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Review

The origin and metabolism of a nascent pre-β high density lipoprotein involved in cellular cholesterol efflux

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The pre-β HDL fraction constitutes a heterogeneous population of discoid nascent HDL particles. They transport from 1 to 25% of total human plasma apo A-I. Pre-β HDL particles are generated *de novo* by interaction between ABCA1 transporters and monomolecular lipid-free apo A-I. Most probably, the binding of apo A-I to ABCA1 initiates the generation of the phospholipid-apo A-I complex which induces free cholesterol efflux. The lipid-poor nascent pre-β HDL particle associates with more lipids through exposure to the ABCG1 transporter and apo M. The maturation of pre-β HDL into the spherical α-HDL containing apo A-I is mediated by LCAT, which esterifies free cholesterol and thereby forms a hydrophobic core of the lipoprotein particle. LCAT is also a key factor in promoting the formation of the HDL particle containing apo A-I and apo A-II by fusion of the spherical α-HDL containing apo A-I and the nascent discoid HDL containing apo A-II. The plasma remodelling of mature HDL particles by lipid transfer proteins and hepatic lipase causes the dissociation of lipid-free/lipid-poor apo A-I, which can either interact with ABCA1 transporters and be incorporated back into pre-existing HDL particles, or eventually be catabolized in the kidney. The formation of pre-β HDL and the cycling of apo A-I between the pre-β and α-HDL particles are thought to be crucial mechanisms of reverse cholesterol transport and the expression of ABCA1 in macrophages may play a main role in the protection against atherosclerosis.

Keywords: HDL, pre-β HDL, ABCA1, apo A-I

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INTRODUCTION

Numerous epidemiological studies have shown that the concentration of high density lipoprotein cholesterol (HDL-C) is inversely related to coronary heart disease (Abbott *et al.*, 1988; Wilson *et al.*, 1988; Gotto, 2001; Gotto & Brinton, 2004). The anti-atherogenic properties of HDLs are attributed mainly to their central role in reverse cholesterol transport (RCT), in which the HDLs accept cholesterol from cell membranes and transport it to the liver for catabolism. Additionally, the HDLs exert anti-inflammatory, anti-oxidant and anti-thrombotic actions (Gotto, 2001). The 'gold' standard for the isolation of lipoprotein fractions is density gradient ultracentrifugation (Havel *et al.*, 1955). The HDLs of human plasma are conventionally defined as lipoproteins isolated in the density range of 1.063–1.21 g/ml. However, the HDL fraction constitutes a heterogeneous group of particles that may be separated into discrete subclasses on the basis of the different physicochemical properties. Indeed, ultracentrifugation distributes the HDLs into two subfractions, HDL_2 and HDL_3, of different densities, sizes and composition (Table 1). They can be further divided into several classes of particles of different sizes by means of non-denaturing polyacrylamide gradient gel electrophoresis (Table 2).

Ultracentrifugation should, however, be recognized as an 'invasive' method for the isolation of HDLs from plasma, which violates natural protein–particle interactions. Moreover, the ultracentrifuged HDL fraction of density of 1.063–1.21 g/ml does not contain all the HDL particles types that occur in the plasma. Oram *et al.* (1981) discovered that serum fraction which has a density range of 1.21–1.25 g/ml comprises apolipoprotein (apo) A-I-containing structures that are effective acceptors for cellular free cholesterol (FC). Using agarose gel electrophoresis, which is probably less disruptive than ultracentrifugation, Kunitake *et al.* (1985) showed the presence in the serum of a small, apo A-I-containing HDL subfraction which was characterized by pre-β mobility (pre-β HDL), unlike the majority of HDL particles migrating with α mobility (α-HDL). A slower migration in the electric field results from a weaker negative surface charge, which is related to the lack of the neutral lipid core, which also causes a flattened, discoid shape of the particles (Davidson *et al.*, 1994). Asztalos *et al.* (1993) demonstrated that the density of pre-β HDL is higher than 1.21 g/ml. Furthermore, the HDL apo E-containing particles (HDL LpE) that lack apo A-I also float at a density higher than 1.21 g/ml (Krimbou *et al.*, 2003).

Pre-β HDL aroused great interest when Castro and Fielding (1988) demonstrated that it is the initial acceptor of cell-derived FC. Shortly afterwards, it was shown that 60% of cellular cholesterol efflux depends on the pre-β HDL subfraction and may involve a cell-surface protein (Kawano *et al.*, 1993). The discovery of the participation of pre-β HDL in the acceptance of cholesterol from cell membranes gave a new insight into HDL metabolism. This review presents selected current information on the origin and metabolism of this unique HDL subpopulation.

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Abbreviations: ABCA1, ATP-binding cassette A1; apo, apolipoprotein; CE, esterified cholesterol; CETP, cholesteryl ester transfer protein;. FC, free cholesterol; HDL-C, HDL cholesterol; HDLs, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; PL, phospholipids; PLTP, phospholipid transfer protein; POPC, palmitoyl-oleoyl phosphatidylcholine; RCT, reverse cholesterol transport; rHDL, reconstituted HDL; TG, triacylglycerols

Table 1. Characteristics of human HDL_2 and HDL_3 subclasses

Fraction	HDL_2	HDL_3	Reference
Density [g/ml]	1.063–1.125	1.125–1.210	(Lindgren *et al*., 1951)
Particle diameter [nm]	8.8–12.9	7.2–8.8	(Anderson *et al*., 1977)
Chemical composition [% wt]			(Patsch *et al*., 1987)
— Total protein	38.6	50.9	
— PL	32.5	29.0	
— FC	5.2	2.5	
— CE	19.3	15.1	
— TG	4.5	2.6	
Apo A-I [% of total protein]	80.1	65.7	(Patsch *et al*., 1987)
Apo A-II [% of total protein]	12.0	25.7	(Patsch *et al*., 1987)

PL, phospholipids; FC, unesterified (free) cholesterol; CE, esterified cholesterol; TG, triacylglycerols

HETEROGENEITY OF PLASMA PRE-β HDL

Apo A-I and apo A-II are major HDL proteins (Table 1); however, only apo A-I has been detected in pre-β HDL. According to a number of studies, Apo A-I-containing serum fraction migrating with pre-β mobility represents from 1% to 25% of the total apo A-I in normolipidemic subjects (Kunitake *et al*., 1985; Castro & Fielding, 1988; Asztalos *et al*., 1993). These discrepancies may reflect both biological variation and differences in the techniques used to quantify the pre-β HDL. It should be noted that the presence of lipid-free apo A-I in human plasma has been reported and that it also has a pre-β mobility when subjected to agarose gel electrophoresis (Daerr *et al*., 1986; Neary & Gowland, 1987). The term 'pre-β HDL' thus includes both monomolecular lipid-free apo A-I and apo A-I–lipid complexes. Using two-dimensional agarose/non-denaturing polyacrylamide gradient gel electrophoresis, pre-β HDL may be divided into several subspecies of different particle sizes and composition (Table 3).

The presence of a third type of pre-β HDL particles, with a molecular mass in excess of 300 kDa, has also been reported (Francone *et al*., 1989). This subfraction contains only 0.2% of total plasma apo A-I; but, unlike the others, apart from apo A-I, pre-β_3 HDL includes also lecithin : cholesterol acyltransferase (LCAT), cholesterol ester transport protein (CETP) and apo D. The first experiments on the uptake of cell-derived cholesterol from labelled cultured cells indicated a precursor-product relationship between the pre-β_1 and pre-β_2 HDL subclasses (Castro & Fielding, 1988). The composition of pre-β_3 HDL suggests participation in esterification and transfer of cholesterol (Francone *et al*., 1989); however, the detailed function of pre-β_3 has not been recognized yet.

Table 2. Distribution of human HDL_2 and HDL_3 subclasses according to the criteria of density and particle size
Blanche ***et al***., 1981)

Subfraction	Mean density [g/ml]	Mean particle size [nm]
HDL_{2b}	1.085	10.57
HDL_{2a}	1.115	9.16
HDL_{3a}	1.136	8.44
HDL_{3b}	1.154	7.97
HDL_{3c}	1.171	7.62

PREDICTED STRUCTURE OF PRE-β HDL PARTICLE

Apo A-I is the only protein detected in pre-β_1 and pre-β_2 HDL and is considered a key factor in modulating both the structure and function of pre-β HDL. The apo A-I molecule is a 243-amino-acid (28 kDa) polypeptide. It belongs to the exchangeable apolipoproteins that are capable of moving from one lipoprotein particle to another. Pivotal roles in the structure of these apolipoproteins are played by amphipathic α helices, with clearly distinguished polar and hydrophobic faces (Segrest *et al*., 1992; Frank & Marcel, 2000). The molecule of apo A-I contains the N-terminal globular region (residues 1–43) and eight 22- and two 11-mer repeats that are usually separated by proline residues (Davidson & Thompson, 2007). Isolated apo A-I combines spontaneously with phospholipids (PL) to form complexes of different PL/apo A-I ratios which are known as reconstituted HDL (rHDL) and resemble pre-β HDL (Atkinson & Small, 1986; Jonas *et al*., 1989; Brouillette & Anantharamaiah, 1995). Lipid binding is predicted to be mediated by the C-terminus of apo A-I, while the N-terminal and central regions are primarily responsible for structural integrity (Saito *et al*., 2004). In the lipid-free state, the N-terminal domain forms a helix bundle, whereas the C-terminal domain is less organized (Saito *et al*., 2003). Lipidation of apo A-I is accompanied by a transition from a compact to an extended structure of stable conformation, with an increase in the α helical content from 40–50% to 70–80% (Jonas *et al*., 1989; 1990; Marcel & Kiss, 2003; Saito *et al*., 2003).

It is widely believed that the pre-β_1 HDL particle in the plasma contains two copies of apo A-I (Barrans *et al*., 1996; Rye & Barter, 2004; Wu *et al*., 2007). However, the study conducted by Chau *et al*. (2006) shows that the pre-β_1 particle may contain only one molecule of apo A-I. The pre-β_1 particles containing one or two molecules of apo A-I may represent successive stages of nascent HDL generation (Chau *et al*., 2006; Duong *et al*., 2008).

Experiments with rHDL led to two basic models of discoid HDL particles, composed of two molecules of apo A-I and about 160 PL molecules. The first was the 'picket fence model' in which the amphipathic α-helices of each apo A-I molecule, separated by reverse turns at the proline residues, were arrayed around the periphery of the lipid core in an anti-parallel fashion (Nolte & Atkinson, 1992; Phillips *et al*., 1997). The picket fence model took into account the presence in the central region of apo A-I of a hinge domain loosely associated with lipids and responding to changes in the lipoprotein particle diameter by folding or unfolding (Frank & Marcel, 2000; Marcel & Kiss, 2003; Davidson & Silva, 2005).

