were also found to benefit most effectively from gemfibrozil treatment.[259] The HHS trial showed that in middle aged men with an average total cholesterol level of 7 mmol/L and an average HDL cholesterol of 1.22 mmol/L, treatment with gemfibrozil increased HDL cholesterol by 11%, which was significantly associated with a reduction in fatal and non-fatal coronary event rates. Gemfibrozil also produced a significant increase in HDL_3 cholesterol in the secondary prevention trial VA-HIT which was related to a reduction of CHD events.[237] VA-HIT selected men with low HDL-cholesterol levels (average 0.82 mmol/L) and low LDL cholesterol (average 2.9 mmol/L).[237] Multivariate analysis calculated that CHD events were reduced by 11% for every 0.13 mmol/L increase in HDL cholesterol; however, mean HDL cholesterol increased by only 0.05 mmol/L.[237]

Furthermore, men with low HDL cholesterol (average 0.9 mmol/L) and moderately elevated cholesterol (LDL cholesterol average 3.8 mmol/L) did not benefit from bezafibrate treatment in the BIP secondary prevention trial.[238] This lack of reduction in CHD events was observed despite an 18% increase of HDL cholesterol and a 21% decrease in triglycerides.[238] The difference between the outcomes of the VA-HIT and the BIP trial may have resulted from differences in study design and populations examined. The BIP trial recruited a smaller proportion of hypertriglyceridaemic individuals than the VA-HIT trial. Interestingly, a subgroup of the BIP trial with triglycerides >2.3 mmol/L benefited from bezafibrate treatment. Moreover, a *post-hoc* analysis of the VA-HIT trial revealed that men with diabetes or increased fasting glucose benefited more from gemfibrozil treatment than euglycaemic men. Hence, it may well be that several metabolic mechanisms lead to low HDL cholesterol and that these differ in their response to fibrates.

5.3 Nicotinic Acid (Niacin)

Treatment with nicotinic acid leads to favourable changes of all major lipid fractions and exerts the strongest HDL increasing effect of all commercially available drugs with increments of up to 30%.[261,262]

In vivo kinetic studies attributed the raise of HDL cholesterol by nicotinic acid to a decreased fractional catabolic rate of apoA-I.[263] This conclusion was corroborated by cell culture experiments, showing that nicotinic acid did not increase apoA-I expression but inhibited the uptake of HDL-apoA-I without blocking the uptake of HDL cholesterol esters.[264] Such a mechanism would improve RCT possibly through SR-BI–mediated cholesterolester uptake.[265]

Two randomised, double-blind studies revealed that daily intake of nicotinic acid 2–3g increased HDL cholesterol by 25–29% in individuals with low baseline HDL cholesterol.[266,267] There is only one controlled secondary prevention trial which assessed the effect of nicotinic acid on clinical endpoints, the Coronary Drug Project.[268] During the first 6 years of follow-up, treatment with nicotinic acid had no effect on coronary and total morbidity. However, after an additional follow up of 9 years, death rates were lower among individuals who received nicotinic acid than among those who received placebo.

5.4 Combination Therapy

Monotherapy with some of the agents discussed in this section (5) is often successful in achieving

target levels of LDL cholesterol or triglycerides. However, severe or refractory mixed hyperlipidaemia frequently associated with low HDL cholesterol and isolated hypoalphalipoproteinaemia often requires combination therapy to achieve treatment goals. Combination therapy helps to normalise HDL cholesterol in some patients with low HDL cholesterol.

5.4.1 Statin/Nicotinic Acid

In a controlled angiographic trial, patients with CHD and normal LDL cholesterol (<3.8 mmol/L), low HDL cholesterol (<0.9 mmol/L for men and <1.03 mmol/L for women) and with triglycerides below 4.5 mmol/L received simvastatin 10–20mg and up to 1g of nicotinic acid daily for 3 years.[269] Compared with baseline lipoprotein levels, treatment lowered LDL cholesterol and triglycerides by 42% and 38%, respectively, and increased HDL cholesterol by 26%. These changes in lipid metabolism were associated with a slight but significant regression in coronary stenosis and a reduction in coronary event rates.[269] Similar favourable modification of lipid levels were observed in an open-label, multicentre study where patients with low HDL cholesterol received a combined nicotinic acid/lovastatin formulation.[270] In this trial, 10% of the patients withdrew from the study because of adverse effects, of which flushing was the most common. However, there is an indication that a lower dose nicotinic acid supplementation to statin therapy may also increase HDL cholesterol with fewer adverse effects.[271]

