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REVIEW

Understanding the Mechanism of LCAT Reaction May Help to Explain the High Predictive Value of LDL/HDL Cholesterol Ratio

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Summary

Traditionally, lecithin:cholesterol acyltransferase (LCAT) role in the reverse cholesterol transport (RCT) has been considered "antiatherogenic" as the cholesterol esterification is the prerequisite for the formation of mature high density lipoprotein (HDL) particles and may create a gradient necessary for the flow of unesterified cholesterol (UC) from tissues to plasma. However, newer data suggest that a higher esterification rate is not necessarily protective. Here we review the available data on the role of LCAT in RCT and propose that the LCAT-mediated esterification of plasma cholesterol promotes RCT only in the presence of sufficient concentrations of HDL_2 while this reaction may be atherogenic in the presence of high concentration of plasma low density lipoprotein (LDL) cholesterol. Thus, the "protective" or potentially "atherogenic" role of LCAT depends on the quality of HDL and concentration of LDL. This hypothesis is consistent with the known high predictive value of LDL/HDL cholesterol ratio.

Key words

Reverse cholesterol transport – LCAT – Atherosclerosis – LDL/HDL cholesterol ratio – FER_{HDL}

Introduction

There is now ample evidence that plasma high density lipoproteins (HDL) are protective against atherosclerosis (Miller 1987, Castelli *et al.* 1986). The concept of reverse cholesterol transport (RCT), mostly based on *in vitro* studies, has been used to explain the protective properties of HDL (Glomset 1968). It has been hypothesized that RCT starts by transfer of unesterified cholesterol (UC) from cell membranes to the small HDL particles where it is esterified *via* lecithin:cholesterol acyltransferase (LCAT) to produce cholesteryl esters (CE). Next steps in CE transport then include their transfer to very low density (VLDL) and low density (LDL) lipoproteins and uptake by the hepatic apo B, E receptors.

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In the above process, LCAT has been considered the "engine" that created a gradient for transfer of cholesterol from cell membranes and the protective role of HDL was related to the rate of cholesterol esterification. In addition, the esterification of UC has been associated with remodelling of HDL subpopulations and with the formation of larger HDL particles (Eisenberg 1984) that are more protective against coronary artery disease (CAD) (Miller 1987). However, the actual measurements of LCAT activity in the plasma have never confirmed this view. On the contrary, lower esterification rates were observed in normal (healthy) populations compared to patients with CAD (Dobiášová *et al.* 1991, 1992, Murakami *et al.* 1995). Even persons with either partial or complete LCAT deficiency do not appear to be at a markedly increased risk of CAD compared to, for instance, those with familial hypercholesterolaemia (Kuivenhoven *et al.* 1997). Also, the relative concentrations of HDL_{2b} are higher in those with lower LCAT activity (Dobiášová *et al.* 1991, 1992) whereas the current RCT hypothesis predicts higher activity based on the above remodelling postulate.

More recently, it has become apparent that the route of cell derived UC is more complicated than originally suggested and that LDL is a major mediator in this process (Francone *et al.* 1990, Fielding *et al.* 1991).

Even if high levels of plasma HDL are protective against CAD, the HDL activity as a carrier of either cellular or particularly foam cell cholesterol can not explain such an antiatherogenic effect by itself. Additional roles may include HDL ability to inhibit the cytokine-induced expression of adhesion molecules by endothelial cells and prevention of endothelial cell-mediated LDL oxidative modification (Quintao 1995, Barter and Rye 1996).

In this review we have summarized current data on the relations between HDL subspecies, LDL and LCAT, point out some inconsistencies in the hypothesis on reversed transport of cholesterol and suggest several possible roles of LCAT in the development of atherosclerosis.

Heterogeneity of HDL and its clinical relevance to CAD

Members of the HDL family of particles differ in structure and function; their differences in density, size and charge have been thoroughly studied. Studies based on subfractionation of plasma lipoproteins by ultracentrifugation indicate that the antiatherogenic properties of HDL are mostly associated with the HDL_2 subclass (Miller 1987).