Current data support the alternative 'double-belt' model (Fig. 1) in which two anti-parallel apo A-I molecules, adopting an extended helical structure, wrap around the edge of the disc (Segrest *et al*., 1999; Davidson & Thompson, 2007; Wu *et al*., 2007).

Table 3. Characteristics of pre-β HDL subspecies in human plasma

	Pre-β_1	Pre-β_2	Reference
Fraction of total apo A-I in healthy subjects [%]	4.6–6.3	0.9–4.3	(Asztalos *et al*., 1993; O'Connor *et al*., 1998; Xu & Fu, 2003)
Molecular mass [kDa]	65–80	325	(Ishida *et al*., 1987; Castro & Fielding, 1988; Barrans *et al*., 1996; O'Connor *et al*., 1998; Chau *et al*., 2006)
Apo A-I content [wt %]	47.5	21.5	(Castro & Fielding, 1988)
PL content [wt %]	44.6	73.8%	(Castro & Fielding, 1988)
FC content [wt %]	7.6	5.7	(Castro & Fielding, 1988)
Sub-fraction, particle diameter [nm]	Pre-β_{1a}, 5.79 Pre-β_{1b}, 5.37	Pre-β_{2a}, 13.74 Pre-β_{2b}, 12.58 Pre-β_{2c}, 12.17	(Asztalos *et al*., 1993)

FC, unesterified (free) cholesterol; PL, phospholipids

Presumably the stabilization of the lipid-bound conformation is ensured by salt bridges between the two adjacent apo A-I molecules. The conformational adaptability of apo A-I in relation to the changing lipid content is consistent with the recently presented 'looped-belt' or 'solar flares' model of discoid HDL particles (Martin *et al*., 2006; Wu *et al*., 2007; Thomas *et al*., 2008). In this model, the central apo A-I regions are more solvent-exposed and protrude from the edge of the disc. These loops could be LCAT interaction sites and are crucial for LCAT activation (Wu *et al*., 2007).

THE ABCA1 TRANSPORTER IS A KEY PROTEIN IN PRE-β HDL BIOGENESIS

Tangier disease is a rare genetic disorder which was described in the early 1960s (Fredrickson, 1964). It is characterized by a lack of the plasma HDL fraction, extremely low concentrations of apo A-I, and accumulation of cholesteryl esters (CE) in macrophages. It was suggested that the clinical and biochemical features of Tangier disease result from a defective efflux of cholesterol and phospholipids (Francis *et al*., 1995). In 1999, the association between Tangier disease and genetic defects in the ATP-binding cassette transporter A1 (ABCA1) gene was discovered by teams led by Michael Hayden, Gerd Schmitz and Gerd Assmann (Bodzioch *et al*., 1999; Brooks-Wilson *et al*., 1999; Rust *et al*., 1999). ABCA1 is a member of a superfamily of 49 mammalian ATP-binding cassette transporters that transport metabolites across membranes at the expense of ATP hydrolysis (Kielar *et al*., 2001; Oram & Heinecke, 2005). *ABCA1* is expressed in the liver, macrophages, brain and other tissues (Kielar *et al*., 2001). It localizes to intracellular compartments and the plasma membrane (Oram & Heinecke, 2005). ABCA1 promotes the transport of PL and FC to lipid-free apo A-I and other exchangeable apolipoproteins (apo A-II, apo E, apo CI, apo CII, apo CIII, apo A-IV) containing amphipathic α-helices (Fitzgerald *et al*., 2004; Oram & Heinecke, 2005). The interaction between ABCA1 and apo A-I leads to the lipidation of apo A-I and generation of the discoid nascent pre-β_1 HDL particle (Vedhachalam *et al*., 2007a). In Tangier disease patients the lack of functional ABCA1 transporters halts the PL and FC efflux, which results in the accumulation of CE in the cells and rapid degradation of apo A-I in the kidney (Timmins *et al*., 2005). Experiments on sophisticated animal models bearing target-inactivating mutations of *ABCA1* gene in specific tissues have proved that it is predominantly the liver and, to a lesser extent, the intestine which contribute to the biogenesis of circulating HDL particles (approximately 70% and 30%, respectively) (Lee *et al*., 2005; Timmins *et al*., 2005; Brunham *et al*., 2006). There is a widespread belief that, although the expression of ABCA1 in macrophages does not influence the HDL concentration significantly, it may be a key mechanism in preventing the development of atherosclerosis (Cuchel & Rader, 2006; Attie, 2007). Recently, a positive relationship has been demonstrated between the ABCA1-dependent macrophage cholesterol efflux and the concentration of pre-β_1 HDL in the serum of healthy human subjects (de la Llera-Moya *et al*., 2010).

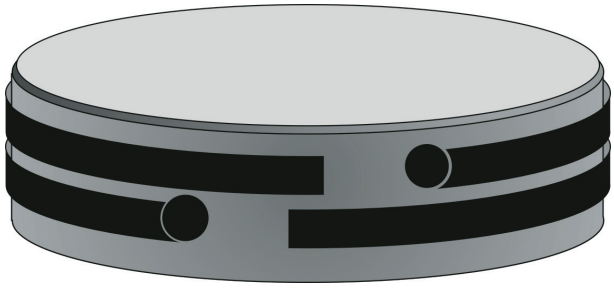

Figure 1. Double-belt model of pre-β HDL particle
Each of the two ring-shaped apo A-I proteins wraps around a lipid bilayer disc in an anti-parallel orientation. After: Segrest *et al*., 1999; Davidson & Thompson, 2007; Wu *et al*., 2007.

However, worthy of note is that a fraction of apo A-I may be lipidated in non-ABCA1-dependent process. This thesis is consistent with a study presented by Kiss *et al*. (2003), who demonstrated that ABCA1 deficiency does not completely block the lipidation of apo A-I in primary mouse hepatocytes, expressing human apo A-I (hapo A-I) or incubated with exogenously added hapo A-I.

The primary substrate for ABCA1 is lipid-free monomolecular apo A-I. The C-terminal domains of apo A-I, which are characterized by the greatest affinity for lipids, seem to play a crucial role in the interaction between apo A-I and ABCA1 (Favari *et al*., 2002; Liu *et al*., 2003; Hassan *et al*., 2007). Studies with synthetic peptides which mimic apolipoproteins have indicated that the interaction between apo A-I and ABCA1 is not sequence-specific; instead, amphipathic helices of apo A-I were found to be the key structural motif (Remaley *et al*., 2003; Gonzal-

ez *et al*., 2008). Apo A-I directly interacts with residues located in two extracellular loops of ABCA1 (Fitzgerald *et al*., 2002).

A number of observations imply that ABCA1 targets specific cholesterol-rich membrane domains (Oram *et al*., 2000; Mendez *et al*., 2001; Wang *et al*., 2001; Hassan *et al*., 2007; Vedhachalam *et al*., 2007b). The properties of these domains remain unclear. Caveolae are among the likely plasma membrane domains where ABCA1-mediated cholesterol efflux takes place. Caveolae are free-cholesterol-rich invaginated microdomains at the surface of most peripheral cells (Fielding & Fielding, 2000). The main structural component of caveolae — caveolin-1 — binds free cholesterol. In aortic endothelial cells, ABCA1 interacts directly with both caveolin-1 and apo A-I and may act as a structural platform between caveolin-1 and HDL (Chao *et al*., 2005).

On the other hand, there is some evidence that lipid rafts which lack structural protein components may also be involved in cholesterol efflux from macrophages (Gaus *et al*., 2004). However, they are thought not to be a source of lipid for apo A-I, serving instead as a functional platform enabling stimulation of ABCA1-dependent cholesterol efflux. A recently presented biophysical study confirms that ABCA1 destabilizes raft domains and redistributes FC to cell surface domains readily accessible to apolipoproteins (Zarubica *et al*., 2009).

Finally, it is also possible that ABCA1 associates with membrane domains distinct from both caveolae and lipid rafts (Mendez *et al*., 2001; Liu *et al*., 2003).

PUTATIVE MECHANISM OF EXTRACELLULAR PRE-β HDL FORMATION

The minimal functional unit required for the biogenesis of HDL particles is thought to be the homotetrameric ABCA1 complex (Denis *et al*., 2004a). The molecular mechanism of ABCA1-mediated lipid efflux to apo A-I is not fully understood. Chau *et al*. (2006) demonstrated that human liver or colon-derived cells secrete apo A-I in a globular form with a Stokes diameter of 5.2 nm that binds neither PL nor FC and migrates in agarose gel with a pre-α mobility. This inactive in binding lipids form is converted by ABCA1 transporter into a product with a diameter of 7.2 nm, which probably results from the refolding of the spherical apo A-I particle into a tubular form. This process is ABCA1-dependent but is not accompanied by PL transfer. Lipids are subsequently transferred to the refolded apo A-I, resulting in the generation of the biologically active pre-β_1 lipid-poor particle (Fig. 2). Chau *et al*. (2006) hypothesized that the inability to bind lipids by the newly produced apo A-I may promote the distribution of lipid-free apo A-I to the extracellular space of peripheral tissues.

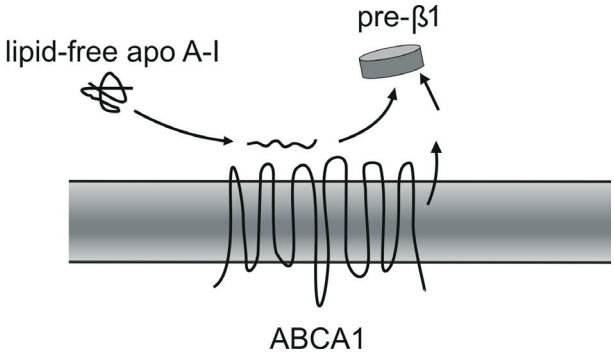

Figure 2. Processing of newly secreted apo A-I into PL-receptive pre-β1 HDL
After: Chau *et al*., 2006. PL, phospholipids; ABCA1, ATP-binding cassette transporter A1.

An important issue is whether the ABCA1-mediated transport of FC and PL to lipid-poor apo A-I is simultaneous or sequential. The 'microsolubilization' model presented by Gillotte *et al*. (1998) assumed that pre-β_1 particles result from a simultaneous transfer of FC and PL to lipid-free apo A-I. However, experimental evidence supports the idea that ABCA1 does not bind cholesterol directly, but acts primarily as a PL translocase. Nowadays, therefore, a two-stage transfer model of pre-β HDL generation is taken into consideration, according to which the binding of apo AI to ABCA1 would lead first to the generation of a PL-apo A-I complex that would subsequently promote FC efflux (Fielding *et al*., 2000; Wang *et al*., 2001). The separation of PL and cholesterol efflux has recently been confirmed by studies carried out on humans with severe HDL deficiency (Rashid *et al*., 2009).