5.4.2 Fibrate/Nicotinic Acid

Combined therapy with fibrates and nicotinic acid has also shown beneficial lipoprotein changes in patients with isolated hypoalphalipoproteinaemia. Two studies reported significant differences between monotherapy with either agent and the combination of fibrate-nicotinic acid which lowered LDL cholesterol by 18–31% and increased HDL-cholesterol levels by 45–48% from baseline.[272,273]

5.4.3 Statin/Fibrate

Cerivastatin was withdrawn worldwide after several deaths occurred from rhabdomyolysis, of which 25% were related to gemfibrozil-cerivastatin combination therapy.[274] This coupled with the increasing use of statin-fibrate combination therapy has focused attention on the safety profile of these combinations. Long-term intervention studies with such combinations have not been reported; however, a recent review calculated the increased risk of muscle damage to be 0.12% when statins are combined with fibrates.[275] Several randomised, statin-fibrate combination therapies were shown to produce beneficial lipoprotein changes in patients with mixed hyperlipidaemia.[275] In patients with refractory combined hyperlipidaemia with an average HDL cholesterol of 0.9 mmol/L, pravastatin-gemfibrozil, simvastatin-gemfibrozil, and simvastatin-ciprofibrate combinations increased HDL cholesterol by 14–25%.[276,277] Similar results were reported with an atorvastatin-fenofibrate and a simvastatin-bezafibrate combination in two small, open-label studies in patients with familial combined hyperlipidaemia which increased HDL cholesterol by 25–28% from a baseline of 0.7 and 0.9 mmol/L, respectively.[278,279]

5.5 Sex Steroids

5.5.1 Estrogens and Estrogen Receptor Modulators

Estrogens increase HDL cholesterol by increasing apoA-I production and by decreasing HDL catabolism through inhibition of hepatic lipase and SR-BI.[280-283] Intriguingly, estrogen treatment decreased SR-BI expression in hepatic tissue but increased SR-BI expression in steroidogenic tissue of rats.[231,232] Oral hormone replacement therapy with estrogens increases HDL cholesterol in postmenopausal women and hypogonadal men by about 8%.[284,285] In non-hysterectomised women, estrogens must be combined with progestins to reduce the risk of endometrial cancer. The 17-hydroxypro-

gesterone derivatives, such as medroxyprogesterone (acetate), possess no or little androgen activity, and partly antiandrogenic activities, whereas the nortestosterone derivatives have an androgenic potency. Androgenic progestins were shown to diminish the HDL-elevating effect of estrogens in combined estrogen/progestin hormone replacement therapy. However, low dose progestin and novel progestins with primarily antiandrogenic activity, such as medroxyprogesterone, do not offset the HDL-elevating effect of estrogens.[284,286,287]

Meta-analyses of observational studies suggested that hormone replacement therapy lowers risk for CHD in post-menopausal women. However, two recent controlled intervention studies revealed that a combination of unconjugated estrogen and medroxyprogesterone acetate did not reduce or even increased coronary event rates in post-menopausal women despite their beneficial effects on lipoprotein levels, which included a 14% decrease in LDL cholesterol, a 10% increase in triglycerides and an 8% elevation of HDL cholesterol.[239,244,288] HERS was a controlled 4.1 year trial with an unblinded follow-up of 2.7 years in 2763 postmenopausal women with CHD,[243] while the WHI was a controlled trial in 16 608 healthy post-menopausal women with a follow-up of 5.2 years.[244] The WHI trial was stopped early because health risks exceeded health benefits in women using the combined estrogen/progestin treatment. Women receiving estrogen plus progestin experienced significant increased incidences of coronary events, venous thromboembolism and breast cancer.[244]

These data motivated the search for alternative hormone replacement therapy regimens. Selective estrogen receptor modulators act as estrogen agonists or antagonists depending on the target tissue. This may explain why their influence on lipid metabolism diverges significantly. Raloxifene has been shown to competitively inhibit estrogen action in the breast and the endometrium, and to act as an agonist on bone and lipid metabolism. Follow-up data from two controlled trials in 1145 post-menopausal women showed decreased LDL levels with unaltered HDL levels after 3 years.[289] The large, 3-year, controlled Multiple Outcomes of Raloxifene Evaluation (MORE) trial showed the benefit of raloxifene for breast cancer prevention but with no difference in overall mortality; however, there was an increase in venous thromboembolism.[290]

