Atherogenic Impact of Lecithin-Cholesterol Acyltransferase and Its Relation to Cholesterol Esterification Rate in HDL (FER\textsubscript{HDL}) and AIP [\log(TG/HDL-C)] Biomarkers: The Butterfly Effect?

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Summary
The atherogenic impact and functional capacity of LCAT was studied and discussed over a half century. This review aims to clarify the key points that may affect the final decision on whether LCAT is an anti-atherogenic or atherogenic factor. There are three main processes involving the efflux of free cholesterol from peripheral cells, LCAT action in intravascular pool where cholesterol esterification rate is under the control of HDL, LDL and VLDL subpopulations, and finally the destination of newly produced cholesteryl esters either to the catabolism in liver or to a futile cycle with apoB lipoproteins. The functionality of LCAT substantially depends on its mass together with the composition of the phospholipid bilayer as well as the saturation and the length of fatty acyls and other effectors about which we know yet nothing. Over the years, LCAT puzzle has been significantly supplemented but yet not so satisfactory as to enable how to manipulate LCAT in order to prevent cardiometabolic events. It reminds the butterfly effect when only a moderate change in the process of transformation free cholesterol to cholesteryl esters may cause a crucial turn in the intended target. On the other hand, two biomarkers – FER\textsubscript{HDL} (fractional esterification rate in HDL) and AIP [\log(TG/HDL-C)] can offer a benefit to identify the risk of cardiovascular disease (CVD). They both reflect the rate of cholesterol esterification by LCAT and the composition of lipoprotein subpopulations that controls this rate. In clinical practice, AIP can be calculated from the routine lipid profile with a help of AIP calculator www.biomed.cas.cz/fgu/aip/calculator.php.

Key words
Lecithin-cholesterol acyltransferase (LCAT) • Atherosclerosis • FER\textsubscript{HDL} (fractional esterification rate in HDL) • AIP (atherogenic index of plasma, log(TG/HDL-C)) • Biomarkers of cardiometabolic risk • Lipoprotein particle size

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Introduction

The butterfly effect expresses the sensitive dependence of the development of a system on initial conditions whose small changes can result in large variations...

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Lecithin cholesterol acyltransferase (LCAT, EC 2.3.1.43) is a plasma enzyme which catalyzes the transfer of fatty acids from 2n- lecithin to 3-hydroxy group of free cholesterol (FC) to form cholesteryl esters (CE) and lysolecithin (LPC). LCAT as the producer of a majority of cholesteryl esters in the human plasma has a unique role in the removal of unesterified cholesterol from peripheral cells, including macrophages into plasma lipoproteins in the pathway of reverse cholesterol transport (RCT) (Glomset \textit{et al.} 1966, Glomset 1968). This was the basis for considering its role in the atherogenic process. There was a long-term attempt for finding the evidence that would support the manipulation of LCAT in the treatment of diseases of atherogenic
origin. Even more than 50 years of monitoring on pro-
and anti-atherogenic LCAT impact did not provide
a reliable answer. Since 1962, when the LCAT
mechanism was first described (Glomset 1962), Google
has monitored about 660 000 records of LCAT. Among
them 86 000 records followed the relationship between
LCAT and atherosclerosis or cardiovascular diseases
(CVD). During this time thousands of details have been
discovered that complement the pathway of FC from
peripheral tissues to the intravascular pool, being
transformed by the action of LCAT into cholesteryl esters
which leave the pool. However, further knowledge about
the characteristics and modus operandi of LCAT, it
turned out that RCT pathway needs revising and
supplementing with other data. Plenty of reviews have
dealt with the issue of LCAT and its atherogenic aspects
(Glomset 1968, Hodh et al. 1980, Doβišová 1983,
Dobišová and Frohlich 1999, Jonas 2000, Roussel et al.
2009, Rader 2009, Calabresi and Franceschini 2010,
Verger et al. 2010, Roussel et al. 2011, Kunnen and Van
Eck 2012, Ng 2012, Calabresi et al. 2012, Levinson and
Wagner 2015, Ossoli et al. 2016). Some authors have
found a protective effect of LCAT in patients with
coronary heart disease (Solajic-Božič et al. 1991,
Solajic-Božičević et al. 1994, Hovingh et al. 2005, Sethi
et al. 2010). Others, and probably majority of them,
reported the increase in atherogenic risk by increasing the
activity of LCAT or cholesterol esterification rate
(Dobišová et al. 1991, Takao et al. 2001, Guerin et al.
2001, Santos et al. 2003, Frohlich and Dobišová 2003,
Dullaart et al. 2008, Dullart et al. 2010, Holleboom et
al. 2010, Khara et al. 2011, Dobišová et al. 2011, Li et
al. 2013, Kim et al. 2014).