Cells expressing *ABCA1* secrete heterogeneous nascent pre-β HDL subpopulations (Mulya *et al*., 2007; Duong *et al*., 2008). This diversity may be related to the multi-stage process of nascent HDL formation. *In vitro*, the binding of small amounts of PL and cholesterol to apo A-I results in the formation of rHDL containing one apo A-I molecule while the incorporation of a greater number of PL molecules promotes the generation of a particle with two apo A-I molecules (Sparks *et al*., 1999). Duong *et al*. (2008) have shown that primary pre-β_1 particles with the diameter of 7.5 nm generated during the interaction between apo A-I and ABCA1, contained three or four PL molecules and one molecule of apo A-I. These lipid-poor complexes were subsequently converted into discs containing 100–200 PL molecules and two, three or four apo A-I molecules. Thus, the small nascent particles could serve as precursors of a larger nascent HDL. This leads us to the conclusion that pre-β_1 is both a product and a substrate for ABCA1-mediated lipid efflux and raises the question as to whether there exists a limit of apo A-I lipidation that inactivates the interaction between ABCA1 and apo A-I. Mulya *et al*. (2007) demonstrated that even a minimal association of PL with apo A-I (5–9 molecules of PL per one molecule of apo A-I) significantly reduces its binding to ABCA1. On the other hand, experiments with rHDL composed of apo A-I and polmitoyl-oleoyl phosphatidylcholine (POPC) proved that up to 82 PL molecules can bind to apo A-I without abolishing its functionality as a ligand for ABCA1 (Favari *et al*., 2009). This indicates that a conformational rearrangement of the apo A-I molecule, rather than lipidation, may be a key factor preventing lipid-poor pre-β_1 from interacting with ABCA1.

The multi-stage model of HDL biogenesis is consistent with experiments demonstrating that ABCA1 activity creates two types of apo A-I-binding sites on the cell surface (Hassan *et al*., 2007; Vedhachalam *et al*., 2007b). The first site is formed by a direct interaction between apo A-I and ABCA1. It is characterized by low specificity, most probably because it occurs between the amphipathic α-helices of apo A-I and hydrophobic regions of the largest ABCA1 extracellular loop. The low capacity site binds about 10% of the apo A-I which is present on the surface of cells (Vedhachalam *et al*., 2007b). The binding with apo A-I protects ABCA1 from proteolytic

degradation (Martinez *et al.*, 2003; Wang *et al.*, 2003) and independently activates Janus kinase 2 (Tang *et al.*, 2006). Thus, the low capacity site may have a regulatory function. The second, high capacity site is a membrane lipid site. The high affinity results from the penetration of the space between the PL polar groups in the highly curved membrane bilayer by the amphipathic α-helices of apo A-I.

A three-step model of the interaction between ABCA1 and apo A-I proposed by Vedhachalam *et al.* (2007a) explains the contribution of the interaction of apo A-I with ABCA1 and membrane lipids, as well as the heterogeneous nature of the nascent HDL particles (Fig. 3). Step 1 involves direct binding of a small number of apo A-I molecules to a specific ABCA1 site. The binding of apo A-I by ABCA1 would induce signalling responses that would stabilize ABCA1 and enhance PL translocase activity. This would lead to the compression of the PL molecules in the exofacial leaflet of the membrane, which would bulge in the direction of the extracellular space. In step 2, more apo A-I would bind to the membrane protrusions. Step 3 would involve membrane microsolubilization and the creation of discoid nascent HDL particles. This stage would limit the rate of the entire ABCA1 — apo A-I interaction. The variations in the lipid composition of membrane curvatures created in different membrane environments would cause the heterogeneity of nascent HDLs, particularly in terms of FC content.

It is also likely that ABCA1-dependent lipidation of apo A-I occurs by retroendocytosis (Neufeld *et al.*, 2004; Azuma *et al.*, 2009). This concept is consistent with the traffic of early endosomes containing ABCA1 between the plasma membrane and other endocytic compartments (Takahashi & Smith, 1999; Neufeld *et al.*, 2001; Oram & Heinecke, 2005). According to this hypothesis, the interaction between ABCA1 and apo A-I would be followed by endocytosis of vesicles containing ABCA1 and apo A-I to intracellular lipid deposits. There, ABCA1 would pump the lipids to the vesicle lumen for release by exocytosis.

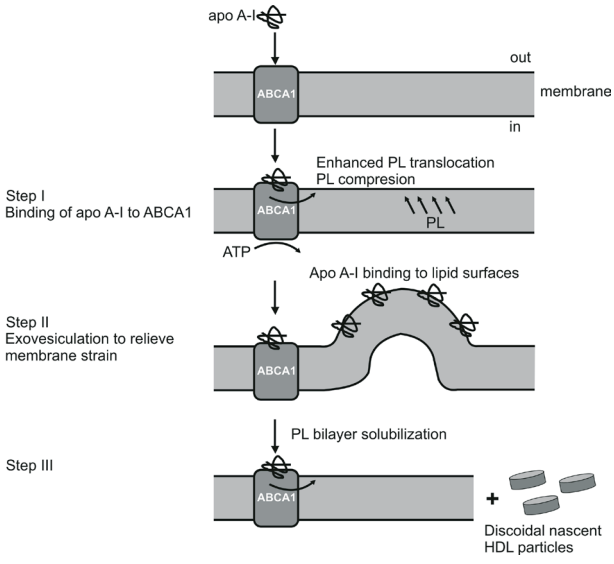

Figure 3. Three-step mechanism of ABCA1-mediated efflux of cellular lipids to apo A-I
Step 1: binding of a small amount of apo A-I to ABCA1 increases ABCA1 activity. Membrane PL actively translocate from the cytoplasmic to the exofacial leaflet of the membrane. The accumulation of lipid molecules in the exofacial leaflet and the net depletion of PL molecules in the cytoplasmic leaflet induce strain in the membrane. **Step 2:** highly curved exovesiculated domain relieves the membrane strain. Apo A-I molecules bind with high affinity to the PL bilayer curvature. **Step 3:** discoidal HDL particles are formed as a result of spontaneous solubilization of membrane PL and cholesterol by apo A-I. After: Vedhachalam *et al.*, 2007a. PL, phospholipids; ABCA1, ATP-binding cassette transporter A1.

INTRACELLULAR ASSEMBLY OF HDL PARTICLES

Apo A-I is synthesized in the liver and intestine (approximately 80% and 20%, respectively) (Wu & Windmueller, 1979; Timmins *et al.*, 2005). In the past, HDL was thought to be formed through an intracellular assembly of discoid particles in the Golgi (Hamilton *et al.*, 1976; Banerjee & Redman, 1983; McCall *et al.*, 1988). Nowadays, there is no doubt that the extracellular lipidation of newly secreted lipid-free apo A-I through interaction with ABCA1 is an important source of pre-β HDL (Chau *et al.*, 2006). However, from 20% to 50% of newly synthesized apo A-I may be lipidated intracellularly prior to secretion (Chisholm *et al.*, 2002; Maric *et al.*, 2005). A study by Maric *et al.* (2005) showed that the initial phospholipidation of apo A-I in primary mouse hepatocytes occurred in the endoplasmic reticulum (ER), probably immediately after translation, and did not depend on ABCA1. It was postulated that the affinity of newly synthesized apo A-I to PL is related to proper protein folding. Apo A-I would then transit from ER to the Golgi where it would interact with ABCA1 and bind more PL. Finally, the apo A-I would leave the Golgi and accumulate in the media.

ABCG1 AND APO M — NEW FACTORS INVOLVED IN PRE-β HDL FORMATION

While ABCA1 mediates lipids efflux to free/lipid-poor apo A-I, subsequent lipidation of nascent lipid-poor pre-β HDL most probably occurs in a non-ABCA1-mediated process. A partner of ABCA1 in the removal of cholesterol from cells was recently identified as the ABCG1 transporter (Gelissen *et al.*, 2006; Favari *et al.*, 2009). ABCG1 is widely expressed in macrophages, adipocytes, lung, brain and other tissues. Although the ABCG1 proteins are predominantly intracellular, associated with the endoplasmic reticulum and Golgi membranes, their movement to the plasma membrane of macrophages has also been demonstrated (Klucken *et al.*, 2000; Vaughan & Oram, 2005). Little is known about the mechanism of the ABCG1-mediated transport of FC. It may proceed by aqueous diffusion, since the cholesterol acceptor does not necessarily bind to a donor cell (Wang *et al.*, 2004; Sankaranarayanan *et al.*, 2009). The role of ABCG1 could be the redistribution of cholesterol to a cell surface pool that is accessible for enzymatic oxidation and removal by HDL.

The most important feature of ABCG1 is probably its ability to promote lipidation not only of nascent pre-β_1 HDL, but also of mature HDL particles (Wang *et al.*, 2004; Vaughan & Oram, 2005; Sankaranarayanan *et al.*, 2009) that constitute the majority of circulating HDLs. Disruption of the *ABCG1* gene in mice causes massive accumulation of neutral lipids in macrophages and the liver, whereas overexpression of *ABCG1* prevents it (Kennedy *et al.*, 2005). Thus, the ABCG1 transporter may also be involved in tissue lipid homeostasis. ABCG1 proteins may have a significant share in mediating cholesterol efflux from macrophage foam cells and thus play an important role in preventing against atherosclerosis (Wang *et al.*, 2004).

Apo M was discovered in 1999 (Xu & Dahlback, 1999) and soon emerged as a new player in of pre-β HDL formation and also thereby in RCT (Hu *et al.*, 2010). Human apo M is an about 25 kDa protein that belongs to the lipocalin superfamily (Luo *et al.*, 2004, Nielsen *et al.*, 2009). Thus, it is thought to possess a hydrophobic pocket binding lipophilic molecules. Apo M is highly expressed in the kidney and liver. Kidney-derived apo M is probably catabolized in proximal tubules, while apo M synthesized in hepatocytes is secreted to the blood, where it associates predominantly with HDL particles (Nielsen *et al.*, 2009). The binding of apo M to HDL has a unique nature, because this protein is anchored there by its retained signal peptide. However, it should be stressed that apo M is a component of only 5% of the HDLs in the plasma. The mean plasma concentration of apo M is about 23 mg/L (Nielsen *et al.*, 2009). In mice, a lack of apo M led to the disappearance of pre-β HDL in the plasma and a significant decrease of HDL-C (Wolfrum *et al.*, 2005). This suggests that apo M is critical for pre-β HDL formation *in vivo*. In turn, overexpression of the apo M gene caused a two-fold increase of HDL-C concentration in the plasma and a reduction of atherosclerotic lesions. Using human kidney-derived cells, Mulya *et al.* (2010) recently proved that apo M expression is not necessary for the generation of nascent HDL by ABCA1, but it promotes the formation of larger nascent HDL particles. This may result from both the transfer of lipids from smaller to larger particles and from the fusion of small pre-β_1 particles. It is also likely that apo M-mediated lipidation of pre-β HDL particles occurs at an intracellular site.