Tibolone is a synthetic steroid that is used for treatment of postmenopausal complaints and bone loss. It has three major metabolites which exert estrogenic, gestagenic and androgenic effects. While tibolone exerts beneficial effects on triglycerides and lipoprotein(a) levels, it also decreases HDL cholesterol by 20%, probably by increasing HL activity.[291] The clinical consequences of these changes in lipoprotein metabolism are not yet known, but it is interesting to note that treatment with this drug does not cause significant changes in the cholesterol efflux capacity or paraoxonase activity of plasma.[291,292]

5.5.2 Testosterone

There is an increasing use of testosterone for treatment of male hypogonadism, especially in the elderly. Testosterone was shown to dose- and concentration-dependently lower HDL cholesterol,[293] probably by increasing the expression of hepatic lipase and SR-BI.[233] In the physiological range, testosterone treatment decreases HDL cholesterol by about 0.05–0.13 mmol/L, whereas supraphysiological dosages cause a pronounced decrease in HDL cholesterol by more than 20%. How these changes in HDL cholesterol translate into coronary risk is not known.[294]

5.6 Fish Oil and Omega-3 Fatty Acids

Greenland Eskimos have a lower cardiovascular mortality than Danish co-inhabitants. Comparison of the lifestyle of the two groups revealed that both groups consumed a diet high in fat. However, Es-

kimos mainly ate fish and marine mammals which are rich in the omega (n)-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), whereas Danes ate a diet rich in meat and dairy products which is high in saturated fat and cholesterol.[295] Several other population studies corroborated the initial finding that fish consumption is associated with a reduced CHD incidence.

Combined EPA and DHA containing pharmacological supplementation was consistently shown to reduce triglycerides. In normolipidaemic humans, fish oil supplementation was shown to lower plasma triglycerides by 25% and slightly increase LDL cholesterol and HDL cholesterol by 4% and 3%, respectively.[296] A similar reduction in triglycerides and a similar increase in LDL cholesterol was observed by fish oil supplementation in diabetics with hypertriglyceridaemia but no change in HDL cholesterol was observed.[297]

Combined EPA and DHA supplementation was investigated in a large secondary prevention trial (Gruppo Italiano per o Studio della Sopravvivenza nell 'Infarto Miocardico [GISSI]) in patients surviving a recent MI.[298] Treatment with n-3 fatty acids in addition to optimal pharmacological treatment and life-style advice led to a significant reduction in total mortality, mainly from a reduction in sudden death. Intriguingly, this risk reduction was already significant after 3 months on n-3 fatty acid treatment. The conclusion from this study was not that the lipid changes protected from sudden death but rather the anti-arrhythmic effect of the n-3 fatty acids.[298]

Fish oil was also shown to modulate the activity of several enzymes of lipid metabolism which resulted in increased fatty acid mitochondrial β-oxidation and inhibition of *de novo* fatty acid synthesis.[299] Lipoprotein metabolism is further modified by an increased catabolic rate of HDL,[300] decreased apoC-III expression and an increase in LPL expression, partially mediated by PPARα.[299] PPARα is not rate limiting for fish oil to exert its triglyceride lowering effect since the decrease in apoC-III production is affected in a PPARα independent manner.[301]

Recently, EPA and DHA were shown to inhibit the activation of the transcription factor liver X receptor (LXR)α by oxysterols.[302] LXRs are key sensors of sterol metabolism and maintain normal cholesterol balance by promoting sterol efflux from peripheral cells, increasing circulating HDL cholesterol, increasing hepatic sterol catabolism and excretion, and inhibiting further sterol absorption.[303,304] After activation through binding of oxysterols, LXRs bind as a heterodimer with the retinoic acid receptor to LXR-response elements in the promoter of genes like *SREBP-1c*, *LPL*, *CETP*, *ApoE* and *ABCA1*, and thereby activate transcription of these genes.[302,304] In line with this, fish oil inhibits LXR activation by oxysterols and as a consequence inhibits the binding to the LXR-responsive element which leads to down regulation of *SREBP-1c*, to reduced *de novo* fatty acid synthesis and, subsequently, to reduced plasma triglyceride levels.[305] In contrast, the net effect of fish oil on HDL cholesterol is small, possibly through a counterbalance of PPAR activation and LXR inactivation. For example, *LPL* and *ABCA1* are both up-regulated by PPARs and LXRs.