HDL subclasses as determined by particle size distribution

The particle size of HDL populations has been recently estimated by gradient gel electrophoresis (GGE) which resolves HDL into five distinct subspecies of different sizes (Nichols *et al.* 1986); HDL_{3c} (7.2–7.8 nm), HDL_{3b} (7.8–8.2 nm), HDL_{3a} (8.2–8.8 nm), HDL_{2a} (8.3–9.7 nm), and HDL_{2b} (9.7–12 nm).

Using this method of separation, it has been established that the concentrations of both the largest and the smallest HDL particles are highly significantly correlated with the risk of CAD (Dobiášová *et al.* 1991, Drexel *et al.* 1992, Williams *et al.* 1992, Skinner 1994, von Eckardstein *et al.* 1994). In a segment of population least at risk of CAD, namely premenopausal healthy women, the relative concentration of large HDL_{2b} particles is the highest (around 25 %) (Williams *et al.* 1992, Skinner 1994, von Eckardstein *et al.* 1994, Dobiášová and Frohlich 1996) and that of small $HDL_{3b,c}$ the lowest (around 15 %). On the other hand, in populations with high CAD risk the concentration of HDL_{2b} particles decreases and that of $HDL_{3b,c}$ increases. Thus men (Williams *et al.* 1992, Dobiášová and Frohlich 1996), obese individuals, postmenopausal women (Williams *et al.* 1993), men and women with hypertension (Dobiášová *et al.* 1992, 1995) and survivors of myocardial infarction (Wilson *et al.* 1990, Dobiášová *et al.* 1991, Johansson *et al.* 1991, Drexel *et al.* 1992) all have a lower proportion of large HDL_{2b} (the relative concentration may decrease under 10 %) and increased $HDL_{3b,c}$ (relative concentrations may reach up to 30 % of total HDL). It is of note that, in individuals with major CAD risk factors (such as family history, hypertension or insulin resistance), marked variations in the HDL subspecies pattern can be found between individuals with identical plasma HDL cholesterol concentrations. We have shown that typically "male" (low HDL_{2b}, high $HDL_{3b,c}$) and "female" (high HDL_{2b}, low $HDL_{3b,c}$) patterns can be detected already in childhood (Dobiášová *et al.* 1998).

Whether the formation of large HDL is favoured by high LCAT and lipoprotein lipase activities not only *in vitro* (Eisenberg 1984) remains open to question because the lowest concentrations of HDL_{2b} are found in individuals with the highest esterification rates and highest CAD risk .

In regard to the prediction of CAD risk the measurement of HDL_{2b} correlates well with the measurement of HDL_2 by other methods while the measurement of HDL cholesterol in HDL_3 is of little value, if any, because the potentially atherogenic $HDL_{3b,c}$ subpopulations form only a small component of HDL_3.

HDL subclasses according to apoprotein composition

An alternative approach to the characterization of HDL subpopulations is based on analysis of their apolipoprotein composition as assessed by either immunoaffinity chromatography (Atmeh *et al.* 1983, Cheung and Albers1984, Puchois *et al.* 1987, Ohta *et al.* 1988) or immunoelectrophoresis (Parra *et al.* 1990, Fruchart *et al.* 1993, Dobiášová and Frohlich 1994). Two major types of particles have been identified: those containing apo AI but not apo AII (LpAI) and particles containing both apo AI and apo AII (LpAI/AII). Both types of particles are found within the whole range of HDL subpopulations whether separated by ultracentrifugation or by GGE (Atmeh *et al.* 1983, Cheung and Albers 1984, Puchois *et al.* 1987). While most of the LpAI/AII particles are found within HDL_3 (Ohta *et al.* 1988), approximately 20–50 % of LpAI particles (depending on sex) are located in HDL_{2b} population. The fact that the LpAI are very closely related to the larger HDL particles is suggested not only by the direct correlation between plasma concentrations of LpAI and HDL_{2b} (Dobiášová and Frohlich 1994), but also by the fact that women have the highest amount of both HDL_{2b} and LpAI (Fruchart *et al.* 1993) and that the risk for CAD is increased by both lower LpAI and HDL_{2b} (Parra *et al.* 1990, Luc *et al.* 1991).