ROLE OF LCAT IN PRE-β HDL MATURATION

Discoid HDL is an intermediate between lipid-free apo A-I and the mature, spherical HDL. The critical step in the transformation of a nascent to a mature form of HDL is the esterification of cholesterol catalyzed by LCAT. It is a 416-amino-acid glycoprotein produced mainly by the liver and circulating in the blood reversibly bound to lipoproteins, preferentially to HDL (Jonas, 2000). LCAT transfers a long-chain fatty acid residue from the *sn*-2 position of phosphatidylcholine to the 3-β-hydroxyl group of cholesterol to produce a cholesteryl ester and lysolecithin. LCAT is active on the surface of both HDL (α activity) and LDL (β activity) (Kuivenhoven *et al.*, 1997). Apo A-I is the major activator of LCAT on HDL (Fielding *et al.*, 1972; Jonas, 1986; 2000). The importance of LCAT for HDL maturation is demonstrated by rare total (familial LCAT deficiency) or partial (fish eye disease) deficiency states, which are related to a markedly decreased plasma HDL-C and the accumulation in the plasma of nascent discoid HDL particles forming aggregates (McIntyre, 1988; Asztalos *et al.*, 2007). *In vitro* experiments with both rHDL and native lipoproteins indicated that lipid-poor pre-β particles are direct and preferential substrates for LCAT (Jonas *et al.*, 1990; Kosek *et al.*, 1999; Nakamura *et al.*, 2004; Shih *et al.*, 2009). Jonas *et al.* (1990) demonstrated that LCAT preferred as its substrate discoid rHDL 34-fold over spherical rHDL with a similar particle size. Thus, the rate of cholesterol esterification is the highest in lipid-poor apo A-I followed by discoid HDL and spherical HDL (Jonas, 1986; Neary *et al.*, 1991; Sparks *et al.*, 1999; Nakamura *et al.*, 2004).

It was presumed that the movement of CE to the centre of the disc forms a hydrophobic core and transforms the flattened structure into a sphere (Jonas, 1986). Recent molecular dynamics simulations have confirmed that the discoid HDL spontaneously forms a spherical particle through the gradual incorporation of CE (Shih *et al.*, 2009). The absorption of CE increases the solvent-accessible surface area of PL, which enables the recruitment of additional apo A-I molecules (Shih *et al.*, 2009). This conclusion is consistent with previous studies which demonstrated that LCAT mediates the incorporation of lipid-free apo A-I into rHDL (Liang *et al.*, 1996).

FORMATION OF HDL CONTAINING BOTH APO A-I AND APO A-II (HDL A-I/A-II)

About half of mature HDLs contain also apo A-II. Apo A-II is a homodimer of two 77-amino acid polypeptides linked by a disulfide bond at residue 6 (Blanco-Vaca *et al.*, 2001). Like apo A-I, apo A-II associates spontaneously with phospholipids, forming discoid structures (rHDL A-II) (Atkinson & Small, 1986), and accepts PL and FC from cell membranes, producing discoid nascent HDL-like lipoproteins (Hara & Yokoyama, 1991). In contrast to apo A-I, little is known about the structure of apo A-II in rHDL. Data presented by Silva *et al.* (2007) support the presence of a belt-like structure of apo A-II in POPC rHDL particles.

Apo A-II is mainly produced by the liver (Wu & Windmueller, 1979). This apolipoprotein is lipidated intracellularly and secreted as discoid pre-β-migrating nascent HDL A-II (Gillard *et al.*, 2009). To date, pre-β HDL A-II particles have not been detected in the serum from healthy humans, but have been reported in serum from LCAT-deficient subjects (Rader *et al.*, 1994; Asztalos *et al.*, 2007) and in transgenic mice overexpressing human apo A-II (Fournier *et al.*, 2002). From 5% to 25% of serum apo A-II is associated with PL-rich, spherical particles that do not contain apo A-I (HDL A-II) (Bekaert *et al.*, 1992), which suggests the possibility of a transformation of nascent pre-β HDL A-II into the mature form by unknown mechanisms. A small fraction of apo A-II is associated with very low density lipoproteins (VLDL) and chylomicrons; however, most apo A-II in human serum is found in HDL A-I/A-II particles (Blanco-Vaca *et al.*, 2001). The lack of discoid HDL A-II in serum suggests rapid formation of HDL A-I/A-II. Indeed, experiments with rabbits that are naturally deficient in apo A-II have confirmed the very rapid incorporation of both lipid-free apo A-II and rHDL A-II entering the plasma to spherical HDL particles (Hime *et al.*, 2006). The mechanism mediating the formation of HDL A-I/A-II is unclear. The central role in this process seems to be played by LCAT. It does not convert discoid HDL A-II into spherical forms (Forte *et al.*, 1995); however, it is thought to mediate the fusion of nascent discoid HDL A-II with spherical HDL A-I to form spherical HDL A-I/A-II (Clay *et al.*, 2000; Gillard *et al.*, 2009). Another possible mechanism for the generation of HDL A-I/A-II is based on the ability of apo A-II to displace apo A-I from HDL particles, which is caused by the higher affinity of apo A-II to lipids (van Tornout *et al.*, 1981). The impact of apo A-II on the HDL structure is not clear. The presence of apo A-II may enhance HDL stability (Rye *et al.*, 2003; Boucher *et al.*, 2004).

GENERATION OF PRE-β HDL FROM MATURE PLASMA HDLS

Soon after the participation of pre-β HDL in RCT had been discovered, Kunitake *et al.* (1992) postulated that apo A-I cycles between pre-β HDL and α-HDL in response to the movement of CE through the HDL compartment. Indeed, a fraction of apo A-I may dissociate from HDLs during their plasma remodelling mediated by a number of factors, such as CETP, phospholipid transfer protein (PLTP) and hepatic lipase (HL). Kinetic analysis demonstrated that the generation of pre-β_1 HDL from α-HDL was approximately 10 times slower than the conversion of liver-derived pre-β_1 to α-HDL (Chetiveaux *et al.*, 2004).

CETP and PLTP are lipid-transfer proteins that play a central role in the regulation of HDL level in the blood. CETP is a hydrophobic plasma glycoprotein composed of 476-amino acids with four N-linked glycosylation sites. It is expressed in the liver, small intestine, adipose tissue, spleen and macrophages (Masson *et al.*, 2009). CETP promotes the transfer of CE from HDL to apo B-containing lipoproteins (VLDL and low density lipoproteins, LDL) in exchange for triacylglycerols (TG) (Masson *et al.*, 2009). Thus CETP action depletes the HDL core of CE and enriches it with TG. A genetic deficiency of CETP is related to markedly increased HDL-C concentrations and altered HDL particle distribution — the proportion of larger particles is increased and of small pre-β_1 particles — decreased (Hirano *et al.*, 1997; Arai *et al.*, 2000; Asztalos *et al.*, 2004). In mice, which normally lack CETP (Masson *et al.*, 2009), the expression of human CETP leads to increased pre-β HDL levels (Francone *et al.*, 1996). It has been shown that during *in vitro* HDL remodelling by CETP, up to 36% of apo A-I may be released from HDL in a lipid-free or lipid-poor form (Liang *et al.*, 1994). Experiments with spherical rHDL also suggest that CETP may promote fusion of the HDLs (Rye *et al.*, 1997).

PLTP is a 476-amino acid protein with six N-linked glycosylation sites which shuttles the PL derived from lipolyzed TG-rich lipoproteins (chylomicrons and VLDL) to HDLs (Tall *et al.*, 1985; Masson *et al.*, 2009). PLTP is widely expressed in the organs (Masson *et al.*, 2009). The importance of PLTP to the maintenance of HDL levels is proved by a markedly reduced HDL-C level in mice lacking PLTP (Jiang *et al.*, 1999). Epidemiological studies confirm the relationship between variants of the *PLTP* gene and the regulation of HDL-C in humans (Aouizerat *et al.*, 2006). *In vitro,* PLTP changes human HDL_2 and HDL_3 particle distribution by converting the particles into both larger and smaller subpopulations (Jauhiainen *et al.*, 1993; Marques-Vidal *et al.*, 1997). These changes are accompanied by a loss of apo A-I and the generation of pre-β_1 HDL particles (von Eckardstein *et al.*, 1996; Marques-Vidal *et al.*, 1997). On the basis of experiments carried out with rHDL, Lusa *et al.* (1996) postulated that the interaction of PLTP with HDL at first induces the release of apo A-I from the HDL. This destabilizes the HDL particles, which subsequently undergo fusion into larger, thermodynamically stable particles.

Hepatic lipase is a lipolytic enzyme with a broad substrate specificity, which hydrolyzed PL and TG in HDLs (Thuren, 2000). Epidemiological studies indicate that HL gene polymorphism strongly influences HDL-C levels (Guerra *et al.*, 1997; Murtomaki *et al.*, 1997; Andersen *et al.*, 2003; Hodoglugil *et al.*, 2010). The lipolysis mediated by HL reduces the size of the HDL particles and generates small amounts of pre-β_1 HDL particles (Clay *et al.*, 1991; Barrans *et al.*, 1994). The enrichment of HDL with TG significantly enhances the loss of apo A-I from HDL and pre-β_1 production by HL (Hopkins & Barter, 1986; Clay *et al.*, 1991; Barrans *et al.*, 1994). Clay *et al.* (1992) demonstrated that the combined action of HL and CETP causes the loss of up to 30% of apo A-I from HDLs.

Several metabolic transformations of the lipid-free/lipid-poor apo A-I shed from HDLs during their plasma remodelling are possible. These are: incorporation into pre-existing HDL particles (Liang *et al.*, 1995), fusion with newly secreted discoid HDL A-II and the formation of new generations of HDL A-I/A-II particles (Rye & Barter, 2004), yielding new pre-β HDL particles by incorporation of lipids from other lipoproteins (Clay *et al.*, 1992; Clay & Barter, 1996), and mediation of cellular cholesterol efflux in an ABCA1-dependent manner (Okuhira *et al.*, 2004). However, lipid-poor apo A-I can also be catabolised in the kidney and irreversibly lost (Horowitz *et al.*, 1993; Lamarche *et al.*, 1999).

It is not known whether the remodelling of mature HDLA-I/A-II in the plasma generates lipid-poor apo A-II. An experiment with rHDL demonstrated a substantial difference between apo A-I and apo A-II in respect to their ability to be transferred into the aqueous phase (Pownall *et al.*, 2007; Gao *et al.*, 2009). On the other hand, studies on HDL remodelling mediated by PL have demonstrated the formation of new apo A-II-containing particles resembling nascent HDL (Tall & Green, 1981; Wróblewska *et al.*, 2009; 2010). Experiments *in vitro* proved that both pre-β HDL A-II and purified apo A-II stimulate cholesterol efflux in an ABCA1-dependent manner (Fournier *et al.*, 2002; Fitzgerald *et al.*, 2004). The recently published results of the European Prospective Investigation into Cancer and Nutrition — Norfolk study showed an inverse correlation between serum apo A-II levels and the risk of future coronary artery disease in apparently healthy subjects (Birjmohun *et al.*, 2007). The possible involvement of apo A-II in RCT remains to be established.