5.7 Cholesterol Ester Transfer Protein Inhibitors

Several CETP inhibitors were developed that form disulphide bonds with CETP and reduce its activity.[306] The original rational of CETP inhibition was to mimic the anti-atherogenic lipoprotein profile of CETP deficiency where HDL cholesterol is high and LDL cholesterol is low.[307] Additional arguments for the clinical use of CETP inhibitors was the previous finding that this group of drugs also inhibits PLTP and thereby the hepatic production of apoB-containing lipoproteins.[30]

In rabbits, the orally bioavailable CETP-inhibitor JTT-705 decreased CETP activity in a dose-dependent way, and decreased non-HDL cholesterol and increased HDL cholesterol. In addition, treatment of rabbits for 6 months led to a marked reduction of atherosclerotic lesions.[306] In a recent randomised dose-response study, 4 weeks of treatment with JJT-705 resulted in a 37% reduction in CETP activity, a 34% increase in HDL cholesterol and a 7% decrease in LDL cholesterol with no change in the level of triglycerides.[308] The consequences of these lipoprotein changes are not clear and further research is necessary to investigate long-term benefits and adverse effects of this drug. In this regard it is also important to recall possible risks of CETP inhibition. Subgroups of CETP deficient patients with low hepatic lipase activity or hypertriglyceridaemia, as well as carriers of certain genetic CETP variants, were found to have an increased risk of CHD despite low CETP activity and elevated HDL cholesterol. Thus, it may be that only subgroups of patients benefit from this class of drugs.

5.8 Exogenous ApoA-I or Reconstituted HDL

The beneficial effects of apoA-I and HDL on atherogenesis triggered the investigation of their potential therapeutic use to cure atherosclerosis. Intravenous infusion of apoA-I/lecithin discs (so-called reconstituted HDL) was shown to increase pre-β-HDL concentrations in lymph and to accelerate bile acid excretion in humans.[309] Similar results were obtained following infusion of reconstituted HDL containing pro-apolipoprotein A-I where bile acid and cholesterol excretion were increased by about a third without signs of increased cholesterol synthesis.[310] Hence, metabolic studies in humans support the view that precursors of HDL can stimulate reverse cholesterol transport.

Further support for the use of apoA-I or reconstituted HDL therapy to stimulate RCT and to protect from atherosclerosis comes from animal studies. Infusion of a single high dose of recombinant apoA-I_{Milano} in rabbits was shown to decrease the lipid content and the number of macrophages in atherosclerotic lesions.[311] Importantly, this effect was documented 48 hours after apoA-I administration suggesting a very efficient way of mobilising lipids from atherosclerotic plaques and eventually stabilising them. In line with these findings, repetitive administration of apoA-I_{Milano} halted the progression of atherosclerosis in a hyperlipidaemic mouse model when mice were administered recombinant apoA-I_{Milano} intravenously every second day for 5 weeks.[312]

Effects independent of RCT were also attributed to apoA-I infusion in humans. Infusion of apoA-I/phosphatidylcholine normalised the impaired endothelial function in patients with hypercholesterolaemia. Endothelial-dependent vasodilation upon acetylcholine stimulation was improved within minutes of apoA-I infusion, reaching forearm blood-flow levels similar to normolipidaemic individuals.[100]

In all of the above studies, apoA-I had to be given intravenously, which limits its use for long-term pharmacological therapy in humans. In contrast, an 18 amino acid long apoA-I mimetic peptide synthesised from D-amino acids which forms an amphipathic lipid binding class A α-helix could be given to mice orally. The D-peptide was bioavailable and stable in circulation, whereas the identical peptide from L-amino acids was rapidly degraded.[313] Treatment with the apoA-I mimetic peptide did not affect lipoprotein levels but significantly inhibited the formation of atherosclerotic plaques in hyperlipidaemic mouse models.[313,314] The same peptide was recently shown to increase plasma HDL cholesterol and paraoxonase activity when injected intraperitoneally.[315] Several apoA-I mimetic peptides have previously been synthesised and investigated for their atheroprotective potential. Many of these peptides strongly associated with phospholip-

ids, promoted cholesterol efflux from lipid-laden cells and interacted with lipoproteins.[51,316-319]

In addition to the atheroprotective mechanism, apoA-I mimetic peptides were shown to possess antiviral activity. An orally available apoA-I mimetic peptide protected mice from influenza infection, and other apoA-I mimetic peptides inhibited HIV-induced syncytium formation and HSV-induced cell fusion in cell cultures.[315,320,321]

6. Conclusions

6.1 State of the Art and Current Clinical Consequences

Five lines of evidence motivate physicians, scientists and the pharmaceutical industry to apply or develop therapies to increase HDL-cholesterol levels for the treatment or prevention of atherosclerotic artery disease.

