

Apolipoproteins A-I and B: biosynthesis, role in the development of atherosclerosis and targets for intervention against cardiovascular disease

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Abstract: Apolipoprotein (apo) AI and apoB are the major apolipoproteins of high-density lipoprotein (HDL) and low-density lipoprotein (LDL), respectively. ApoB assembles the precursor of LDL, very-low-density lipoprotein (VLDL), in the liver. The assembly starts with the formation of a primordial particle, which is converted to VLDL2. The VLDL2 particle is then transferred to the Golgi apparatus and can either be secreted or converted to triglyceride-rich VLDL1. We have reviewed this assembly process, the process involved in the storage of triglycerides in cytosolic lipid droplets, and the relationship between these two processes. We also briefly discuss the formation of HDL. ApoB mediates the interaction between LDL and the arterial wall. Two regions in apoB are involved in this binding. This interaction and its role in the development of atherosclerosis are reviewed. ApoB can be used to measure the number of LDL or VLDL particles present in plasma, as there is one molecule of apoB on each particle. By contrast, the amount of cholesterol and other lipids on each particle varies under different conditions. We address the possibility of using apoAI and apoB levels to estimate the risk of development of cardiovascular diseases and to monitor intervention to treat these diseases.

Keywords: Apolipoprotein AI, atherosclerosis, cardiovascular disease

Introduction

Cardiovascular diseases are increasing in prevalence and represent a major public health burden of the 21st century. Atherosclerosis is the major cause of these diseases and several risk factors have been identified that promote the development of premature atherosclerosis. In a large, international, case-control study (INTERHEART), abnormal plasma lipoproteins have been shown to be a major risk factor for myocardial infarction (Yusuf et al 2004).

Several types of lipoprotein have been identified in plasma. They are usually separated according to their hydrated density into high-density lipoprotein (HDL) 2 and 3, low-density lipoprotein (LDL), intermediate-density lipoprotein, very-low-density lipoprotein (VLDL) 1 and 2, and chylomicrons (the plasma lipoproteins and their composition are reviewed in most modern biochemistry textbooks). VLDL is secreted from the liver and is the precursor of LDL. The conversion of VLDL to LDL is a complex process that has been modeled in vivo in humans both under normal conditions and in diseases (reviewed in (Adiels et al 2006)).

Apolipoproteins are the protein components of plasma lipoproteins and several different apolipoproteins have been identified. The major apolipoprotein of LDL is apoB100, which is synthesized in the liver and enters the plasma with VLDL. There is one apoB100 molecule on each LDL particle, and it is used to estimate the number of LDL and VLDL particles present in plasma. In this aspect, apoB100 is superior to LDL cholesterol or non-HDL cholesterol as the cholesterol content on the LDL

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and VLDL particles can vary considerably. The two major proteins in HDL are apoAI and apoAII. Of these, apoAI has most frequently been used to estimate the HDL levels but, in contrast to apoB100, it exchanges between lipoproteins and the number of molecules varies between particles.

In this article, we have reviewed how apoB100 assembles VLDL in the liver and how the protein interacts with the arterial wall during the development of atherosclerosis. We also give a brief summary on the assembly of HDL. Moreover, we discuss the possibility of using apoB and apoAI to predict the risk of cardiovascular diseases and to monitor the effect of intervention to treat such diseases.

Composition of VLDL

VLDL is assembled in and secreted from the liver. There are two forms of VLDL: VLDL1, which is large and rich in triglycerides, and VLDL2, which is smaller and contains less triglycerides. The type of particle that is secreted is highly important for the atherogenicity of the plasma lipoproteins. Secretion of VLDL1 increases the levels of so-called small dense LDL and decreases the levels of HDL, and both of these changes have been shown to promote the development of atherosclerosis (for review, see (Taskinen 2003)). Overproduction of VLDL1 is observed in patients with insulin resistance and type 2 diabetes (Taskinen 2003).

As for all lipoproteins, VLDL consists of a core of neutral lipids (triglycerides and cholesterol esters) surrounded by a monolayer of amphipathic structures such as phospholipids, free cholesterol, and proteins. ApoB100 is a large (4536 amino acid long) amphipathic protein that is present on VLDL (Segrest et al 2001). It has a pentapartite structure consisting of a globular N-terminal domain, two domains of amphipathic β -sheets, and two domains of amphipathic α -helices, one between the two β -sheet domains and one near the C-terminus (Segrest et al 2001). The N-terminal domain is of vital importance for the formation of VLDL as it interacts with the microsomal triglyceride transfer protein (MTP), which catalyzes the transfer of lipids to apoB during the formation of lipoproteins (Dashti et al 2002). The amphipathic β -sheet domains consist of antiparallel β -sheets with a width of approximately 30 Å. They form very strong lipid-binding structures (Segrest et al 2001), which is generally thought to explain why apoB remains associated with the original core structure rather than equilibrating between different lipoproteins.