While LpAI are considered highly antiatherogenic (Roheim and Asztalos 1995), the mechanism of this effect is not completely understood. It appears that it is not based on a specific interaction of this molecular form of the apo AI with cellular cholesterol. In addition, it appears that LpAI's do not specifically influence the efflux of cellular cholesterol as LpAI and LpAI/AII particles are both equally effective in promoting cholesterol efflux from human fibroblasts and from aortic endothelial cells (Oikawa *et al.* 1993). Currently. there is not sufficient evidence to show a clear relationship between apolipoprotein-specific subpopulations of HDL and CAD (Barter and Rye 1996).

HDL subclasses according to surface charge

Another criterion that distinguishes human HDL subspecies is their particle charge: particles with pre-beta mobility have a lower negative surface charge than the bulk of HDL (approximately 90 %) with alpha mobility (Kunitake *et al.* 1985, 1992, Daerr *et al.* 1986, Albers *et al.* 1986, Castro and Fielding 1988, Neary *et al.* 1991, Musliner *et al.* 1991, Barrans *et al.* 1994, Asztalos *et al.* 1993, Davidson *et al.* 1994). With a proper standardization of the method that combines agarose gel electrophoresis and a non-denaturing PAGE, two pre-beta-1-Lp(AI), three pre-beta-2-Lp(AI), three alpha-Lp(AI) and three pre-alpha-Lp(AI) fractions can be distinguished (Kunitake *et al.* 1992). Pre-beta-HDLs are of particular interest as they appear to be the primary acceptor of cellular cholesterol. After exposure to fibroblasts, the cholesterol content of the pre-beta-HDL increased approximately six fold, suggesting that they take up appreciable amounts of cellular cholesterol (Kunitake *et al.* 1992). This pre-beta subpopulation contains phospholipids and free and esterified cholesterol while protein (apo AI) constitutes 90 % of its mass. The protein moiety of this subpopulation exhibits markedly lower helicity than that of HDL isolated by ultracentrifugation (Kunitake *et al.* 1985). The pre-beta-HDL originated probably from plasma triglyceride-rich lipoproteins during lipolysis (Daerr *et al.* 1986, Kunitake *et al.* 1992, Muslinger *et al.* 1991, Barrans *et al.* 1994).

The surface potentials of spherical and discoidal r-HDL are not affected by their apoprotein composition (Asztalos *et al.* 1993). Other data show that cholesterol has a direct effect on apo AI conformation and charge in HDL. Structural changes of this magnitude can affect the interactions between HDL and various plasma proteins and cell surfaces (Davidson *et al.* 1994). The understanding of interaction of HDL particles with cellular surfaces, receptors and plasma proteins in relation to particle charge may contribute to the explanation of variations in their function.

Role of LCAT in reverse cholesterol transport

A number of reviews (Albers *et al.* 1986, Dobiášová 1983, Fielding and Fielding 1995a, Jonas 1991) summarize the studies that followed the original description of LCAT by Glomset (1968). The enzyme produces cholesteryl esters and lysolecithin (LPC) by transferring the acyl group of fatty acid from the 2-sn position of lecithin to the 3-hydroxy group of cholesterol. In human plasma, the LCAT reaction is the major source of CE and plays a crucial role in the remodelling of plasma lipoproteins and in RCT. The reaction takes place mostly on the surface of HDL and is best activated by apo AI; the enzyme is mainly associated with large HDLs and the binding is influenced by the lipid but not by the protein composition of plasma lipoproteins (Applebaum-Bowden 1995).

The esterification rate depends on the composition of the lipoprotein lecithin, particularly on the acyl chain length and its degree of saturation; the ratio of phospholipids to cholesterol determines the optimum fluidity of the phospholipids bilayer and thus the optimal physical chemical state for the reaction. Specific HDL particles determine the reaction rate most likely by their size and charge. In the small HDLs, characterized by specific size ($HDL_{3b,c}$), shape (e.g. discoidal) or by specific apolipoproteins (apo AI) or charge (pre-beta-HDL), the esterification rate is high

(Hamilton *et al.* 1976, Barter *et al.* 1985, Dobiášová *et al.* 1992, Murakami *et al.* 1995, Czarnecka and Yokoyama 1995). On the other hand, large HDL particles inhibit the esterification rate (Barter *et al.* 1984, Dobiášová *et al.* 1992, Ikeda *et al.* 1994). The concentration of the enzyme has little effect on the rate of esterification (Albers *et al.* 1981, Murakami *et al.* 1995). Even at low LCAT concentrations, such as in heterozygotes for LCAT deficiency, the ratio of CE to UC in plasma remains within the normal range (Ohta *et al.* 1995).