- Prospective observational studies identified low HDL cholesterol as an important independent coronary risk factor.
- Some rare inborn errors of HDL metabolism cause low HDL cholesterol and premature atherosclerosis.
- In large, controlled intervention studies, statin and fibrate treatment of patients with low HDL cholesterol increased HDL cholesterol and reduced the incidence of coronary events, and in some of these trials, the increase in HDL cholesterol correlated significantly with the decrease of event rates.
- HDL-associated proteins and lipids exert several potentially anti-atherosclerotic activities.
- Transgenic overexpression of human *apoA-I* or *ABCA1* genes was shown to inhibit the development or even induce regression of atherosclerosis in atherosclerosis-susceptible animal models.

However, all these arguments are limited or questioned by counter-evidence.

- Low HDL cholesterol is frequently confounded with several components of the metabolic syndrome suggesting that low HDL cholesterol may be a surrogate marker of other pro-atherogenic disturbances.
- Some mutations in the *apoA-I* gene, e.g. *apoA-I Milano*, cause low HDL cholesterol and were associated with reduced coronary risk. Likewise some mutations or polymorphisms in the genes of CETP or hepatic lipase increase HDL cholesterol but also CHD risk.
- Fibrates and statins exert a broad spectrum of regulatory and metabolic effects, only one of which is the moderate increase of HDL cholesterol. Moreover, the positive correlation between changes in HDL cholesterol and coronary risk were only made in some of these trials. Thus, it is only justified to say that patients with low HDL cholesterol benefit from treatment with fibrates or statins.
- Several biological activities of HDL were found associated with subclasses or components of HDL rather than with the bulk of HDL. In several situations the level of HDL cholesterol does not reflect the capacity but the result of RCT, i.e. dependent on the drug used, a therapeutically induced rise in HDL cholesterol may indicate a blocked rather than an enhanced RCT.
- There are several examples of genetic mouse models where changes in HDL cholesterol and atherosclerosis were equidirectional rather than the expected inversely directional.

In view of the unresolved questions and contradictory findings, anti-atherosclerotic therapy of cardiovascular patients at high risk with low HDL cholesterol should be focused on life style changes (smoking cessation, increment of physical activity, reduction of overweight) and the correction of additional risk factors, including statin treatment of elevated LDL cholesterol and fibrate treatment of elevated triglycerides.

However, since only half of the coronary events are prevented by the conventional interventions including lipid- and blood pressure lowering therapies, modification of HDL metabolism remains an attractive target for the development of new regimens of anti-atherogenic drug therapy. It is also necessary to develop tests for the clinical assessment of HDL functionality and cholesterol flux through the entire organism.

6.2 Targets for Future HDL Modifying Therapy

From the current knowledge of HDL metabolism and RCT, three major targets for an anti-atherogenic strategy in HDL metabolism have emerged. Stimulation of apoA-I synthesis and secretion, stimulation of ABCA1 expression, and up-regulation of SR-BI are all expected to improve RCT.

Stimulation of apoA-I synthesis and secretion is one key target for an anti-atherosclerotic therapy because of the pivotal role of apoA-I for the formation of HDL and because of the unequivocal finding of reduced atherosclerosis in transgenic animals overexpressing human apoA-I. Stimulation of ABCA1 expression and activity is also an attractive target because this transporter is essential for HDL formation and for the removal of cellular cholesterol from macrophages. Stimulation of both apoA-I and ABCA1 would increase HDL cholesterol, and would be easily accepted as preventive or curative treatment regimens. In contrast, up-regulation of SR-BI would cause a drop rather than an increase of HDL cholesterol and, therefore, create problems in clinical surveillance and acceptance of this therapy. Nevertheless, SR-BI has an important impact on cholesterol efflux from macrophages and on selective uptake of lipids from lipoproteins into the liver, and up-regulation will improve RCT.

The effect of therapeutic modulation of CETP, PLTP and hepatic lipase on atherogenesis is difficult to predict, since they were shown to have complex metabolic consequences and equivocal effects on atherosclerosis in animal models.

In many patients, low HDL cholesterol is not caused by dysregulation of a single pivotal gene but results from dysregulation of several genes. Moreover, low HDL cholesterol is a prototype symptom of the metabolic syndrome that is frequently confounded with other risk factors. Even more so, low HDL cholesterol frequently precedes the manifestation of other sequelae of the metabolic syndrome such as diabetes.[180,181] Therefore, common regulatory pathways appear to be dysregulated in most individuals with low HDL cholesterol which lead to increased coronary risk or even manifest atherosclerosis.