NASCENT HDL PARTICLES MAY EXHIBIT α-ELECTROPHORETIC MOBILITY

Nascent apo A-I-containing particles of pre-β mobility are thought to be the major source of HDL in the plasma. However, it should be remembered that pre-β mobility is only a physical feature and results from the surface electric charge of lipoprotein particles. The ABCA1-dependent formation of nascent HDL A-I particles migrating with α mobility has also been demonstrated (Denis *et al.*, 2004b; Krimbou *et al.*, 2005). These particles also interacted efficiently with LCAT (Krimbou *et al.*, 2005). The α-electrophoretic mobility may have been due to the high content of phosphatidylinositol or to the high number of apo A-I molecules per particle (Denis *et al.*, 2004b). An analysis of nascent apo A-I-containing HDL generated by the incubation of exogenously added lipid-free apo A-I with various cell lines suggested that speciation of nascent HDL into pre-β and/or α-HDL is linked to specific cell lines (Krimbou *et al.*, 2005). So far, little is known about the metabolism of nascent α-HDL and whether its physiological role differs from that of pre-β HDL.

CONCLUDING REMARKS

Reverse cholesterol transport allows the maintenance of cholesterol homeostasis in the body and affords protection against atherosclerosis. The first step of RCT is the efflux of FC from the cell membrane. This occurs as a result of the interaction between lipid-free apo A-I and ABCA1 protein and generates discoid pre-β HDL particles capable of taking up FC from cell membranes. The remodelling of mature HDLs in the plasma, mediated by lipid transfer proteins and hepatic lipase, is also the source of pre-β HDL particles. Research on pre-β HDL has opened up new possibilities for atheroprotective therapies augmenting the RCT. These include such strategies as the use of synthetic pre-β HDL prepared from recombined apo A-I, or small apo A-I mimetic peptides combined with phospholipids (Shah, 2007) and the elevation of *de novo* apo A-I synthesis and, thereby, of HDL formation (Dullens *et al*., 2007).

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REFERENCES

Abbott RD, Wilson PW, Kannel WB, Castelli WP (1988) High density lipoprotein cholesterol, total cholesterol screening, and myocardial infarction. The Framingham Study. *Arteriosclerosis* **8:** 207–211.

Anderson DW, Nichols AV, Forte TM, Lindgren FT (1977) Particle distribution of human serum high density lipoproteins. *Biochim Biophys Acta* **493:** 55–68.

Andersen RV, Wittrup HH, Tybjaerg-Hansen A, Steffensen R, Schnohr P, Nordestgaard BG (2003) Hepatic lipase mutations, elevated high-density lipoprotein cholesterol, and increased risk of ischemic heart disease: the Copenhagen City Heart Study. *J Am Coll Cardiol* **41:** 1972–1982.

Aouizerat BE, Engler MB, Natanzon Y, Kulkarni M, Song J, Eng C, Huuskonen J, Rivera C, Poon A, Bensley M, Sehnert A, Zellner C, Malloy M, Kane J, Pullinger CR (2006) Genetic variation of PLTP modulates lipoprotein profiles in hypoalphalipoproteinemia. *J Lipid Res* **47:** 787–793.

Arai T, Tsukada T, Murase T, Matsumoto K (2000) Particle size analysis of high density lipoproteins in patients with genetic cholesteryl ester transfer protein deficiency. *Clin Chim Acta* **301:** 103–117.

Asztalos BF, Sloop CH, Wong L, Roheim PS (1993) Two-dimensional electrophoresis of plasma lipoproteins: recognition of new apo A-I-containing subpopulations. *Biochim Biophys Acta* **1169:** 291–300.

Asztalos BF, Horvath KV, Kajinami K, Nartsupha C, Cox CE, Batista M, Schaefer EJ, Inazu A, Mabuchi H (2004) Apolipoprotein composition of HDL in cholesteryl ester transfer protein deficiency. *J Lipid Res* **45:** 448–455.

Asztalos BF, Schaefer EJ, Horvath KV, Yamashita S, Miller M, Franceschini G, Calabresi L (2007) Role of LCAT in HDL remodeling: investigation of LCAT deficiency states. *J Lipid Res* **48:** 592–599.

Atkinson D, Small DM (1986) Recombinant lipoproteins: implications for structure and assembly of native lipoproteins. *Annu Rev Biophys Biophys Chem* **15:** 403–456.

Attie AD (2007) ABCA1: at the nexus of cholesterol, HDL and atherosclerosis. *Trends Biochem Sci* **32:** 172–179.

Azuma Y, Takada M, Shin HW, Kioka N, Nakayama K, Ueda K (2009) Retroendocytosis pathway of ABCA1/ApoA-I contributes to HDL formation. *Genes Cells* **14:** 191–204.

Banerjee D, Redman CM (1983) Biosynthesis of high density lipoprotein by chicken liver: nature of nascent intracellular high density lipoprotein. *J Cell Biol* **96:** 651–660.

Barrans A, Collet X, Barbaras R, Jaspard B, Manent J, Vieu C, Chap H, Perret B (1994) Hepatic lipase induces the formation of pre-beta 1 high density lipoprotein (HDL) from triacylglycerol-rich HDL2. A study comparing liver perfusion to *in vitro* incubation with lipases. *J Biol Chem* **269:** 11572–11577.

Barrans A, Jaspard B, Barbaras R, Chap H, Perret B, Collet X (1996) Pre-beta HDL: structure and metabolism. *Biochim Biophys Acta* **1300:** 73–85.

Bekaert ED, Alaupovic P, Knight-Gibson C, Norum RA, Laux MJ, Ayrault-Jarrier M (1992) Isolation and partial characterization of lipoprotein A-II (LP-A-II) particles of human plasma. *Biochim Biophys Acta* **1126:** 105–113.

Birjmohun RS, Dallinga-Thie GM, Kuivenhoven JA, Stroes ES, Otvos JD, Wareham NJ, Luben R, Kastelein JJ, Khaw KT, Boekholdt SM (2007) Apolipoprotein A-II is inversely associated with risk of future coronary artery disease. *Circulation* **116:** 2029–2035.

Blanche PJ, Gong EL, Forte TM, Nichols AV (1981) Characterization of human high-density lipoproteins by gradient gel electrophoresis. *Biochim Biophys Acta* **665:** 408–419.

Blanco-Vaca F, Escola-Gil JC, Martin-Campos JM, Julve J (2001) Role of apoA-II in lipid metabolism and atherosclerosis: advances in the study of an enigmatic protein. *J Lipid Res* **42:** 1727–1739.

Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, Drobnik W, Barlage S, Buchler C, Porsch-Ozcurumez M, Kaminski WE, Hahmann HW, Oette K, Rothe G, Aslanidis C, Lackner KJ, Schmitz G (1999) The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nat Genet* **22:** 347–351.

Boucher J, Ramsamy TA, Braschi S, Sahoo D, Neville TA, Sparks DL (2004) Apolipoprotein A-II regulates HDL stability and affects hepatic lipase association and activity. *J Lipid Res* **45:** 849–858.

Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO, Loubser O, Ouelette BF, Fichter K, Ashbourne-Excoffon KJ, Sensen CW, Scherer S, Mott S, Denis M, Martindale D, Frohlich J *et al.* (1999) Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat Genet* **22:** 336–345.

Brouillette CG, Anantharamaiah GM (1995) Structural models of human apolipoprotein A-I. *Biochim Biophys Acta* **1256:** 103–129.

Brunham LR, Kruit JK, Iqbal J, Fievet C, Timmins JM, Pape TD, Coburn BA, Bissada N, Staels B, Groen AK, Hussain MM, Parks JS, Kuipers F, Hayden MR (2006) Intestinal ABCA1 directly contributes to HDL biogenesis in vivo. *J Clin Invest* **116:** 1052–1062.

Castro GR, Fielding CJ (1988) Early incorporation of cell-derived cholesterol into pre-beta-migrating high-density lipoprotein. *Biochemistry* **27:** 25–29.

Chao WT, Tsai SH, Lin YC, Lin WW, Yang VC (2005) Cellular localization and interaction of ABCA1 and caveolin-1 in aortic endothelial cells after HDL incubation. *Biochem Biophys Res Commun* **332:** 743–749.

Chau P, Nakamura Y, Fielding CJ, Fielding PE (2006) Mechanism of prebeta-HDL formation and activation. *Biochemistry* **45:** 3981–3987.

Chetiveaux M, Ouguerram K, Zair Y, Maugere P, Falconi I, Nazih H, Krempf M (2004) New model for kinetic studies of HDL metabolism in humans. *Eur J Clin Invest* **34:** 262–267.

Chisholm JW, Burleson ER, Shelness GS, Parks JS (2002) ApoA-I secretion from HepG2 cells: evidence for the secretion of both lipid-poor ApoA-I and intracellularly assembled nascent HDL. *J Lipid Res* **43:** 36–44.

Clay MA, Barter PJ (1996) Formation of new HDL particles from lipid-free apolipoprotein A-I. *J Lipid Res* **37:** 1722–1732.

Clay MA, Newnham HH, Barter PJ (1991) Hepatic lipase promotes a loss of apolipoprotein A-I from triglyceride-enriched human high density lipoproteins during incubation *in vitro*. *Arterioscler Thromb* **11:** 415–422.

Clay MA, Newnham HH, Forte TM, Barter PI (1992) Cholesteryl ester transfer protein and hepatic lipase activity promote shedding of apo A-I from HDL and subsequent formation of discoidal HDL. *Biochim Biophys Acta* **1124:** 52–58.

Clay MA, Pyle DH, Rye KA, Barter PJ (2000) Formation of spherical, reconstituted high density lipoproteins containing both apolipoproteins A-I and A-II is mediated by lecithin:cholesterol acyltransferase. *J Biol Chem* **275:** 9019–9025.

Cuchel M, Rader DJ (2006) Macrophage reverse cholesterol transport: key to the regression of atherosclerosis? *Circulation* **113:** 2548–2555.

Daerr WH, Minzlaff U, Greten H (1986) Quantitative determination of apolipoprotein A-I in high-density lipoproteins and 'free' apolipoprotein A-I by two-dimensional agarose gel lipoprotein-'rocket' immunoelectrophoresis of human serum. *Biochim Biophys Acta* **879:** 134–139.

Davidson WS, Sparks DL, Lund-Katz S, Phillips MC (1994) The molecular basis for the difference in charge between pre-beta- and alpha-migrating high density lipoproteins. *J Biol Chem* **269:** 8959–8965.