Free cholesterol meets the LCAT on the way
from peripheral cells to the intravascular space and is
transformed into CEs which leave this space in two ways.
However, a number of small variations on this way may
change atherogenic/anti-atherogenic impact of LCAT.
Recently Philips (2014) reviewed four pathways for
exporting free cholesterol from macrophages to
extracellular HDL. The passive processes include simple
diffusion via the aqueous phase and facilitated diffusion
mediated by scavenger receptor class B, type I (SR-BI).
The data about FC diffusion via aqueous phase were
complemented by Czarnecka and Yokoyama (1995) in
an earlier report on the regulation of cellular cholesterol
eflux. SR-BI has also a significant role in the transfer of
CE into the catabolic process in the liver (Acton et al.
1996, Hoekstra 2017). The active pathways are mediated
by membrane lipid translocases such as the ATP-binding
cassette (ABC) transporters ABCA1 and ABCG1. As
stated by Philips (2014), both transporters mediate efflux
of cholesterol from cells, but the reaction mechanisms are
different. ABCG1 leads to redistribution of FC from cell
interior into the plasma membranes. When activated pool
of plasma membrane is created, it leads to the flux of
cholesterol mass out of the cell by aqueous diffusion
pathway, but not to lipid-free apoA. In contrast, the
ABCA1 activity in the plasma membrane promotes efflux
of phospholipids (with a preference of lecithin) and FC to
lipid-free apoA1 (plasma pre-β1/HDL).

When free cholesterol enters the intravascular
space, it encounters the LCAT. As a relative small
soluble glycoprotein (cca 67 kDa) LCAT operates in
an aqueous plasma medium with less or more
hydrophobic components. Therefore, the effectiveness
of LCAT and cholesterol esterification rate will depend on
many factors such as physicochemical nature of
cholesterol-phospholipid bilayer (Yokoyama et al. 1980,
Parks et al. 2000), the characteristics of the fatty acyls of
lecithin, activator and inhibitor proteins – apoAI and
apoAII (Fielding et al. 1972), apoC (Soutar et al. 1975),
specific cholesterol ATP-binding cassette transporters
ABCA1 and ABCG1 (Philips 2014) and the size of
lipoproteins.

The size of HDL, LDL and VLDL particles and
activity of LCAT probably represent the strongest
connection with atherogenic plasma profile. The
associations of high-density lipoprotein subclasses and
apolipoproteins with ischemic heart disease and coronary
atherosclerosis were found already in the eighties (Miller
1987). Barter et al. (1984, 1985) studied esterification
of cholesterol by LCAT in two HDL subfractions,
HDL-subfraction 3 (HDL3) and HDL-subfraction 2
(HDL2) and found that the two HDL subfractions may
compete for interactions with the enzyme. HDL3
subfraction was highly effective substrate, while
HDL2 subfraction function as an inhibitor of the
cholesterol esterification reaction. Newer method of
gradient gel electrophoresis in various gels (Nichols et al.
1986, Williams et al. 1990) enabled split subfractions
HDL2 and HDL3 into smaller subpopulations, which
confirmed that the smallest particles HDL3<sub>3b,c</sub>
are preferred substrate for LCAT, while the larger HDL2<br>
inhibit the reaction (Dobišová et al. 1991, Dobišová et
al. 1992). The development of new methods has allowed
to discover atherogenic potential of individual
HDL subpopulations. The initial dichotomy of HDL2 and
HDL3 did not bring unambiguous view relative to the CVD, while the NMR spectroscopy is able to do it (Freedman et al. 1998, Jeyarajah et al. 2006, Movva and Rader 2008, Dobiášová et al. 2011, Haﬁane and Genest 2015). These speciﬁc techniques conﬁrmed that the best substrate for LCAT are only small HDL (HDL_{3bc}) particles from the whole population of HDL3. The HDL small particles are substantially correlated with the cardiovascular risk, similarly as small dense LDL particles (Austin et al. 1988) and large VLDL particles. Large LDLS and particularly large HDL have shown reduced CVD risk.