The three-dimensional structure of apoB is not known in detail, but the overall organization on the LDL has been elucidated using immuno-electronmicroscopy (Chatterton

et al 1995). The protein has an elongated structure that encircles the entire particle (Figure 1). The C-terminus folds back over the preceding structure; an arginine (residue 3500) binds to a tryptophan (residue 4396), which prevents the C-terminus from sliding over the binding site for the LDL receptor between residues 3359 and 3369 (Figure 1). Mutation of the arginine (residue 3500) results in reduced binding to the LDL receptor because the arginine–tryptophan interaction is broken (Boren et al 2001a; Boren et al 1998a).

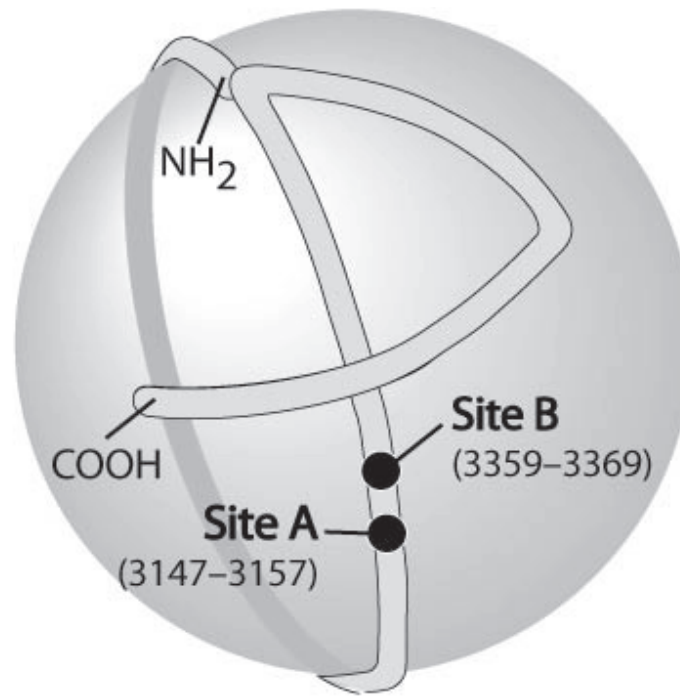
In several species, an additional apoB, apoB48 is also expressed in the liver, where it forms VLDL of the same type as that formed by apoB100. In humans, apoB48 is formed in the intestine and assembles chylomicrons.

The assembly of VLDL

ApoB100 is a secretory protein and is synthesized on ribosomes attached to the surface of the endoplasmic reticulum (Figure 2). During its formation, the “nascent” polypeptide is translocated through a channel from the site of synthesis in the ribosome through the membrane to the lumen of the endoplasmic reticulum (ER). Secretory proteins acquire their tertiary structure in the ER by a folding process that depends on so-called chaperone proteins. Correctly folded proteins are sorted into exit sites to leave the ER by transport vesicles. If the correct tertiary structure is not achieved, the protein is retained in the ER and retracted through the membrane channel and sorted to proteasomal degradation (Ellgaard and Helenius 2001; Ellgaard and Helenius 2003; Ellgaard et al 1999; Johnson and Haigh 2000; Johnson and van Waes 1999; Kostova and Wolf 2003; Lippincott-Schwartz et al 2000).

The formation of vesicles at the exit site depends on the GTPase SAR1 and the coat protein coatamere protein II (COPII). These vesicles carry the secretory products out of the ER and form the ER-Golgi intermediate compartment (ERGIC), an organelle that is involved in sorting of proteins. Thus, proteins that should remain in the ER are returned to this organelle from ERGIC. The sorting process involves the formation of vesicles by the GTPase ARF1 and COPI. ERGIC is transported to the cis-Golgi and fuses to continuously form this compartment. ARF1 is of importance for the ability of ERGIC to mature into cis-Golgi (Nickel et al 1998; Nickel and Wieland 1998; Spang 2002) (Figure 2).

VLDL is formed by a stepwise lipidation of apoB100 occurring as it is transferred through the secretory pathway (Figure 2). The process starts during the entry of the N-terminus of apoB100 in the lumen of the ER by an MTP-dependent addition of lipid to the growing apoB protein (Figure 2). The C-terminal portion of apoB100 breaks this lipidation



Site A	Lys	Ala	Gln	Tyr	Lys	Lys	Asn	Lys	His	Arg	His
Site B	Arg	Leu	Thr	Arg	Lys	Arg	Gly	Leu	Lys	Leu	Ala

Figure 1 The organization of apoB100 on the LDL particle. Two of the sites (A and B) involved in the binding of apoB100 (and LDL) to proteoglycans are indicated in the figure and their primary sequence is given below. Site B is also the binding site for the LDL receptor.

(Stillemark-Billton et al 2005). An underlipidated primordial particle (pre-VLDL) is thus formed that interacts with ER chaperones and is either retained in the cell (and subsequently degraded) or is further lipidated to form VLDL2. We propose that this reflects a need to form a VLDL particle that is large enough to allow apoB to fold correctly in its surface.