Davidson WS, Silva RA (2005) Apolipoprotein structural organization in high density lipoproteins: belts, bundles, hinges and hairpins. *Curr Opin Lipidol* **16:** 295–300.

Davidson WS Thompson TB (2007) The structure of apolipoprotein A-I in high density lipoproteins. *J Biol Chem* **282:** 22249–22253.

de la Llera-Moya M, Drazul-Schrader D, Asztalos BF, Cuchel M, Rader DJ, Rothblat GH (2010) The ability to promote efflux *via* ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein cholesterol to remove cholesterol from macrophages. *Arterioscler Thromb Vasc Biol* **30:** 796–801.

Denis M, Haidar B, Marcil M, Bouvier M, Krimbou L, Genest J (2004a) Characterization of oligomeric human ATP binding cassette transporter A1. Potential implications for determining the struc-

ture of nascent high density lipoprotein particles. *J Biol Chem* **279:** 41529–41536.
Denis M, Haidar B, Marcil M, Bouvier M, Krimbou L, Genest JJr (2004b) Molecular and cellular physiology of apolipoprotein A-I lipidation by the ATP-binding cassette transporter A1 (ABCA1). *J Biol Chem* **279:** 7384–7394.
Dullens SP, Plat J, Mensink RP (2007) Increasing ApoA-I production as a target for CHD risk reduction. *Nutr Metab Cardiovasc Dis* **17:** 616–628.
Duong PT, Weibel GL, Lund-Katz S, Rothblat GH, Phillips MC (2008) Characterization and properties of pre beta-HDL particles formed by ABCA1-mediated cellular lipid efflux to apoA-I. *J Lipid Res* **49:** 1006–1014.
Favari E, Bernini F, Tarugi P, Franceschini G, Calabresi L (2002) The C-terminal domain of apolipoprotein A-I is involved in ABCA1-driven phospholipid and cholesterol efflux. *Biochem Biophys Res Commun* **299:** 801–805.
Favari E, Calabresi L, Adorni MP, Jessup W, Simonelli S, Franceschini G, Bernini F (2009) Small discoidal pre-beta1 HDL particles are efficient acceptors of cell cholesterol *via* ABCA1 and ABCG1. *Biochemistry* **48:** 11067–11074.
Fielding CJ, Fielding PE (2000) Cholesterol and caveolae: structural and functional relationships. *Biochim Biophys Acta* **1529:** 210–222.
Fielding CJ, Shore VG, Fielding PE (1972) A protein cofactor of lecithin:cholesterol acyltransferase. *Biochem Biophys Res Commun* **46:** 1493–1498.
Fielding PE, Nagao K, Hakamata H, Chimini G, Fielding CJ (2000) A two-step mechanism for free cholesterol and phospholipid efflux from human vascular cells to apolipoprotein A-1. *Biochemistry* **39:** 14113–14120.
Fitzgerald ML, Morris AL, Rhee JS, Andersson LP, Mendez AJ, Freeman MW (2002) Naturally occurring mutations in the largest extracellular loops of ABCA1 can disrupt its direct interaction with apolipoprotein A-I. *J Biol Chem* **277:** 33178–33187.
Fitzgerald ML, Morris AL, Chroni A, Mendez AJ, Zannis VI, Freeman MW (2004) ABCA1 and amphipathic apolipoproteins form high-affinity molecular complexes required for cholesterol efflux. *J Lipid Res* **45:** 287–294.
Forte TM, Bielicki JK, Goth-Goldstein R, Selmek J, McCall MR (1995) Recruitment of cell phospholipids and cholesterol by apolipoproteins A-II and A-I: formation of nascent apolipoprotein-specific HDL that differ in size, phospholipid composition, and reactivity with LCAT. *J Lipid Res* **36:** 148–157.
Fournier N, Cogny A, Atger V, Pastier D, Goudouneche D, Nicoletti A, Moatti N, Chambaz J, Paul JL, Kalopissis AD (2002) Opposite effects of plasma from human apolipoprotein A-II transgenic mice on cholesterol efflux from J774 macrophages and Fu5AH hepatoma cells. *Arterioscler Thromb Vasc Biol* **22:** 638–643.
Francis GA, Knopp RH, Oram JF (1995) Defective removal of cellular cholesterol and phospholipids by apolipoprotein A-I in Tangier disease. *J Clin Invest* **96:** 78–87.
Francone OL, Gurakar A, Fielding C (1989) Distribution and functions of lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein in plasma lipoproteins. Evidence for a functional unit containing these activities together with apolipoproteins A-I and D that catalyzes the esterification and transfer of cell-derived cholesterol. *J Biol Chem* **264:** 7066–7072.
Francone OL, Royer L, Haghpassand M (1996) Increased prebeta-HDL levels, cholesterol efflux, and LCAT-mediated esterification in mice expressing the human cholesteryl ester transfer protein (CETP) and human apolipoprotein A-I (apoA-I) transgenes. *J Lipid Res* **37:** 1268–1277.
Frank PG, Marcel YL (2000) Apolipoprotein A-I: structure-function relationships. *J Lipid Res* **41:** 853–872.
Fredrickson DS (1964) The inheritance of high density lipoprotein deficiency (Tangier disease). *J Clin Invest* **43:** 228–236.
Gao X, Yuan S, Jayaraman S, Gursky O (2009) Differential stability of high-density lipoprotein subclasses: effects of particle size and protein composition. *J Mol Biol* **387:** 628–638.
Gaus K, Kritharides L, Schmitz G, Boettcher A, Drobnik W, Langmann T, Quinn CM, Death A, Dean RT, Jessup W (2004) Apolipoprotein A-1 interaction with plasma membrane lipid rafts controls cholesterol export from macrophages. *FASEB J* **18:** 574–576.
Gelissen IC, Harris M, Rye KA, Quinn C, Brown AJ, Kockx M, Cartland S, Packianathan M, Kritharides L, Jessup W (2006) ABCA1 and ABCG1 synergize to mediate cholesterol export to apoA-I. *Arterioscler Thromb Vasc Biol* **26:** 534–540.
Gillard BK, Lin HY, Massey JB, Pownall HJ (2009) Apolipoproteins A-I, A-II and E are independently distributed among intracellular and newly secreted HDL of human hepatoma cells. *Biochim Biophys Acta* **1791:** 1125–1132.
Gillotte KL, Davidson WS, Lund-Katz S, Rothblat GH, Phillips MC (1998) Removal of cellular cholesterol by pre-beta-HDL involves plasma membrane microsolubilization. *J Lipid Res* **39:** 1918–1928.
Gonzalez MC, Toledo JD, Tricerri MA, Garda HA (2008) The central type Y amphipathic alpha-helices of apolipoprotein AI are involved in the mobilization of intracellular cholesterol depots. *Arch Biochem Biophys* **473:** 34–41.
Gotto AM Jr (2001) Low high-density lipoprotein cholesterol as a risk factor in coronary heart disease: a working group report. *Circulation* **103:** 2213–2218.
Gotto AM Jr, Brinton EA (2004) Assessing low levels of high-density lipoprotein cholesterol as a risk factor in coronary heart disease: a working group report and update. *J Am Coll Cardiol* **43:** 717–724.
Guerra R, Wang J, Grundy SM, Cohen JC (1997) A hepatic lipase (LIPC) allele associated with high plasma concentrations of high density lipoprotein cholesterol. *Proc Natl Acad Sci USA* **94:** 4532–4537.
Hamilton RL, Williams MC, Fielding CJ, Havel RJ (1976) Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J Clin Invest* **58:** 667–680.
Hara H, Yokoyama S (1991) Interaction of free apolipoproteins with macrophages. Formation of high density lipoprotein-like lipoproteins and reduction of cellular cholesterol. *J Biol Chem* **266:** 3080–3086.
Hassan HH, Denis M, Lee DY, Iatan I, Nyholt D, Ruel I, Krimbou L, Genest J (2007) Identification of an ABCA1-dependent phospholipid-rich plasma membrane apolipoprotein A-I binding site for nascent HDL formation: implications for current models of HDL biogenesis. *J Lipid Res* **48:** 2428–2442.
Havel RJ, Eder HA, Bragdon JH (1955) The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* **34:** 1345–1353.
Hime NJ, Drew KJ, Wee K, Barter PJ, Rye KA (2006) Formation of high density lipoproteins containing both apolipoprotein A-I and A-II in the rabbit. *J Lipid Res* **47:** 115–122.
Hirano K, Yamashita S, Nakajima N, Arai T, Maruyama T, Yoshida Y, Ishigami M, Sakai N, Kameda-Takemura K, Matsuzawa Y (1997) Genetic cholesteryl ester transfer protein deficiency is extremely frequent in the Omagari area of Japan. Marked hyperalphalipoproteinemia caused by CETP gene mutation is not associated with longevity. *Arterioscler Thromb Vasc Biol* **17:** 1053–1059.
Hodoglugil U, Williamson DW, Mahley RW (2010) Polymorphisms in the hepatic lipase gene affect plasma HDL-cholesterol levels in a Turkish population. *J Lipid Res* **51:** 422–430.
Hopkins GJ, Barter PJ (1986) Role of triglyceride-rich lipoproteins and hepatic lipase in determining the particle size and composition of high density lipoproteins. *J Lipid Res* **27:** 1265–1277.
Horowitz BS, Goldberg IJ, Merab J, Vanni TM, Ramakrishnan R, Ginsberg HN (1993) Increased plasma and renal clearance of an exchangeable pool of apolipoprotein A-I in subjects with low levels of high density lipoprotein cholesterol. *J Clin Invest* **91:** 1743–1752.
Hu YW, Zheng L, Wang Q (2010) Characteristics of apolipoprotein M and its relation to atherosclerosis and diabetes. *Biochim Biophys Acta* **1801:** 100–105.
Ishida BY, Frolich J, Fielding CJ (1987) Prebeta-migrating high density lipoprotein: quantitation in normal and hyperlipidemic plasma by solid phase radioimmunoassay following electrophoretic transfer. *J Lipid Res* **28:** 778–786.
Jauhiainen M, Metso J, Pahlman R, Blomqvist S, van Tol A, Ehnholm C (1993) Human plasma phospholipid transfer protein causes high density lipoprotein conversion. *J Biol Chem* **268:** 4032–4036.
Jiang XC, Bruce C, Mar J, Lin M, Ji Y, Francone OL, Tall AR (1999) Targeted mutation of plasma phospholipid transfer protein gene markedly reduces high-density lipoprotein levels. *J Clin Invest* **103:** 907–914.
Jonas A (1986) Synthetic substrates of lecithin: cholesterol acyltransferase. *J Lipid Res* **27:** 689–698.
Jonas A (2000) Lecithin cholesterol acyltransferase. *Biochim Biophys Acta* **1529:** 245–256.
Jonas A, Kezdy KE, Wald JH (1989) Defined apolipoprotein A-I conformations in reconstituted high density lipoprotein discs. *J Biol Chem* **264:** 4818–4824.
Jonas A, Wald JH, Toohill KL, Krul ES, Kezdy KE (1990) Apolipoprotein A-I structure and lipid properties in homogeneous, reconstituted spherical and discoidal high density lipoproteins. *J Biol Chem* **265:** 22123–22129.
Kawano M, Miida T, Fielding CJ, Fielding PE (1993) Quantitation of pre beta-HDL-dependent and nonspecific components of the total efflux of cellular cholesterol and phospholipid. *Biochemistry* **32:** 5025–5028.
Kennedy MA, Barrera GC, Nakamura K, Baldan A, Tarr P, Fishbein MC, Frank J, Francone OL, Edwards PA (2005) ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. *Cell Metab* **1:** 121–131.
Kielar D, Dietmaier W, Langmann T, Aslanidis C, Probst M, Naruszewicz M, Schmitz G (2001) Rapid quantification of human ABCA1 mRNA in various cell types and tissues by real-time reverse transcription-PCR. *Clin Chem* **47:** 2089–2097.
Kiss RS, McManus DC, Franklin V, Tan WL, McKenzie A, Chimini G, Marcel YL (2003) The lipidation by hepatocytes of human apoli-