These common pathways may organise essential metabolic steps at the level of single cells, distinct organs and the entire organism. The most likely candidate genes for these common denominators are transcription factors that regulate the transcription of several downstream genes and/or kinases or phosphatases that regulate signal transduction cascades involved in RCT. At present, the main targets for anti-atherosclerotic therapy are the transcription factors PPARα, PPARγ, PPARδ, LXRα, RXRα and other orphan members of the nuclear receptor gene family.[322,323]

Agonists of PPARα (fibrates) and PPARγ (glitazones) are already used as drugs for prevention of atherosclerosis and treatment of diabetes, respectively. More potent agonists of PPARα ('superfibrates') are sought and glitazones will have to be investigated for their capacity to prevent diabetes and CHD. The metabolic effects of PPARδ agonists have just started to be explored with indications that selective PPARδ activation improves RCT.[324] Natural agonists of LXRα (oxysterols) and RXRα (retinoids) improve cholesterol efflux from macrophages but also induce hypertriglyceridaemia and are, therefore, not suitable for preventing atherosclerosis.

rosis. However, one may consider synthetic agonists that only exert non-hepatic effects.

In addition to these transcription factors with known ligands, pivotal genes of HDL metabolism are also regulated by orphan receptors with unknown ligands, e.g. HNF1α and ZNF202.[325-327] For these orphan receptors it will be important to identify the physiological ligands. Because geographical differences in the prevalence of low HDL cholesterol, type 2 diabetes and atherosclerotic vessel diseases are frequently paralleled with changes or differences in dietary habits, it is very likely that small molecules taken up with the diet are ligands of these nuclear receptors. Their elucidation may help to develop new compounds needed for the modulation of HDL metabolism. Likewise, several upstream effects of HDL on cell cycle and activation are mediated by its lipid components which can be exploited for drug development.

6.3 Assessment of the Anti-Atherosclerotic Potential of Therapeutical Modification of HDL Metabolism

HDL cholesterol is possibly a non-specific and insensitive marker to assess the effect of an intervention on atherosclerosis. Rather the kinetics of HDL metabolism influences the course of atherosclerosis. Thus, anti-atherosclerotic modification of HDL metabolism does not necessarily result in increased HDL-cholesterol levels. In particular, interference with HDL catabolism at first sight may produce controversial results, namely increased cardiovascular risk despite increased HDL cholesterol and vice versa. Moreover, some drugs (e.g. statins and fibrates) and metabolites (e.g. cholesterol and oxysterols) exert complex effects on HDL metabolism, which do not result in major changes of serum HDL-cholesterol level but have profound effects on HDL turnover and cholesterol flux through the organism.

Hence, metabolic and functional studies are important tools to assess the anti-atherosclerotic effects of HDL modification on a preclinical level. As yet, determination of apolipoproteins A-I and A-II and HDL subclasses (LpA-I and LpA-I, A-II, or HDL_2 and HDL_3 cholesterol) did not prove superior to HDL cholesterol in assessing cardiovascular risk.[183-185] Whether these parameters or the measurement of HDL subclasses, HDL-associated proteins or lipids provide more valuable information for monitoring the efficacy of HDL modifying therapy has yet to be proven.[291,328]

In vitro, the functionality of HDL in RCT may be monitored as the capacity of plasma to remove cholesterol from cells. However, such efflux studies are strongly influenced by the cell type used.[291,329,330] Paraoxonase, arylesterase or platelet activating factor hydrolase can be monitored to assess the antioxidative capacity of HDL.[331,332] *In vivo* measures of RCT assess the removal of radiolabeled cholesterol depots from muscle[333] but this can only be studied in animal models. Furthermore, monitoring of faecal sterol excretion under strongly controlled dietary regimens could provide evidence for RCT.[310] Turnover studies with stable isotopes demonstrate whether changes in HDL cholesterol are as a result of changes in HDL production or HDL catabolism.[334]

All these *in vivo* studies appear suitable only in a strongly controlled clinical research setting but are not practical in a routine clinical setting. Moreover, it is clear that these are all surrogate markers for a potential anti-atherosclerotic effect of any HDL cholesterol modifying therapy. Therefore, controlled interventional clinical outcome studies are needed at an early stage to prove the efficacy of HDL modifying drugs in preventing CHD.

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