The ability of apoB100 to form pre-VLDL is dependent on the sequence between residues 3265 and 4082 (Stillemark-Billton et al 2005), which introduces a shift in lipidation of the protein. This sequence is capable of temporarily switching off the lipidation awaiting, for example, the availability of MTP and/or lipids. It is possible that this switch is involved in the lipid-dependent sorting of apoB100 to secretion or degradation. Moreover, it seems that the effect of this sequence is to promote the binding of chaperones to pre-VLDL (Stillemark-Billton et al 2005), which are most likely involved in the retention of the particle in the cell. The observation that pre-VLDL is converted to VLDL2 by the addition of more lipids implies that MTP is also active after the completion of apoB100. This fits with our finding that MTP is needed

for a period after the completion of apoB100 to allow the formation of a secretable apoB100 containing lipoproteins (Rustaeus et al 1998). Thus, during this period, MTP may convert pre-VLDL to VLDL2.

The lipidation of VLDL2 is dependent on the size of apoB, and there is an inverse relationship between the density of the particle formed and the length of the protein (Stillemark-Billton et al 2005). A *bona fide* VLDL2 is only formed by apoB100 while truncated forms of apoB form more dense particles that are VLDL2 analogues. For example, apoB48, which is the natural occurring truncated form of apoB100, forms a dense “HDL-sized” particle that is mature and secreted from the cell, unlike the primordial immature particle formed by apoB100. The apoB48-containing VLDL2 analogue is formed in the ER (Stillemark et al 2000), but it is not known whether the apoB100-containing VLDL2 is formed in the ER or after apoB has exited this compartment. Assembly of VLDL2 (and VLDL2 analogues) in the ER would fit with the paradigm that only correctly folded proteins are allowed to be transferred to the later part of the secretory pathway (Figure 2). (For quality

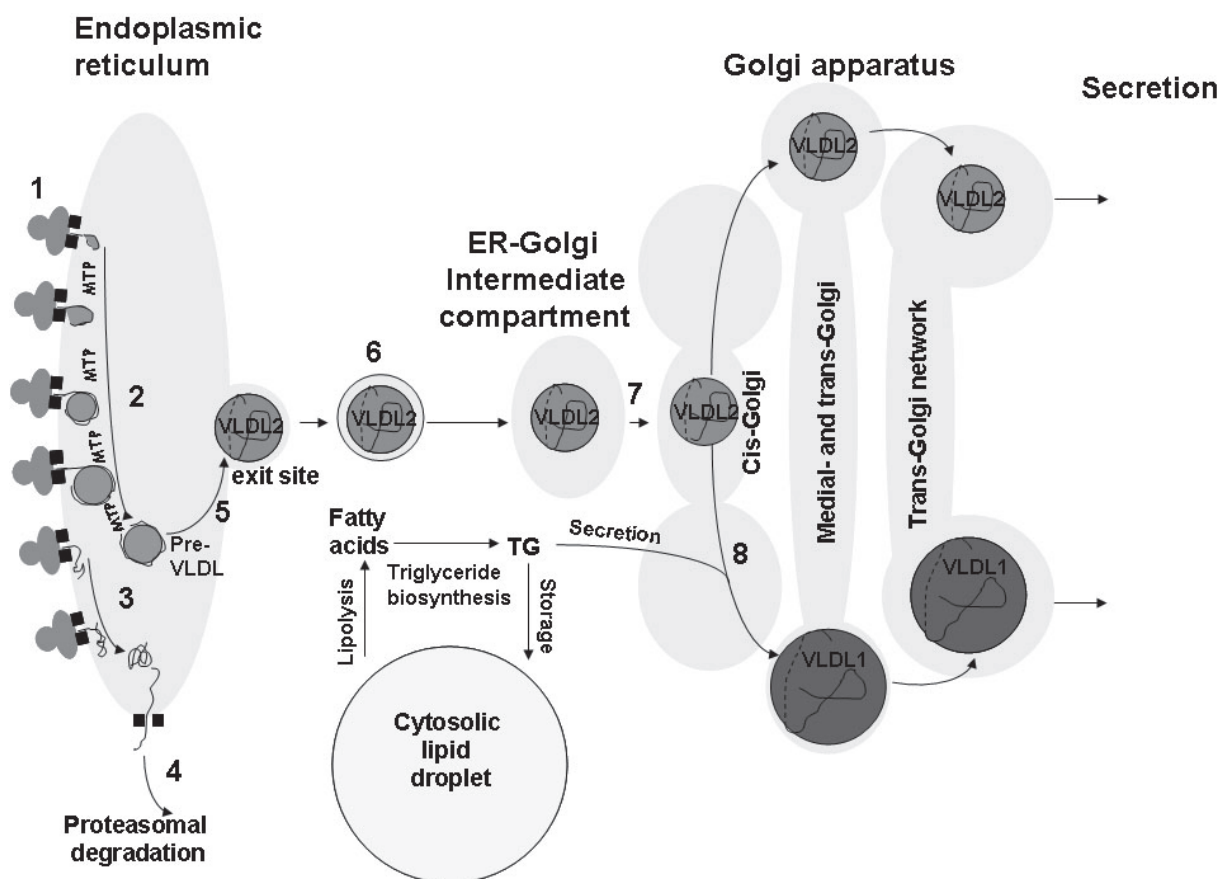


Figure 2 The assembly of VLDL. During its biosynthesis, apoB100 (1) is translocated to the lumen of the endoplasmic reticulum and lipidated by MTP to form a pre-VLDL particle (2). Pre-VLDL is further lipidated to form VLDL2 (5). Alternatively, pre-VLDL and misfolded apoB100 (3) can be retained and degraded in the cell (4). The VLDL2 is transferred to the Golgi apparatus (6, 7) and is either secreted or further lipidated to form VLDL1 (8). Fatty acids are released from cytosolic lipid droplets and used for the formation of triglycerides, which are assembled into VLDL (8).