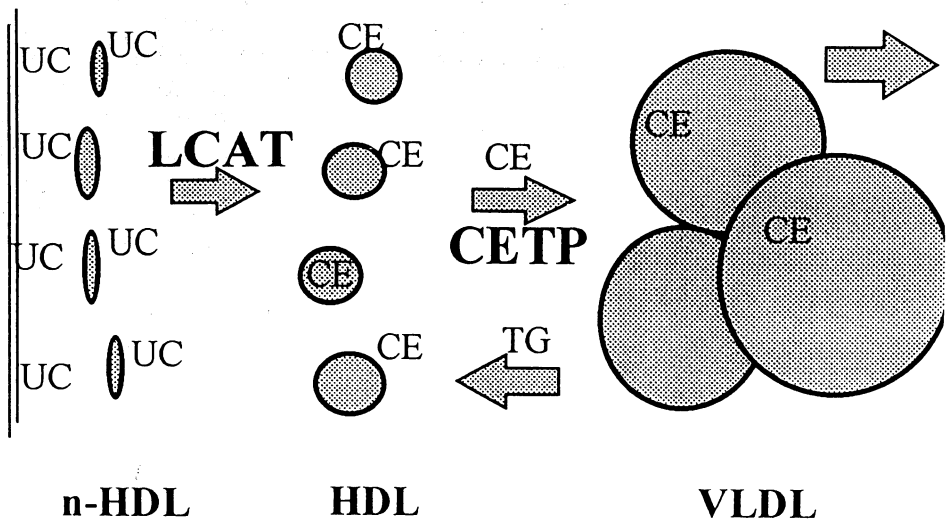

Fig. 1. *The original concept of reversed cholesterol transport.*

The original concept of RCT suggests the following steps (Fig. 1)

1. Cellular UC is accepted by nascent HDL (n-HDL) of discoidal shape that mostly contain UC.
2. LCAT esterifies UC and produces CE and LPC.
3. Discoidal HDL become spherical as CE's are incorporated into the core of the particles.
4. CETP transfers CE to VLDL; VLDL is catabolized to LDL *via* lipoprotein lipase. Triglycerides from VLDL are exchanged for CE of HDL. As in the case of LCAT, CETP activity is related to the availability of triglyceride-rich acceptor particles, not to CETP plasma concentration (Mann *et al.* 1991).
5. LDL transfers CE to the receptors on the surface of liver cells.

The current concept of RCT

1. Cell-derived UC is initially taken up by the lipid-poor pre-beta-1-HDL and subsequently transferred in the sequence: pre-beta-2-HDL to pre-beta-3-HDL to pre-alpha-HDL to LDL (Fig. 2). During the first five minutes of this process, only 5 % of cellular cholesterol is esterified in the pre-beta-3-HDL and alpha-HDL. The remainder reaches LDL as UC (Huang *et al.* 1993). Therefore, the major mediator in the transfer of cellular UC is LDL. Thus LDL becomes the major source of UC for the LCAT reaction; practically all the cholesterol substrate for LCAT originates from VLDL and LDL particles (Francone *et al.* 1990, Fielding *et al.* 1991, Huang *et al.* 1993).