Moreover, the removal of highly hydrophobic cholesteryl esters out of the intravascular space is also subjected to the regulation by lipoproteins of different size. In the first case the efﬂux of cholesteryl esters associated with the HDL2 and SR-BI is directed into liver size. In the second case the efﬂux of cholesteryl esters subjected to the regulation by lipoproteins is the method of “cold labeled substrate” (Dobiášová and Schützová 1986, Dobiášová and Frohlich 1987), LCAT activities in 120 human sera showed 4.4- to 5.4-fold higher values obtained by self-substrate radioassay method. Not only the speciﬁcity of different substrates determines values obtained but in radioassays with 3H-cholesterol based upon the original publication of Stokke and Norum (1971). However, in the recent years, ﬂuorometric methods prevail (Parks et al. 2000, Homan et al. 2013). The differences in LCAT activity in a variety of substrates can be enormous. For example, using DMPC (dimyristylphosphatidylcholine)-cholesterol liposomes as the substrate (Manabe et al. 1987), LCAT activities in 120 human sera showed 4.4- to 5.4-fold higher values obtained by self-substrate radioassay method. Not only the speciﬁcity of different substrates determines values obtained but in radioassays it is essential how the radioactive label (1H-cholesterol) is incorporated into the substrate (Dobiášová et al. 2000). Perfect equilibration of the cholesterol label with lipoproteins is the method of “cold labeled substrate” (Dobiášová and Schützová 1986, Dobiášová and Frohlich 1996) which allows the transfer of radioactive label from the small ﬁlter paper disc into a solution of lipoprotein in the cold without the intervention of chemicals, heat or non-standard emulsion with albumin.

**LCAT activity**

The purpose of determining the activity of LCAT is to establish the efficiency of autologous enzyme relative to the substrate which provides FC and which stimulates or limits its capacity. Many different substrates were used, such as liposomes, proteoliposomes (Vaisman and Remaley 2013) or plasma lipoproteins. The methods for measuring the activity of LCAT *in vitro* are focused on the direct measurement of a decrease of free cholesterol, on CE increase in a mixture of the enzyme and variety substrates, and on the ﬂuorometric assay of LCAT phospholipase activity. The decrease of FC is measured by gas-liquid chromatography (Marcel and Vezina 1973) or by enzymatic methods (Patsch et al. 1976). The determination of the activity LCAT as CE production is carried out in countless variations of radioassays with 1H-cholesterol based upon the original publication of Stokke and Norum (1971). However, in the recent years, ﬂuorometric methods prevail (Parks et al. 2000, Homan et al. 2013). The differences in LCAT activity in a variety of substrates can be enormous. For example, using DMPC (dimyristylphosphatidylcholine)-cholesterol liposomes as the substrate (Manabe et al. 1987), LCAT activities in 120 human sera showed 4.4- to 5.4-fold higher values obtained by self-substrate radioassay method. Not only the speciﬁcity of different substrates determines values obtained but in radioassays it is essential how the radioactive label (1H-cholesterol) is incorporated into the substrate (Dobiášová et al. 2000). Perfect equilibration of the cholesterol label with lipoproteins is the method of “cold labeled substrate” (Dobiášová and Schützová 1986, Dobiášová and Frohlich 1996) which allows the transfer of radioactive label from the small ﬁlter paper disc into a solution of lipoprotein in the cold without the intervention of chemicals, heat or non-standard emulsion with albumin.

**CER – cholesterol esterification rate and FER_{HDL} – fractional cholesterol esterification rate in apoB-lipoprotein depleted plasma**

The problem of a method for the determination
of cholesterol esterification rate (CER) is in the origin of free cholesterol that enters the LCAT reaction in the whole plasma. Since the esterification takes place mostly in HDL, a source of free cholesterol may be peripheral cells (including macrophages and foam cells), which are mostly not able to metabolize cholesterol. However, another source of free cholesterol for the esterification can be FC transported into HDL via aqueous diffusion from other lipoproteins. The paradox is that during the first five minutes of the process, only 5% of cellular cholesterol is esterified in the pre-beta-3-HDL and alpha-HDL before it reaches LDL (Miida et al. 1990). As the remainder of free cholesterol first reaches other lipoproteins containing apoB and then it comes freely transferred into HDL subpopulations that specifically regulate the rate of its esterification, it was concluded that practically all free cholesterol substrate for the LCAT procedure originates from LDL and VLDL particles (Huang et al. 1993).

Plans for use of LCAT procedures in treatment of CVD

The measurement of LCAT mass, LCAT activity and LCAT esterification rate produced the hypothesis that by increasing the concentration of LCAT might be achieved a support of the RCT and the FC efflux from peripheral cells with resulting therapeutic effect in patients with atherosclerosis. However, this hypothesis in animal models has not proven. Over-expression of human LCAT in transgenic rabbits prevented diet-induced atherosclerosis (Hoeg et al. 1996, Brousseau et al. 1997). On the other hand, over-expression of human LCAT in mice did not prevent diet-induced atherosclerosis (Francone et al. 1997, Mehmum et al. 1997). On the contrary, high plasma HDL concentrations were associated with enhanced atherosclerosis in this animal model.