poprotein A-I occurs by both ABCA1-dependent and -independent pathways. *J Biol Chem* **278:** 10119–10127.
Klucken J, Buchler C, Orso E, Kaminski WE, Porsch-Ozcurumez M, Liebisch G, Kapinsky M, Diederich W, Drobnik W, Dean M, Allikmets R, Schmitz G (2000) ABCG1 (ABC8), the human homolog of the *Drosophila* white gene, is a regulator of macrophage cholesterol and phospholipid transport. *Proc Natl Acad Sci USA* **97:** 817–822.
Kosek AB, Durbin D, Jonas A (1999) Binding affinity and reactivity of lecithin cholesterol acyltransferase with native lipoproteins. *Biochem Biophys Res Commun* **258:** 548–551.
Krimbou L, Marcil M, Chiba H, Genest J Jr (2003) Structural and functional properties of human plasma high density-sized lipoprotein containing only apoE particles. *J Lipid Res* **44:** 884–892.
Krimbou L, Hajj Hassan H, Blain S, Rashid S, Denis M, Marcil M, Genest J (2005) Biogenesis and speciation of nascent apoA-I-containing particles in various cell lines. *J Lipid Res* **46:** 1668–1677.
Kuivenhoven JA, Pritchard H, Hill J, Frohlich J, Assmann G, Kastelein J (1997) The molecular pathology of lecithin:cholesterol acyltransferase (LCAT) deficiency syndromes. *J Lipid Res* **38:** 191–205.
Kunitake ST, La Sala KJ, Kane JP (1985) Apolipoprotein A-I-containing lipoproteins with pre-beta electrophoretic mobility. *J Lipid Res* **26:** 549–555.
Kunitake ST, Mendel CM, Hennessy LK (1992) Interconversion between apolipoprotein A-I-containing lipoproteins of pre-beta and alpha electrophoretic mobilities. *J Lipid Res* **33:** 1807–1816.
Lamarche B, Uffelman KD, Carpentier A, Cohn JS, Steiner G, Barrett PH, Lewis GF (1999) Triglyceride enrichment of HDL enhances in vivo metabolic clearance of HDL apo A-I in healthy men. *J Clin Invest* **103:** 1191–1199.
Lee JY, Timmins JM, Mulya A, Smith TL, Zhu Y, Rubin EM, Chisholm JW, Colvin PL, Parks JS (2005) HDLs in ApoA-I transgenic Abca1 knockout mice are remodeled normally in plasma but are hypercatabolized by the kidney. *J Lipid Res* **46:** 2233–2245.
Liang HQ, Rye KA, Barter PJ (1994) Dissociation of lipid-free apolipoprotein A-I from high density lipoproteins. *J Lipid Res* **35:** 1187–1199.
Liang HQ, Rye KA, Barter PJ (1995) Cycling of apolipoprotein A-I between lipid-associated and lipid-free pools. *Biochim Biophys Acta* **1257:** 31–37.
Liang HQ, Rye KA, Barter PJ (1996) Remodelling of reconstituted high density lipoproteins by lecithin : cholesterol acyltransferase. *J Lipid Res* **37:** 1962–1970.
Lindgren FT, Elliott HA, Gofman JW (1951) The ultracentrifugal characterization and isolation of human blood lipids and lipoproteins, with applications to the study of atherosclerosis. *J Phys Colloid Chem* **55:** 80–93.
Liu L, Bortnick AE, Nickel M, Dhanasekaran P, Subbaiah PV, Lund-Katz S, Rothblat GH, Phillips MC (2003) Effects of apolipoprotein A-I on ATP-binding cassette transporter A1-mediated efflux of macrophage phospholipid and cholesterol: formation of nascent high density lipoprotein particles. *J Biol Chem* **278:** 42976–42984.
Luo G, Zhang X, Nilsson-Ehle P, Xu N (2004) Apolipoprotein M. *Lipids Health Dis* **3:** 21.
Lusa S, Jauhiainen M, Metso J, Somerharju P, Ehnholm C (1996) The mechanism of human plasma phospholipid transfer protein-induced enlargement of high-density lipoprotein particles: evidence for particle fusion. *Biochem J* **313** (Part 1)**:** 275–282.
Marcel YL, Kiss RS (2003) Structure-function relationships of apolipoprotein A-I: a flexible protein with dynamic lipid associations. *Curr Opin Lipidol* **14:** 151–157.
Maric J, Kiss RS, Franklin V, Marcel YL (2005) Intracellular lipidation of newly synthesized apolipoprotein A-I in primary murine hepatocytes. *J Biol Chem* **280:** 39942–39949.
Marques-Vidal P, Jauhiainen M, Metso J, Ehnholm C (1997) Transformation of high density lipoprotein 2 particles by hepatic lipase and phospholipid transfer protein. *Atherosclerosis* **133:** 87–95.
Martin DD, Budamagunta MS, Ryan RO, Voss JC, Oda MN (2006) Apolipoprotein A-I assumes a "looped belt" conformation on reconstituted high density lipoprotein. *J Biol Chem* **281:** 20418–20426.
Martinez LO, Agerholm-Larsen B, Wang N, Chen W, Tall AR (2003) Phosphorylation of a pest sequence in ABCA1 promotes calpain degradation and is reversed by ApoA-I. *J Biol Chem* **278:** 37368–37374.
Masson D, Jiang XC, Lagrost L, Tall AR (2009) The role of plasma lipid transfer proteins in lipoprotein metabolism and atherogenesis. *J Lipid Res* **50** (Suppl): S201–6.
McCall MR, Forte TM, Shore VG (1988) Heterogeneity of nascent high density lipoproteins secreted by the hepatoma-derived cell line, Hep G2. *J Lipid Res* **29:** 1127–1137.
McIntyre N (1988) Familial LCAT deficiency and fish-eye disease. *J Inherit Metab Dis* **11** (Suppl 1)**:** 45–56.
Mendez AJ, Lin G, Wade DP, Lawn RM, Oram JF (2001) Membrane lipid domains distinct from cholesterol/sphingomyelin-rich rafts are involved in the ABCA1-mediated lipid secretory pathway. *J Biol Chem* **276:** 3158–3166.
Mulya A, Lee JY, Gebre AK, Thomas MJ, Colvin PL, Parks JS (2007) Minimal lipidation of pre-beta HDL by ABCA1 results in reduced ability to interact with ABCA1. *Arterioscler Thromb Vasc Biol* **27:** 1828–1836.
Mulya A, Seo J, Brown AL, Gebre AK, Boudyguina E, Shelness GS, Parks JS (2010) Apolipoprotein M expression increases the size of nascent pre beta HDL formed by ATP binding cassette transporter A1. *J Lipid Res* **51:** 514–524.
Murtomaki S, Tahvanainen E, Antikainen M, Tiret L, Nicaud V, Jansen H, Ehnholm C (1997) Hepatic lipase gene polymorphisms influence plasma HDL levels. Results from Finnish EARS participants. European Atherosclerosis Research Study. *Arterioscler Thromb Vasc Biol* **17:** 1879–1884.
Nakamura Y, Kotite L, Gan Y, Spencer TA, Fielding CJ, Fielding PE (2004) Molecular mechanism of reverse cholesterol transport: reaction of pre-beta-migrating high-density lipoprotein with plasma lecithin/cholesterol acyltransferase. *Biochemistry* **43:** 14811–14820.
Neary RH, Gowland E (1987) Stability of free apolipoprotein A-1 concentration in serum, and its measurement in normal and hyperlipidemic subjects. *Clin Chem* **33:** 1163–1169.
Neary R, Bhatnagar D, Durrington P, Ishola M, Arrol S, Mackness M (1991) An investigation of the role of lecithin:cholesterol acyltransferase and triglyceride-rich lipoproteins in the metabolism of pre-beta high density lipoproteins. *Atherosclerosis* **89:** 35–48.
Neufeld EB, Remaley AT, Demosky SJ, Stonik JA, Cooney AM, Comly M, Dwyer NK, Zhang M, Blanchette-Mackie J, Santamarina-Fojo S, Brewer HB Jr (2001) Cellular localization and trafficking of the human ABCA1 transporter. *J Biol Chem* **276:** 27584–27590.
Neufeld EB, Stonik JA, Demosky SJ Jr, Knapper CL, Combs CA, Cooney A, Comly M, Dwyer N, Blanchette-Mackie J, Remaley AT, Santamarina-Fojo S, Brewer HB Jr (2004) The ABCA1 transporter modulates late endocytic trafficking: insights from the correction of the genetic defect in Tangier disease. *J Biol Chem* **279:** 15571–15578.
Nielsen LB, Christoffersen C, Ahnstrom J, Dahlback B (2009) ApoM: gene regulation and effects on HDL metabolism. *Trends Endocrinol Metab* **20:** 66–71.
Nolte RT, Atkinson D (1992) Conformational analysis of apolipoprotein A-I and E-3 based on primary sequence and circular dichroism. *Biophys J* **63:** 1221–1239.
O'Connor PM, Zysow BR, Schoenhaus SA, Ishida BY, Kunitake ST, Naya-Vigne JM, Duchateau PN, Redberg RF, Spencer SJ, Mark S, Mazur M, Heilbron DC, Jaffe RB, Malloy MJ, Kane JP (1998) Pre-beta-1 HDL in plasma of normolipidemic individuals: influences of plasma lipoproteins, age, and gender. *J Lipid Res* **39:** 670–678.
Okuhira K, Tsujita M, Yamauchi Y, Abe-Dohmae S, Kato K, Handa T, Yokoyama S (2004) Potential involvement of dissociated ApoA-I in the ABCA1-dependent cellular lipid release by HDL. *J Lipid Res* **45:** 645–652.
Oram JF, Heinecke JW (2005) ATP-binding cassette transporter A1: a cell cholesterol exporter that protects against cardiovascular disease. *Physiol Rev* **85:** 1343–1372.
Oram JF, Albers JJ, Cheung MC, Bierman EL (1981) The effects of subfractions of high density lipoprotein on cholesterol efflux from cultured fibroblasts. Regulation of low density lipoprotein receptor activity. *J Biol Chem* **256:** 8348–8356.
Oram JF, Lawn RM, Garvin MR, Wade DP (2000) ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. *J Biol Chem* **275:** 34508–34511.
Patsch JR, Prasad S, Gotto AM Jr, Patsch W (1987) High density lipoprotein2. Relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. *J Clin Invest* **80:** 341–347.
Phillips JC, Wriggers W, Li Z, Jonas A, Schulten K (1997) Predicting the structure of apolipoprotein A-I in reconstituted high-density lipoprotein disks. *Biophys J* **73:** 2337–2346.
Pownall HJ, Hosken BD, Gillard BK, Higgins CL, Lin HY, Massey JB (2007) Speciation of human plasma high-density lipoprotein (HDL): HDL stability and apolipoprotein A-I partitioning. *Biochemistry* **46:** 7449–7459.
Rader DJ, Ikewaki K, Duverger N, Schmidt H, Pritchard H, Frohlich J, Clerc M, Dumon MF, Fairwell T, Zech L (1994) Markedly accelerated catabolism of apolipoprotein A-II (ApoA-II) and high density lipoproteins containing ApoA-II in classic lecithin: cholesterol acyltransferase deficiency and fish-eye disease. *J Clin Invest* **93:** 321–330.
Rashid S, Marcil M, Ruel I, Genest J (2009) Identification of a novel human cellular HDL biosynthesis defect. *Eur Heart J* **30:** 2204–2212.
Remaley AT, Thomas F, Stonik JA, Demosky SJ, Bark SE, Neufeld EB, Bocharov AV, Vishnyakova TG, Patterson AP, Eggerman TL, Santamarina-Fojo S, Brewer HB (2003) Synthetic amphipathic helical peptides promote lipid efflux from cells by an ABCA1-dependent and an ABCA1-independent pathway. *J Lipid Res* **44:** 828–836.
Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, Deleuze JF, Brewer HB, Duverger N, Denefle P, Assmann G (1999) Tangier

disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nat Genet* **22:** 352–355.
Rye KA, Barter PJ (2004) Formation and metabolism of prebeta-migrating, lipid-poor apolipoprotein A-I. *Arterioscler Thromb Vasc Biol* **24:** 421–428.
Rye KA, Hime NJ, Barter PJ (1997) Evidence that cholesteryl ester transfer protein-mediated reductions in reconstituted high density lipoprotein size involve particle fusion. *J Biol Chem* **272:** 3953–3960.
Rye KA, Wee K, Curtiss LK, Bonnet DJ, Barter PJ (2003) Apolipoprotein A-II inhibits high density lipoprotein remodeling and lipid-poor apolipoprotein A-I formation. *J Biol Chem* **278:** 22530–22536.
Saito H, Dhanasekaran P, Nguyen D, Holvoet P, Lund-Katz S, Phillips MC (2003) Domain structure and lipid interaction in human apolipoproteins A-I and E, a general model. *J Biol Chem* **278:** 23227–23232.
Saito H, Lund-Katz S, Phillips MC (2004) Contributions of domain structure and lipid interaction to the functionality of exchangeable human apolipoproteins. *Prog Lipid Res* **43:** 350–380.
Sankaranarayanan S, Oram JF, Asztalos BF, Vaughan AM, Lund-Katz S, Adorni MP, Phillips MC, Rothblat GH (2009) Effects of acceptor composition and mechanism of ABCG1-mediated cellular free cholesterol efflux. *J Lipid Res* **50:** 275–284.
Segrest JP, Jones MK, De Loof H, Brouillette CG, Venkatachalapathi YV, Anantharamaiah GM (1992) The amphipathic helix in the exchangeable apolipoproteins: a review of secondary structure and function. *J Lipid Res* **33:** 141–166.
Segrest JP, Jones MK, Klon AE, Sheldahl CJ, Hellinger M, De Loof H, Harvey SC (1999) A detailed molecular belt model for apolipoprotein A-I in discoidal high density lipoprotein. *J Biol Chem* **274:** 31755–31758.
Shah PK (2007) High-density lipoprotein mimetics: focus on synthetic high-density lipoprotein. *Am J Cardiol* **100:** S62–S67.
Shih AY, Sligar SG, Schulten K (2009) Maturation of high-density lipoproteins. *J R Soc Interface* **6:** 863–871.
Silva RA, Schneeweis LA, Krishnan SC, Zhang X, Axelsen PH, Davidson WS (2007) The structure of apolipoprotein A-II in discoidal high density lipoproteins. *J Biol Chem* **282:** 9713–9721.
Sparks DL, Frank PG, Braschi S, Neville TA, Marcel YL (1999) Effect of apolipoprotein A-I lipidation on the formation and function of pre-beta and alpha-migrating LpA-I particles. *Biochemistry* **38:** 1727–1735.
Takahashi Y, Smith JD (1999) Cholesterol efflux to apolipoprotein AI involves endocytosis and resecretion in a calcium-dependent pathway. *Proc Natl Acad Sci USA* **96:** 11358–11363.
Tall AR, Green PH (1981) Incorporation of phosphatidylcholine into spherical and discoidal lipoproteins during incubation of egg phosphatidylcholine vesicles with isolated high density lipoproteins or with plasma. *J Biol Chem* **256:** 2035–2044.
Tall AR, Krumholz S, Olivecrona T, Deckelbaum RJ (1985) Plasma phospholipid transfer protein enhances transfer and exchange of phospholipids between very low density lipoproteins and high density lipoproteins during lipolysis. *J Lipid Res* **26:** 842–851.
Tang C, Vaughan AM, Anantharamaiah GM, Oram JF (2006) Janus kinase 2 modulates the lipid-removing but not protein-stabilizing interactions of amphipathic helices with ABCA1. *J Lipid Res* **47:** 107–114.
Thomas MJ, Bhat S, Sorci-Thomas MG (2008) Three-dimensional models of HDL apoA-I: implications for its assembly and function. *J Lipid Res* **49:** 1875–1883.
Thuren T (2000) Hepatic lipase and HDL metabolism. *Curr Opin Lipidol* **11:** 277–283.
Timmins JM, Lee JY, Boudyguina E, Kluckman KD, Brunham LR, Mulya A, Gebre AK, Coutinho JM, Colvin PL, Smith TL, Hayden MR, Maeda N, Parks JS (2005) Targeted inactivation of hepatic ABCA1 causes profound hypoalphalipoproteinemia and kidney hypercatabolism of apoA-I. *J Clin Invest* **115:** 1333–1342.
van Tornout P, Caster H, Lievens MJ, Rosseneu M, Assmann G (1981) In vitro interaction of human HDL with human apolipoprotein A-II. Synthesis of apolipoprotein A-II-rich HDL. *Biochim Biophys Acta* **663:** 630–636.
Vaughan AM, Oram JF (2005) ABCG1 redistributes cell cholesterol to domains removable by high density lipoprotein but not by lipid-depleted apolipoproteins. *J Biol Chem* **280:** 30150–30157.
Vedhachalam C, Duong PT, Nickel M, Nguyen D, Dhanasekaran P, Saito H, Rothblat GH, Lund-Katz S, Phillips MC (2007a) Mechanism of ATP-binding cassette transporter A1-mediated cellular lipid efflux to apolipoprotein A-I and formation of high density lipoprotein particles. *J Biol Chem* **282:** 25123–25130.
Vedhachalam C, Ghering AB, Davidson WS, Lund-Katz S, Rothblat GH, Phillips MC (2007b) ABCA1-induced cell surface binding sites for ApoA-I. *Arterioscler Thromb Vasc Biol* **27:** 1603–1609.
von Eckardstein A, Jauhiainen M, Huang Y, Metso J, Langer C, Pussinen P, Wu S, Ehnholm C, Assmann G (1996) Phospholipid transfer protein mediated conversion of high density lipoproteins generates pre beta 1-HDL. *Biochim Biophys Acta* **1301:** 255–262.
Wang N, Silver DL, Thiele C, Tall AR (2001) ATP-binding cassette transporter A1 (ABCA1) functions as a cholesterol efflux regulatory protein. *J Biol Chem* **276:** 23742–23747.
Wang N, Chen W, Linsel-Nitschke P, Martinez LO, Agerholm-Larsen B, Silver DL, Tall AR (2003) A PEST sequence in ABCA1 regulates degradation by calpain protease and stabilization of ABCA1 by apoA-I. *J Clin Invest* **111:** 99–107.
Wang N, Lan D, Chen W, Matsuura F, Tall AR (2004) ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. *Proc Natl Acad Sci USA* **101:** 9774–9779.
Wilson PW, Abbott RD, Castelli WP (1988) High density lipoprotein cholesterol and mortality. The Framingham Heart Study. *Arteriosclerosis* **8:** 737–741.
Wolfrum C, Poy MN, Stoffel M (2005) Apolipoprotein M is required for prebeta-HDL formation and cholesterol efflux to HDL and protects against atherosclerosis. *Nat Med* **11:** 418–422.
Wróblewska M, Kortas-Stempak B, Szutowicz A, Badzio T (2009) Phospholipids mediated conversion of HDLs generates specific apoA-II pre-beta mobility particles. *J Lipid Res* **50:** 667–675.
Wróblewska M, Czyżewska M, Wolska A, Kortas-Stempak B, Szutowicz A (2010) Apo A-II participates in HDL-liposome interaction by the formation of new pre-beta mobility particles and the modification of liposomes. *Biochim Biophys Acta* **1801:** 1323–1329.
Wu AL, Windmueller HG (1979) Relative contributions by liver and intestine to individual plasma apolipoproteins in the rat. *J Biol Chem* **254:** 7316–7322.
Wu Z, Wagner MA, Zheng L, Parks JS, Shy JM 3rd, Smith JD, Gogonea V, Hazen SL (2007) The refined structure of nascent HDL reveals a key functional domain for particle maturation and dysfunction. *Nat Struct Mol Biol* **14:** 861–868.
Xu N, Dahlback B (1999) A novel human apolipoprotein (apoM). *J Biol Chem* **274:** 31286–31290.
Xu Y, Fu M (2003) Alterations of HDL subclasses in hyperlipidemia. *Clin Chim Acta* **332:** 95–102.
Zarubica A, Plazzo AP, Stockl M, Trombik T, Hamon Y, Muller P, Pomorski T, Herrmann A, Chimini G (2009) Functional implications of the influence of ABCA1 on lipid microenvironment at the plasma membrane: a biophysical study. *FASEB J* **23:** 1775–1785.