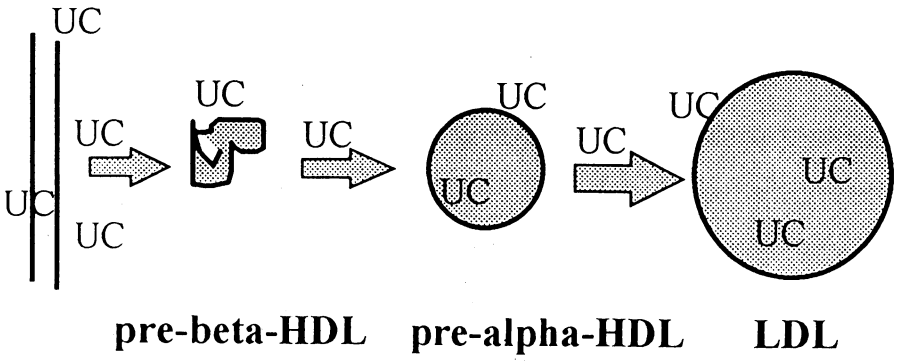

Fig. 2. *Transfer of cellular unesterified cholesterol.*

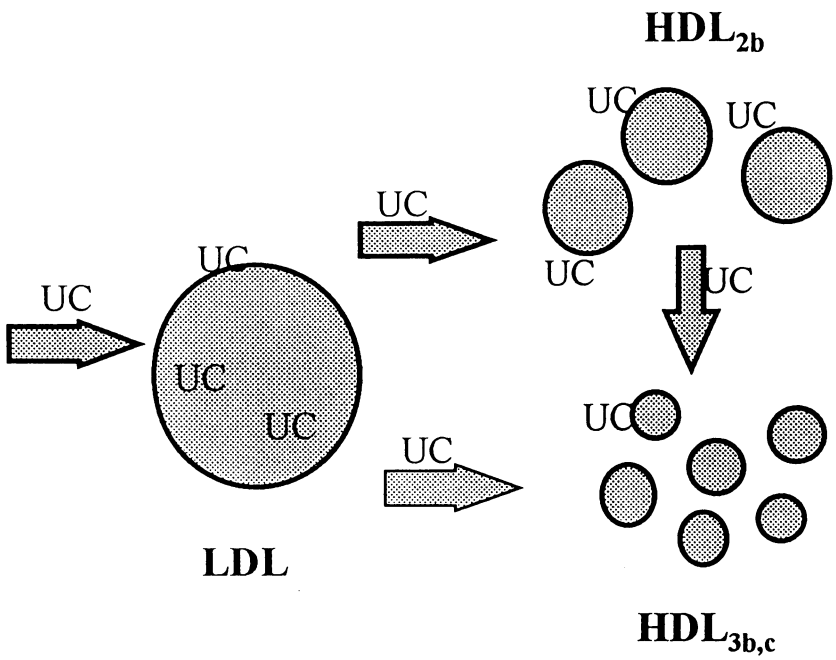

Fig. 3. *Redistribution of unesterified cholesterol from LDL.*

2. Cellular UC is mostly transferred from LDL to HDL (Fig. 3) into its largest fraction, namely the major alpha-migrating class (HDL_{2b}) (Miida *et al.* 1990). The largest fraction of the major alpha-migrating class of HDL(HDL_{2b}) is thus the major initial acceptor of LDL-derived cholesterol.

3. Kinetic analysis indicates a rapid secondary transfer of UC from HDL_{2b} to small alpha-HDL (particularly HDL_3) driven enzymatically by LCAT reaction (Fig. 3). The rates of transfer among alpha-HDLs were most rapid from the largest alpha-HDL fraction (HDL_{2b}) suggesting possible protein-mediated facilitation (Miida *et al.* 1990). When the LCAT reaction is inhibited, the transfer of UC from LDL to HDL ceases; this transfer therefore appears to be LCAT-dependent (Huang *et al.* 1993).