control of folded proteins, see the following reviews (Ahner and Brodsky 2004; Ellgaard and Helenius 2001; Ellgaard and Helenius 2003; Kleizen and Braakman 2004; Schröder and Kaufman 2005; Sitia and Braakman 2003)). We therefore propose that the primordial particle that is formed by lipidation of the growing apoB100 must be converted to VLDL2 before it can leave the ER, and that VLDL2 must reach the Golgi apparatus before it can be converted to VLDL1. Both VLDL2 and VLDL1 are secreted while the primordial particle (pre-VLDL) is retained and eventually degraded.

The formation of VLDL1 involves a bulk lipidation (ie, the addition of a major load of triglycerides) of VLDL2 or the VLDL2 analogues, and the apoB acceptor is only required to have a minimum size of apoB48 (Stillemark-Billton et al 2005). Both apoB and VLDL2 (or its analogues) must be transported to the Golgi apparatus to allow VLDL1 formation (Asp et al 2005; Stillemark et al 2000). The time gap necessary for apoB to be transferred to the Golgi apparatus to obtain its major lipidation is in agreement with our recent

observation in humans injected with stable amino acid and glycerol isotopes: labeled triglycerides were secreted 15 min before labeled apoB100 (Adiels et al 2005b). Furthermore, we have found evidence of a precursor-product relationship between VLDL2 and VLDL1 in clinical turnover studies using stable isotopes. Thus, in insulin-clamp studies, we observed that insulin decreased the assembly of VLDL1 and at the same time increased the assembly and secretion of VLDL2 (Adiels et al Manuscript in preparation).

Cytosolic lipid droplets

The assembly of VLDL is highly dependent on the amount of triglycerides in the hepatocytes. Several authors have demonstrated that the fatty acids used for the biosynthesis of VLDL triglycerides are derived from triglycerides stored in cytosolic lipid droplets (Gibbons et al 2000; Salter et al 1998; Wiggins and Gibbons 1992). The droplets are now recognized as dynamic organelles rather than passive stores of lipids (Martin and Parton 2006).

The structure of cytosolic lipid droplets is very similar to that of lipoproteins, with a core of neutral lipids surrounded by a monolayer of amphipathic structures such as phospholipids, cholesterol, and proteins. The most well known of these proteins are the PAT proteins (Londos et al 1999) perilipin, which is present in adipocytes and cells involved in the biosynthesis of steroid hormones, adipocyte differentiation-related protein (ADRP), which is ubiquitously expressed, and tail-interacting protein 47 (TiP47). There are several other proteins with important functions present on the droplets (Brasaemle et al 2004; Liu et al 2004).

The lipid droplets are formed as small structures (0.1–0.4 μm) in or close to the ER and/or Golgi membranes (Marchesani et al 2003). Insulin promotes the formation of lipid droplets via the activation of phospholipase D1 (PLD1) and extracellular signal-related kinase 2 (ERK2) (Andersson et al 2006). ERK2 phosphorylates the motor protein dynein, which is then targeted to lipid droplets allowing them to transfer to microtubules and increase in size by fusion (Andersson et al 2006; Bostrom et al 2005). A model for the assembly of lipid droplets is shown in Figure 3.

The importance of triglycerides for the assembly and secretion of VLDL is supported by our turnover studies *in vivo*, which demonstrate that the secretion of VLDL1 apoB100 increases with increasing concentrations of liver

lipids (Adiels et al 2005a). However, the relationship between stored triglycerides and the secretion of VLDL is complex.

On the basis of our results, we conclude that there are at least two ways of influencing the pool of lipid droplets in the cell: (i) by promoting the formation of small primordial droplets and (ii) by promoting the rate of fusion between the droplets. We have demonstrated that both mechanisms also influence the assembly of VLDL. An increase in ADRP results in an increase in the formation of the droplets and the pool of the droplets and a subsequent decrease in the assembly of VLDL, in particular VLDL1 (Magnusson et al 2006). This is because ADRP promotes the storage of newly formed triglycerides in cytosolic droplets, diverting lipids from the VLDL assembly pathway. We also observed that epigallocatechin gallate, which is present in green tea, increases the rate of fusion between droplets, resulting in an increased pool of cytosolic lipid droplets, and decreases the assembly and secretion of VLDL, in particular VLDL1 (Li et al 2006). Together, these results indicate that factors that promote the assembly of lipid droplets and increase their cellular pool can in fact deplete the VLDL assembly pathway of lipids and thus decrease the secretion of the lipoprotein.