Not even 10 years later, large clinical trials found no protective effect of high concentration of HDL induced by the inhibition of cholesteryl ester transfer protein with torcetrapib (Nissen et al. 2007). Neither the further studies with torcetrapib derivatives have brought a clinical benefit (Eyvazian and Frishman 2017). These experiences support the idea that a moderate changes in the process of transformation free cholesterol to cholesteryl esters may cause a butterfly effect by a change of the intended target.

FER_{HDL} – fractional esterification rate in HDL

We have tried to compare LCAT esterification rate in normal population of men and women, in those with risks of cardiometabolic diseases and in those who already suffer of them. We studied that part of LCAT process (FER_{HDL}), which directly focuses on a change of free cholesterol into cholesteryl esters that takes place in HDL (Dobiášová and Frohlich 1996). The principle of the method is a radioassay of cholesterol esterification in the HDL labeled with 3H-FC (Dobiášová et al. 1992) in plasma depleted of apoB containing lipoproteins. This depletion makes it possible to omit a secondary source of FC from LDL and VLDL. Thus, FER_{HDL} measures autologous capacity of LCAT and HDL in the natural plasma environment, which allows to eliminate the effect of other reaction products such as lyssolecithin, whose increased concentration was observed in cardiovascular diseases and is able to inhibit the LCAT reaction in vitro (Wells et al. 1986). FER_{HDL} depends only slightly on the concentration of plasma total or LDL-cholesterol but considerably correlates with a rise of triglycerides and a fall in HDL-cholesterol. FER_{HDL} is controlled by a relative occurrence of large and small HDL particles in which LCAT reaction takes place. Surprisingly, there is also a highly positive correlation between FER_{HDL} and small-dense LDL particles and large VLDL particles, which had been removed prior to the reaction. This suggests an intimate relationship between apoA and apoB lipoproteins in the actual plasma (Ohta et al. 1995, Dobiášová et al. 2011). FER_{HDL} may therefore be regarded as a marker of plasma phenotype, since it reflects the distribution of differently-sized particles of lipoproteins.

It was shown that FER_{HDL} is significantly elevated in humans at risk of atherosclerosis, e.g. higher in men than in women (those before menopause), higher in hypertensives and diabetics type 2 or in patients with positive findings on coronary angiography (Frohlich and Dobiášová 2003, Dobiášová et al. 2011) and those with risk factors of CVD (Rašlová et al. 2011). Although FER_{HDL} can predict the particle size in HDL and LDL, which, in turn, predicts CVD risk, it is not applicable to routine clinical examinations.

AIP – atherogenic index of plasma [log(TG/HDL-C)]

We looked for a clinically available marker that could at least partially replace the FER_{HDL} test, which takes into account lipoprotein particle size distribution. Since Gaziano et al. (1997) reported that the ratio of triglycerides to HDL was a strong predictor of myocardial infarction, additional findings have been
made regarding the relationship between HDL-C and TGs. Although an independent, inverse relationship between HDL-C and cardiovascular risk was demonstrated beyond any doubt (Miller 1987), the contribution of triglycerides to cardiovascular risk was underestimated. However, triglycerides play a role in the regulation of lipoprotein interactions but they cannot serve convincingly as an independent marker of CVD. Among the first, who described the importance of linking triglycerides with HDL to predict CVD risk, was Treatment Panel III, referring this combination as an atherogenic dyslipidemia (Grundy et al. 2004). This claim is supported by the evidence that an increased plasma concentration of TGs is associated with increased populations of small dense LDL and large VLDL, with elevated cholesteryl ester transfer from HDL to apoB containing lipoproteins (Guerin et al. 2001) and also with an increased incidence of coronary artery disease (Hokanson and Austin 1996). Triglycerides have been proposed to be a major determinant of cholesterol esterification/transfer and HDL remodeling in human plasma (Murakami et al. 1995).

Increased ratio TG/HDL-C thus represents a cardiometabolic risk which is determined mainly by the increase in risk subpopulations – large VLDL and small LDL and small HDL. Higher concentrations of total HDL cholesterol contain more cardioprotective HDL_{3b} subpopulation and large LDL (Jeyarajah et al. 2006). For better expression of the relationship between triglycerides and HDL-C, we adopted the log-transformed molar ratio of TG/HDL-C, which we called atherogenic index of plasma (AIP). AIP is used as a logarithmically transformed value because in this form it produced better correlations and normal probability plots (Tan et al. 2004) and thus, it is more suitable from the statistical perspective than simple TG/HDL-C ratio (Urbina et al. 2011). The correlation plot between simple TG/HDL-C ratio and HDL and LDL particle sizes is curvilinear, while with log-transformed TG/HDL-C ratio is linear (Dobiášová et al. 2011).