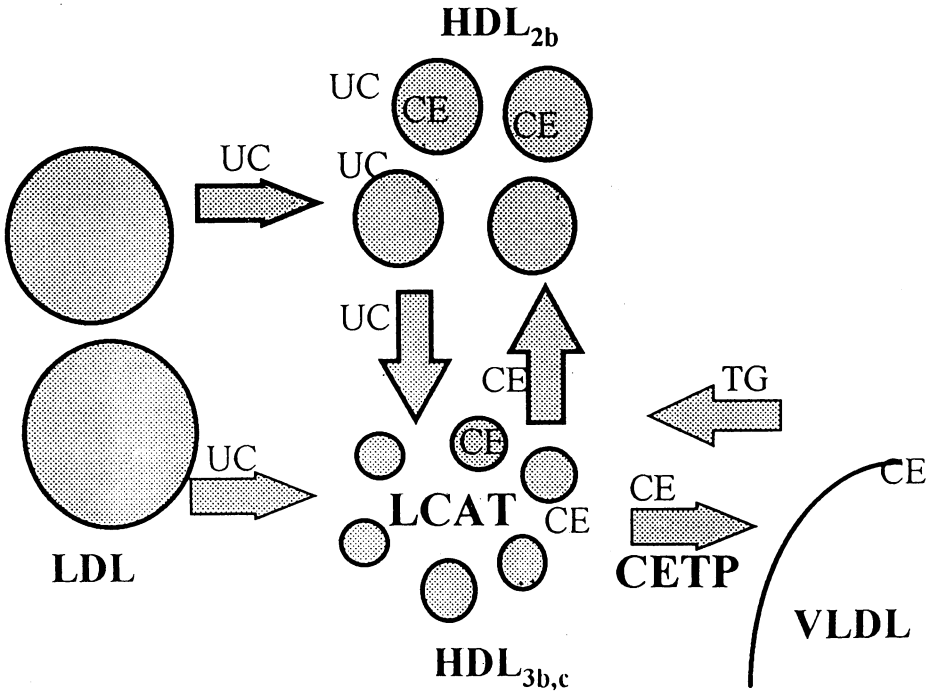

Fig. 4. *Unesterified cholesterol and cholesteryl ester transport in plasma with normal LDL/HDL ratio.*

4. The large HDL_{2bs} also take up most of the newly formed CE as VLDL and LDL accept only about 10 % of them (Francone *et al.* 1989) (Fig. 4). As mentioned above, HDL_{2b} particles inhibit LCAT activity and regulate the esterification rate in HDL (Karpe *et al.* 1990).

5. HDL may selectively deliver its cholesterol and cholesteryl esters to specific (SR-BI) liver and steroidogenic tissue receptors (Rigotti *et al.* 1997). While LDL catabolism involves cellular uptake and degradation of the entire particle by a well-characterized receptor, HDL bind to the cell surface, transfer their cholesterol into the cell, and the lipid-depleted HDL are released into the extracellular fluid (Pittman *et al.* 1987).

LDL concentrations and LCAT activity affect cellular cholesterol transport

1. LDL inhibits the efflux of cellular cholesterol; the esterification of labelled cellular cholesterol was completely inhibited by the addition of excess unlabeled LDL but not by excess of HDL (Huang *et al.* 1993, Nakamura *et al.* 1993). This may be related to the activities of plasma membrane caveolae that serve both as preferred acceptors of LDL derived cholesterol and also donors of UC to extracellular acceptors (Fielding and Fielding 1995b).

2. LCAT is not directly involved in the transfer of cellular UC as it only acts after the latter stage of transfer *via* LDL to large HDL and to small HDL.

3. LCAT activity is determined by the ratio of large and small HDL particles which in turn is probably determined by the activities of hepatic and lipoprotein lipases and cholesteryl ester transfer protein (CETP).

4. The increased esterification rate does not necessarily result in transfer of cellular cholesterol; it has been shown that higher esterification on HDL does not enhance cellular cholesterol efflux (Czarnacke and Yokoyama 1995, Huang *et al.* 1993, Sviridov and Fidge 1995). Conversely, complete LCAT deficiency does not prevent the UC efflux (Kuivenhoven *et al.* 1997).