It is known that the secretion of VLDL1 is reduced by insulin (see Taskinen 2003) for review). Moreover, a failure of this insulin effect may be of importance for the formation of the dyslipidemia seen in insulin resistance and type 2 diabetes. The mechanism is not understood, but it is possible that promotion of lipid droplet assembly by insulin (Andersson et al 2006) promotes a shift of triglycerides from the VLDL assembly pathway to storage in cytosol. There are, however, other tentative mechanisms to explain the influence of insulin on VLDL assembly (for review, see (Taskinen 2003)).

Post-translational regulation of the secretion of apoB100

The secretion of apoB100 is regulated post-transcriptionally by co- and post-translational degradation. It has long been known that apoB100 undergoes intracellular degradation (for review, see (Olofsson et al 1999; Davidson and Shelness 2000; Shelness and Sellers 2001)). The intracellular degradation of apoB100 occurs at three different levels (Fisher and Ginsberg, 2002; Fisher et al 2001):

- (i) Close to the biosynthesis of apoB (co- or post-translationally) by a mechanism that involves retraction of the apoB molecule from the lumen of the ER to the cytosol, a ubiquitination and a subsequent proteasomal degradation (Mitchell et al 1998; Liang et al 2000; Fisher et al 2001; Pariyarath et al 2001). The co-translational degradation

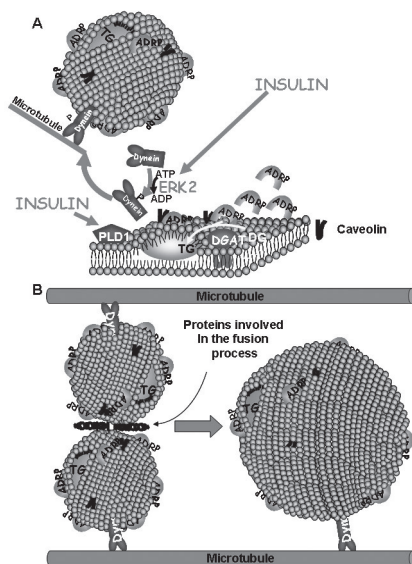


Figure 3 (A) Lipid droplets are formed as primordial droplets at the microsomal membranes. Insulin stimulates the droplet formation by activating PLD1 and ERK2. ERK2 phosphorylates the motor protein dynein, which is then recruited to the droplets to promote their formation and fusion. Diacylglycerol acyltransferase (DGAT) catalyzes the formation of triglycerides (TG) from diglycerides (DG) and fatty acids (FA). It has been suggested that the lipid droplet is initiated by the oiling out of triglycerides between the leaflets of the membrane. (B) Primordial droplets increase in size by fusion. This process depends on dynein and its interaction with microtubules. Moreover it involves specific proteins that catalyze fusion between the droplets.

of apoB is influenced by the availability of lipids and the activity of MTP. Thus, when the amount of lipids or the MTP activity is limited, apoB100 remains associated with the translocon and is sorted to proteasomal degradation (for review, see (Fisher and Ginsberg 2002)). It is well known that secretory proteins that misfold undergo proteasomal degradation (see above; Figure 2). A lack of lipids may remove one of the prerequisites for the correct folding of apoB, thus promoting its degradation.

- (ii) Post-translationally by an unknown mechanism that can be promoted by culturing the cells in the presence of polyunsaturated fatty acids (Fisher et al 2001). This degradation seems to occur in a compartment separate from the rough ER, and has therefore been referred to as post-ER presecretory proteolysis (PERPP) (Fisher et al 2001). Nothing is known about the enzyme systems involved or the sorting of apoB100 for this degradation. The process is promoted by ω -3 fatty acids and by increased levels of reactive oxygen species (Jiang et al 2005). It has also been demonstrated that apoB100 interacts with the protease/chaperone ER 60 (Adeli et al 1997; Taghibiglou et al 2002), and that this interaction is linked to the intracellular degradation of newly synthesized apoB100. The role of this interaction for PERPP has not been elucidated.
- (iii) By reuptake from the unstirred water layer around the outside of the plasma membrane (Williams et al 1990) via the LDL receptor. The LDL receptor has been shown to have an important role in regulation of the secretion of apoB100-containing lipoproteins (Horton et al 1999; Twisk et al 2000). There is even evidence that the effect of the receptor is not only due to the interaction with apoB100 on the cell surface, but that the receptor and apoB100 interact early in the secretory pathway and that this interaction is of importance for the post-translational degradation of apoB100 (Gillian-Daniel et al 2002; Larsson et al 2004; Twisk et al 2000)}.