Table 1. Atherogenic impact of LCAT, FER_{HDL}, and AIP biomarkers: the correlation between AIP and the size of lipoprotein subpopulations (adapted from Dobiášová et al. 2011).

| AIP  | r   | AIP  | r   | AIP  | r   |
|------|-----|------|-----|------|-----|
| HDL large | -0.597<sup>a</sup> | LDL large | -0.670<sup>a</sup> | VLDL large | 0.816<sup>a</sup> |
| HDL small | 0.272<sup>b</sup> | LDL small | 0.477<sup>a</sup> | VLDL small | -0.184<sup>c</sup> |

The bivariate correlation coefficients (r) between AIP and size of lipoprotein subpopulations. <sup>a</sup> p<0.0001, <sup>b</sup> p<0.002, <sup>c</sup> p<0.01.

We found that both tests – esterification rate in HDL (FER_{HDL}) as well as AIP – are controlled by the same size of the lipoproteins and highly correlate with each other (Fig. 1). The significance of lipoprotein particle size for determining AIP is shown also by the respective correlation coefficients r (Table 1) (Dobiášová et al. 2011). AIP correlates inversely with large HDLs and large LDL, which may be considered as anti-atherogenic effect, while AIP correlates positively with small dense LDLs and large VLDLs (atherogenic
effect). The values for both $\text{FER}_{\text{HDL}}$ and AIP increased significantly with increasing atherogenic risk (Dobiášová and Frohlich 2001, Frohlich and Dobiášová 2003, Rašlová et al. 2011).

During the past years AIP was frequently used for the examination of different risk subjects (Dobiášová et al. 2001, Di Castelnuovo et al. 2007, Onat et al. 2010, Nwagha et al. 2010, Soška et al. 2011, Soška et al. 2012, Stefanović et al. 2012, Dos Santos-Weiss et al. 2013, Onyedum et al. 2014, Essiarab et al. 2014, Hermans et al. 2012, Akbas et al. 2014, Vrablík et al. 2014, Niroumand et al. 2015, Zhu et al. 2015, Nunes et al. 2015, Shen et al. 2017). When using readily available biochemical assays, AIP provides valuable information about the atherogenicity of plasma and may quantify the response to therapeutic intervention. The calculator of AIP, which sequesters investigated subjects into three categories of CVD risk (AIP Calculator on line), may be helpful in clinical practice.

Conclusions

The final atherogenic or antiatherogenic effects of LCAT depend on a balance between the efflux of free cholesterol from peripheral cells to the extracellular space, its esterification and destinations of CE produced either into a futile excessive cycle together with apoB lipoproteins back to the plasma membranes or together with HDL$_{2b}$ and SR-BI into the protective catabolism in the liver. Even if we comprehensively consider all LCAT activities when planning the therapy of cardiovascular diseases, it still remains a space for the butterfly effect when just a tiny deviation in a set of steps will change the sign of the impact. This does not detract from the importance of LCAT in maintaining the cholesterol metabolic balance and opens next possibilities for meaningful research. Biomarkers $\text{FER}_{\text{HDL}}$ and AIP can help to reveal the risk of CVD having in mind that it reflects not only the lipid concentrations but also the interaction of LCAT with tiny changes in lipoprotein subpopulations.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

ABCA1 and ABCG1, ATP-binding cassette transporters; AIP, atherogenic index of plasma ($\log(\text{TG}/\text{HDL-C})$); apoA, apoprotein A; apoB, apoprotein B; apoC, apoprotein C; C, cholesterol; CE, cholesteryl ester; CETP, cholesteryl ester transferring protein; CVD, cardiovascular disease; FER$_{\text{HDL}}$, fractional esterification rate in HDL; FC, free cholesterol; HDL, high density lipoprotein; HDL-C, HDL-cholesterol; LCAT, lecithin cholesterol acyltransferase; LDL, low density lipoprotein; LDL-C, LDL-cholesterol; NMR, nuclear magnetic resonance; RCT, reverse cholesterol transport; SR-BI, scavenger receptor class B type I; TG, triglycerides; VLDL, very low density lipoprotein; VLDL-C, VLDL-cholesterol.

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