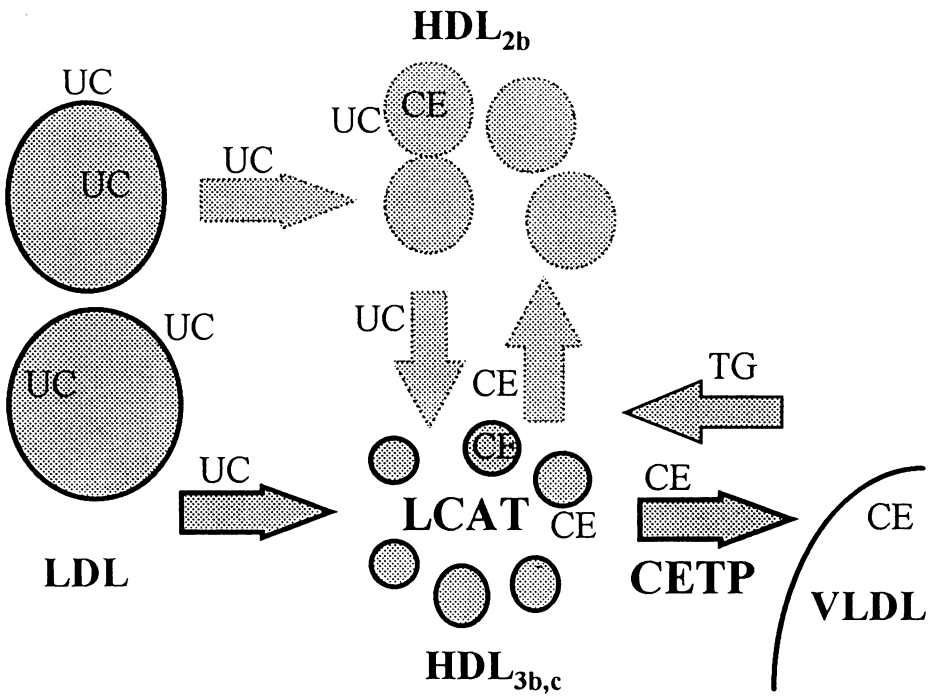

Fig. 5. *Unesterified cholesterol and cholesteryl ester transport in plasma with excess of LDL and lack of large HDL.*

How then does the LCAT reaction fit into the concept of RCT?

We suggest three possible scenarios that determine the role of LCAT in RCT:

1. Balanced ratio HDL and LDL (Fig. 4): LDL contains both cell-derived and other UC. Of the LDL-UC that is transferred to HDL a major part remains on large HDL_{2b} particles with a smaller amount transferred to and esterified on the small $HDL_{3b,c}$. Part of the newly formed CE is transferred to HDL_{2b} and part is exchanged for triglycerides with VLDL. In this scenario there is sufficient HDL_{2b} to regulate the rate of LCAT reaction and accept LDL-UC.

2. Excess of plasma LDL and sufficient amount of HDL_{2b}: The efflux of cellular UC is almost inhibited. However, a steady-state of LDL-UC transfer is maintained in the presence of sufficient HDL_{2b}.

3. Excess of LDL combined with a deficit of HDL_{2b} (Fig. 5): Not only is the efflux of cellular UC blocked but LDL-UC is taken up directly by the small $HDL_{3b,c}$. The esterification rate is high and, in the absence of HDL_{2b}, most newly formed CE are ultimately taken up by LDL (*via* VLDL and TG exchange). Thus, a decrease in HDL_{2b} not only increases the esterification rate but also results in a higher CE content of LDL.

The ratio of plasma concentration of LDL and HDL (particularly HDL_{2b}) plays a crucial role in the efflux and subsequent fate of cellular cholesterol. When LDL is high and HDL_{2b} is low, LCAT may paradoxically create a "reverse gradient" towards increased LDL-CE and thus to enhanced atherogenicity of the plasma.

Structural and functional assessment of HDL heterogeneity: measurement of esterification rate of cholesterol in plasma depleted of apo B lipoproteins (FER_{HDL})

It follows from the above arguments that the measurement of production of CE in individual plasma samples does not provide information on the direction of CE flow. The outcome of esterification may be different in the following scenarios: when there is a high level of large HDL (that give up only a small proportion of their CE to VLDL and LDL), a high rate of production of CE may be beneficial. However, when there is a lack of these particles, most of the produced CE is channelled into the futile cycle between VLDL and LDL resulting in an increase of CE in potentially atherogenic particles.