The apoB100 primordial particle (pre-VLDL) is not secreted from the cell to any significant extent, but appears to be retained and degraded (unless converted to VLDL2; see above) (Stillemark-Billton et al 2005)

The development of atherosclerosis: different hypotheses

Although it is well documented that elevated levels of LDL and other apoB-containing lipoproteins cause increased atherosclerosis, the molecular and cellular mechanisms for the pathobiological changes that lead to the disease are still

poorly understood. Several hypotheses have been articulated to explain the events that initiate atherogenesis: the response-to-injury hypothesis states that endothelial injury leads to an inflammatory response as a part of a healing process in the arterial wall (Ross 1999; Ross et al 1977); the response-to-oxidation hypothesis proposes that lipoprotein oxidation is the important link in atherosclerosis (Steinberg et al 1989); and the response-to-retention hypothesis suggests that subendothelial retention of lipoproteins is the initiating step and leads to oxidation, inflammation, and endothelial dysfunction (Williams and Tabas 1995; Williams and Tabas 1998). This hypothesis is based on pioneering work from the 1970s and 1980s showing that lipoproteins can interact with the arterial wall (Iverius 1972; Camejo et al 1975; Vijayagopal et al 1981).

While these hypotheses are by no means mutually exclusive, and may even be considered mutually compatible with differences in emphasis, a growing body of recent evidence supports the response-to-retention hypothesis. This hypothesis states that the subendothelial retention of lipoproteins determines the concentration and the susceptibility to modification of lipoproteins in the arterial wall. Several lines of evidence indicate that the retention of arterial lipoproteins involves the extracellular matrix, and proteoglycans in particular appear to play an important role.

Mechanism for retention of atherogenic lipoproteins in the artery wall

The binding of atherogenic lipoproteins to artery wall proteoglycans is mediated by ionic interactions between clustered basic amino acids in the apoB100 that bind to the negatively charged glycosaminoglycans (GAGs) of the proteoglycans. *In vitro* studies of delipidated apoB100 identified eight specific regions of clustered basic amino acids in the apoB moiety of LDL that bind to the negatively charged GAGs (Hirose et al 1987; Weisgraber and Rall Jr, 1987; Camejo et al 1988). However, it was not known which of these sites were functional on LDL particles. To identify the main proteoglycan-binding sites on LDL, specifically mutated forms of the human apoB gene were expressed in transgenic mice, and the ability of the recombinant human LDL isolated from the mice to bind to artery wall proteoglycans *in vitro* was assessed (Boren et al 1998b). These experiments implicated the basic amino acids in Site B (residues 3359–3369; Figure 1), the site in apoB100 that binds to the LDL receptor, as the main site on apoB100 that interacts with proteoglycans (Boren et al 1998b). In addition, a mutant recombinant LDL

with a single amino acid mutation (K3363E) was generated that bound normally to the LDL receptor but defectively to proteoglycans (Boren et al 1998b). This finding showed that it is possible to discriminate between the LDL receptor-binding activity and proteoglycan-binding activity, even though their binding sites coincide (Boren et al 1998a; Boren et al 1998b). These and other experiments also indicate that the LDL receptor binding is conformational dependent, whereas proteoglycan-binding is charge dependent.

Atherosclerosis studies in mice expressing recombinant LDL

The atherogenic potential of the interaction between apoB100 and artery wall proteoglycans was investigated in transgenic mice expressing human control LDL and recombinant proteoglycan-binding-defective LDL (ie, LDL with a Site B mutation that abolishes the binding to proteoglycan) (Skalen et al 2002). The study was designed to ensure that any differences in atherosclerosis were due to weak binding of the proteoglycan-binding-defective LDL to proteoglycans and not to some other attribute of the mutated LDL, such as the inability to bind to LDL receptors.

The transgenic mice were fed a Western diet for 20 weeks. The results showed that the extent of the vessel wall covered by atherosclerotic lesions correlated with the plasma cholesterol level in both groups of transgenic mice. However, the extent of atherosclerosis differed dramatically. Transgenic mice expressing the proteoglycan-binding-defective LDL had strikingly less atherosclerosis than mice expressing wild-type recombinant LDL. These findings show that proteoglycan-binding-defective LDL has a greatly reduced atherogenic potential and provide direct experimental evidence that binding of LDL to artery wall proteoglycans is an early step in atherogenesis (Skalen et al 2002).

A tentative explanation for why some atherosclerosis developed in mice that express proteoglycan-binding-defective apoB is that LDL from mice on a high-fat diet contains apoE, which can mediate binding to proteoglycans and thus retention. Indeed, proteoglycan-binding-defective LDL isolated from the mice in the atherosclerosis study contained apoE and displayed approximately 20% of normal proteoglycan binding (Skalen et al 2002).

Is it possible to modulate the proteoglycan binding of LDL?

The conformation of apoB100 on the surface of the LDL particle is dependent on the composition of the core lipids,

the surface phospholipid content, and the diameter of the lipoprotein particle. Thus, binding sites other than Site B may become functional in modified LDL. LDL that has been modified by secretory group IIA phospholipase A₂ (sPLA₂), a strong risk factor for coronary heart disease (Kugiyama et al 1999), binds to proteoglycans more avidly than unmodified LDL (Sartipy et al 1998). Recent studies have shown that Site A (residues 3147–3157 in apoB100) becomes functional in modified forms of LDL and that it then acts cooperatively with Site B in the association with proteoglycans (Flood et al 2004). Furthermore, the core lipids of LDL also influence the folding of apoB100 on the LDL particle. Recent studies have shown that the triglyceride content of LDL influences the conformation of apoB and decreases the affinity for GAGs. This mechanism is mediated by a conformational change of Site B and is, in contrast to sPLA₂-modified LDL, independent of Site A (Flood et al 2004). The finding that the triglyceride content of LDL decreases the binding of LDL to artery wall proteoglycans may explain why triglyceride-rich apoB LDL are not as atherogenic as the triglyceride poor (Willner et al 2003).

Indirect interactions of LDL to the extracellular matrix

In addition to the direct interaction between apoB100 and artery wall proteoglycans, retention of LDL also appears to involve an indirect interaction between LDL and artery wall proteoglycans facilitated by “bridging molecules”. ApoE functions as a potent bridging molecule between recombinant LDL and artery wall proteoglycans, and apoE enrichment of proteoglycan-binding-defective LDL substitutes for the defective direct interaction between RK3359–3369SA LDL and artery wall proteoglycans (Skalen et al 2002).

In addition to apolipoproteins, several lipases have been shown to mediate bridging between lipoproteins and heparin sulfate proteoglycans (HSPGs) on the cell surface, which results in increased cellular uptake and degradation of lipoproteins. Lipoprotein lipase (LPL), which is secreted by smooth muscle cells and macrophages in atherosclerotic lesions (Boren et al 2001b), has been shown to act as a bridge between GAG and extensively oxidized LDL, which is sufficiently depleted of positive charges to inhibit direct binding to GAG (Olin et al 1999; Pentikainen et al 2000).

Thus, the proteoglycan-binding activity of LDL depends on the diameter and lipid composition of the LDL particle and on the presence of apolipoproteins other than apoB on the LDL particle. Several pathological conditions with

increased risk of cardiovascular diseases, such as diabetes and rheumatoid arthritis, are characterized by LDL particles with enhanced interaction with the subendothelium (Hurt-Camejo et al 2001; Nesto and Rutter 2002)

The assembly and metabolism of HDL

Another important player in atherogenesis is HDL and its major apolipoprotein apoA1. Amphipathic α -helices anchor apoA1 in the surface of the HDL particles. In contrast to apoB100, apoA1 can transfer between particles.

The assembly and metabolism of HDL have recently been extensively reviewed (see for example (Lewis and Rader 2005; Yokoyama 2005; Krimbou et al 2006)), and here we will only deal briefly with this subject. A groundbreaking event in the understanding of the assembly of HDL was the identification of the gene for Tangiers disease (Bodzioch et al 1999; Brooks-Wilson et al 1999; Marcil et al 1999; Remaley et al 1999; Rust et al 1999), a condition in which HDL is almost completely lacking. This gene was shown to encode the ATP-binding cassette transporter (ABCA1), which is involved in supplying lipid-poor apoA1 or pre- β -HDL with cholesterol.

It is now believed that apoA1 is secreted from liver and intestine as lipid-poor apoA1 (for recent reviews, see for example (Krimbou et al 2006; Lewis and Rader 2005; Yokoyama 2005)). Lipid-poor apoA1 seems to be the major target for the lipidation by ABCA1; the transporter is critical for the initial lipidation of apoA1, protecting it from being rapidly degraded in the liver, kidney, and steroidogenic cells. The lipidation of apoA1 by ABCA1 is a complex process that is not understood in detail, but is thought to involve an interaction between the apolipoprotein and the transporter and the formation of a high-affinity binding site for apoA1 from which the nascent pre- β -HDL is formed. The process involves intracellular cAMP release induced by apoA1 and phosphorylation of ABCA1 by protein kinase A. It also appears that the apoA1-ABCA1 complex can be internalized and transported through early and late endosomes (and possibly through other unknown compartments), during which time nascent HDL is formed and secreted.

In addition to ABCA1, transporters of the ABCG family play an essential role in the efflux of cellular cholesterol to HDL (Klucken et al 2000; Kennedy et al 2005). ABCG1 has been reviewed recently (Baldan et al 2006b; Oram and Vaughan 2006) and it seems to primarily influence the cholesterol content of macrophages. In *ABCG1*^{-/-} mice, high levels of neutral lipids accumulated particularly in pulmonary

macrophages, but also in Kupffer cells and hepatocytes; by contrast, little or no accumulation was observed in these cells in human *ABCG1* transgenic mice (Baldan et al 2006b).

Interestingly, ABCG1 is important for the exit of cholesterol to HDL but not to lipid-poor apoA-1 (for review, see (Baldan et al 2006b)). Indeed, it has been suggested that ABCA1 and ABCG1 work in concert. Thus, ABCA1 is suggested to lipidate lipid-poor apoA-I while ABCG1 promotes the efflux of cholesterol to the already preformed lipid-protein complexes (Gelissen et al 2006; Vaughan and Oram 2006). However, there are suggestions that ABCA1 and ABCG1 control separate cholesterol efflux pathways (for review, see (Baldan et al 2006b)). There are diverging opinions about the importance of ABCG1 for the development of atherosclerosis (Baldan et al 2006a; Out et al 2006; Ranalletta et al 2006), but a recent paper seems to favour the idea that ABCG1 protects against the development of this disease (Out et al 2007).