Thus, the information about the absolute rates of production of cholesteryl esters is useful when interpreted with the knowledge of the composition of HDL subpopulations. Therefore, a method of measurement of esterification rate in the plasma depleted of apo B lipoproteins (FER_{HDL}) (Dobiášová and Schützová 1986, Dobiášová *et al.* 1992, Dobiášová and Frohlich 1996), which reflects the distribution of HDL subpopulation, is a good indicator of the atherogenic potential of the plasma. We previously described the method in detail (Dobiášová and Frohlich 1996). It reflects the ability of the individual plasma HDL-LCAT complexes to esterify UC that had been transported by diffusion from the surfaces of other lipoproteins. Our studies showed that FER_{HDL} discriminates well between patients with major risk factors for atherosclerosis, those with symptomatic CAD and those without any risk factors. FER_{HDL} is significantly different in men and women with comparable plasma lipid concentrations (16.8 ± 4.5 % per hour and 10.6 ± 3.6 % per hour, respectively; $p < 0.001$) and correlate well with the HDL particle size distribution in the individual plasma. Plasma samples with a high relative content of HDL_{2b} (typical for women and healthy controls) have low FER_{HDL}, while men and persons with major risk factors for CAD have high FER_{HDL}, low HDL_{2b} and high HDL_{3b} (Dobiášová *et al.* 1991, 1992, 1995, Dobiášová and Frohlich 1996, Tan *et al.* 1998).

These reports also show that the FER_{HDL} value correlates well with the known lipid risk factors for CAD, such as the plasma level of triglycerides and HDL cholesterol concentration as well as with body mass. Recently, Ohta *et al.* (1997a) reported that FER_{HDL} also closely correlates with the relative concentration of small dense LDL, whereas high FER_{HDL} values reflects the atherogenic lipoprotein profile (Ohta *et al.* 1997b).

Experiments with LCAT transgenic animals

The differences in the effects of human LCAT gene transfer in cholesterol-fed mice (deleterious) (Francone *et al.* 1997, Mehlum *et al.* 1997, Berard *et al.* 1997) or rabbits and monkeys (protective) (Hoeg *et al.* 1996, Brousseau *et al.* 1997) have been explained by the formation of "dysfunctional" HDL in mice because of their lack of CETP.

On the other hand, Watanabe rabbits that lack LDL receptors have not benefitted from the expression of human LCAT (Brousseau *et al.* 1997). Interestingly. the LCAT transgenic animals appear to benefit not only from increased HDL but also from a decrease in LDL which appears to be receptor-mediated and the mechanism of which is unknown.

While these experiments added an invaluable new dimension to LCAT research they also stressed the following question: if increased LCAT activity is beneficial as suggested by these data, why do some homozygotes for LCAT deficiency have low LDL and apo B levels? On the other hand, these data agree with the observation of an atherogenic lipid profile in heterozygotes in many but not all LCAT-deficient families.

Furthermore, the effect of diet, including the fatty acid composition of lecithin, may be more profound in man than in experimental animals. Thus, we believe that it would be premature to consider an increase in LCAT activity to be an antiatherogenic factor in men.

Conclusions

The context in which the interaction between cellular UC, HDL and LCAT occurs determines if the esterification is "beneficial" or "harmful". In circumstances where LDL level is high and HDL_{2b} level is low, the LCAT reaction may further promote the flux of CE toward atherogenic LDL and VLDLs. A recent study dealing with the determinants of plasma

HDL cholesterol concentration in hypertriglyceridaemic patients (Brousseau *et al.* 1997) confirms this suggestion: hypertriglyceridaemic patients have approximately three times higher transfer rates of cholesteryl esters into VLDL despite little, if any, differences in the production rates of CE (measured as LCAT activity) from normotriglyceridaemic controls.

We suggest that the RCT hypothesis should be revised in that LCAT may function not only as an antiatherogenic (the "engine" of RCT) but also, in the above mentioned scenario, as an atherogenic factor. Whether LCAT activity is proatherogenic or antiatherogenic will depend on whether the products of the reaction, namely CE, are taken up by large HDL particles and partly delivered to the HDL receptors in the liver and steroidogenic tissues or by VLDL and LDL particles. The direction of this transfer is influenced by the concentration of LDL which inhibits the efflux of cellular cholesterol and increases the substrate availability for LCAT; similarly, the composition of HDL subpopulations plays a crucial role, with excess of small HDL particles accelerating the esterification rate, while the large HDLs serve as a scavenger for UC from LDL and for CE resulting from the LCAT reaction. These data also help to explain the very high predictive value of LDL/HDL cholesterol ratio (Castelli *et al.* 1986),

Finally, the factors that determine the composition of the HDL pool, namely the activities of hepatic and lipoprotein lipases and CETP, should be assessed in conjunction with LCAT activity and FER_{HDL} to better understand the mechanism of RCT.

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