Nascent HDL is modified in plasma by several factors. The most well known is lecithin cholesterol acyltransferase (LCAT), which esterifies cholesterol allowing the formation of a core in the lipoprotein. The core of HDL is also modified by cholesterol ester transfer protein (CETP), which exchanges cholesterol and triglycerides between HDL and triglyceride-rich lipoproteins such as VLDL. Phospholipids are also transferred from the surface of triglyceride-rich lipoprotein (during triglyceride hydrolyses) to HDL.

The catabolism of HDL can occur in at least two ways: (i) by selective removal of cholesterol and other lipids without uptake of the whole particle (this involves the scavenger receptor B1); and (ii) by uptake and degradation of the whole particle, which involves endocytosis and lysosomal degradation.

ApoA1 and HDL are highly linked to the so-called reverse cholesterol transport, ie, the transfer of cholesterol from peripheral cells to the liver to be secreted as bile acids. The roles of ABCA1, ABCG1 and the scavenger receptor B1 in the turn over of HDL seem to fit very well into such a role of HDL. However, the process is far from elucidated and may be complex. In humans, radiolabeled HDL cholesterol that is eventually secreted into the bile is almost entirely transported to apoB-containing lipoproteins (presumably through CETP).

Apolipoprotein levels in plasma as a tool for clinical judgements

In most clinical trials on lipid lowering for prevention of cardiovascular disease, LDL cholesterol has been used both

as a target for treatment and for the identification of treatment success. This relates to trials using drugs as well as dietary intervention. Consequently, the different guidelines for prevention of cardiovascular disease have LDL levels as their primary target (2005; Conroy et al 2003; Grundy et al 2004). With the increased efficiency of lipid-lowering drugs, it has also become evident that targets far below the earlier suggested goals for LDL cholesterol may further reduce the morbidity and mortality in cardiovascular disease.

Recently, apoB as a target for treatment has been widely discussed and also supported by authorities in some guidelines (Grundy 2002; Genest et al 2003). ApoB in plasma is regarded as a marker for the total number of atherogenic lipoproteins in plasma, and the equivalent in lipid terms would be non-HDL cholesterol. Non-HDL cholesterol is also given as an alternative treatment goal in the National Cholesterol Education Program (NCEP) (Clearfield 2003). Although strongly correlated, however, apoB and non-HDL cholesterol are not identical, and several studies have found that apoB is a better predictor of risk for cardiovascular disease than non-HDL cholesterol (for review, see (Sniderman et al 2003).

It has been suggested that apoB could substitute for LDL cholesterol and non-HDL cholesterol for risk prediction (Pischon et al 2005). However, this idea has been questioned because the currently available population and disease correlation data and the cholesterol education program are based on LDL cholesterol and non-HDL cholesterol (Denke 2005). It is important to note that apoB is a direct measure of particle number rather than lipid content, which is a crucial point for patients with the metabolic syndrome or type 2 diabetes. These subjects often have increased levels of small dense LDL, which are not detected by a simple lipid analysis. Thus, the combination of hypertriglyceridemia and high apoB is a new important risk algorithm (Sniderman 2004). Few intervention studies have been designed to use apoB levels for inclusion or as treatment target. However, data from some of the large statin trials indicate that apoB could also serve as an important treatment goal. For example, in the AFCAPS/TexCAPS trial, apoB in subjects on treatment was a strong predictor for major coronary events, both in the actively treated and the placebo groups (Gotto et al 2000).

Levels of ApoAI in plasma are strongly correlated with HDL levels. In agreement with the studies on HDL, low apoAI has been shown to be equivalent or better than low HDL cholesterol as a risk marker for atherosclerosis or cardiovascular events (Francis and Frohlich 2001; Luc et al 2002; Walldius and Jungner 2006). Several mechanisms

to explain the strong association between low apoAI and risk have been suggested (Barter and Rye 2006). The most common is that apoAI has an important role in the reversed cholesterol transport, and an alternative suggestion is that apoAI is anti-inflammatory or an antioxidant (for review see (Barter and Rye 2006). However, the process is not convincingly understood.

It is more complex to determine whether elevation of apoAI also can be used as a target for treatment. ApoAI levels in plasma reflect both production and clearance. Interference in these processes may have opposing effects on the reverse cholesterol transport. In recent studies in humans and animal models, infusions of HDL or apoAI resulted in reduced atherosclerosis (Nissen et al 2003; Nicholls et al 2005), suggesting that increased plasma levels of apoAI or HDL have the potential to treat and prevent atherosclerosis.

A lot of enthusiasm has recently been focused on the apoB/apoAI ratio as a risk marker or treatment target. Several large epidemiological studies strongly support this concept by finding the apoB/apoAI ratio superior to lipid parameters as a risk marker (for review see (Walldius and Jungner 2006). Whether the ratio apoB/apoAI also can be used as a general treatment target still has to be proven. It seems unlikely that a high ratio caused by low apoAI should be treated the same way as a low ratio caused by high apoB. Furthermore, we do not know if a change in the ratio caused by apoB reduction is equivalent to an increase in apoAI. This has to be further studied, but the treatment should be based on levels of both apoB and apoAI, and probably also on LDL and HDL lipid levels.

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