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STATIN-PARAOXONASE INTERACTIONS DURING STATIN TREATMENT

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I. INTRODUCTION

Over the last few decades, our understanding of the basic mechanisms involved in atherosclerosis has progressed significantly. The pathological effects of atherosclerosis occur over decades. A subtle injury to the endothelium initiates the atherosclerotic process. Endothelial dysfunction underlies many stages in the progression of atherosclerosis from earliest onset to the lesions that result in coronary heart disease (CHD).

Foam cells may infiltrate the vessel, progressing to a fatty streak. As the lesion progresses, small pools of extracellular lipid form within the smooth muscle layers, disrupting the intimal lining of the vessel. Progression to an advanced lesion, or atheroma, occurs when accumulated lipid, cells, and other plaque components disrupt the arterial wall. (Pepine et al 1998) (**Fig.1.**)

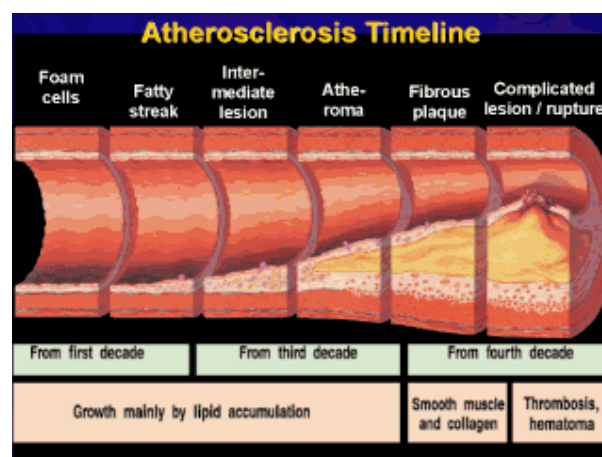


Fig. 1. Progression of atherosclerosis
Pepine CJ. Am J Cardiol. 1998.

Progression of atheroma involves accumulation of smooth muscle cells that elaborate extracellular matrix macromolecules. Once the plaque becomes fibrous, the danger of rupture increases. The clinically important complication of atheroma usually involve thrombosis. Arterial stenoses by themselves seldom cause acute unstable angina or acute myocardial infarction. Indeed, sizable atheroma may remain silent for decades or produce only stable symptoms, such as angina, precipitated by increased demand. Thrombus formation usually occurs because of the physical disruption of atherosclerotic

plaque. The majority of coronary thromboses result from a rupture of the plaque's protective fibrous cap, which permits contact between blood and the highly thrombogenic material located in the lesion's lipid core. The endothelium participates in the atherosclerotic process and remodeling through secretion of specific compounds. (Pepine et al 1998, **Fig.1.**)

The concentration of serum low-density lipoprotein (LDL) is directly related to the likelihood of developing atherosclerosis. One current theory to explain the development of the foam cell-laden fatty streaks in the arterial wall, which are believed to initiate atherosclerosis, proposes that oxidative modification of LDL is critical. Oxidation of LDL initiates the atherosclerotic process in the vessel wall by acting as a potent stimulus for the induction of inflammatory gene products in vascular endothelial cells. By activating the nuclear factor κ B (NF κ B) transcription factor, oxidized LDL (oxLDL) stimulates increased expression of cellular adhesion molecules. There are several different types of adhesion molecules with specific functions in the endothelial leukocyte interaction: The selectins trap monocytes and other leukocytes. Importantly, vascular cell adhesion molecules (VCAMs) and intercellular adhesion molecules (ICAMs) mediate firm attachment of these leukocytes to the endothelial layer (**Fig.2.**).

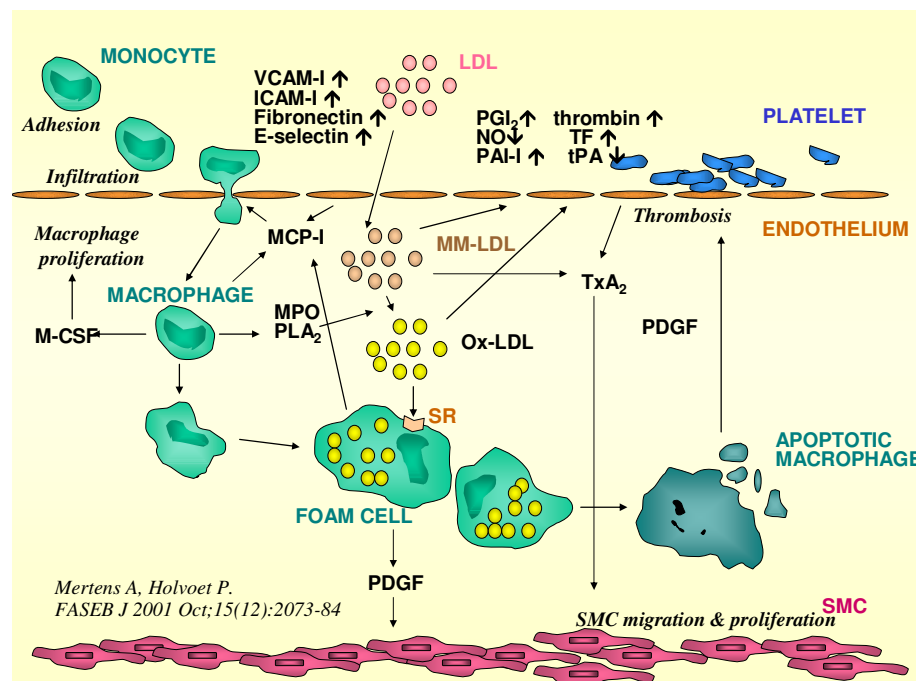


Fig. 2. Effect of oxLDL on monocyte infiltration.
Mertens A, Holvoet P. FASEB J. 2001 Oct;15(12):2073-84

OxLDL also augments expression of monocyte chemoattractant protein 1 (MCP-1) and macrophage colony stimulating factor (M-CSF). MCP-1 mediates the attraction of monocytes and leukocytes and their diapedesis through the endothelium into the intima. M-CSF plays an important role in the transformation of monocytes to macrophage foam cells. Macrophages express scavenger receptors and take up and internalize oxLDL in their transformation into foam cells. Migration of smooth muscle cells (SMCs) from the intima into the media is another early event initiating a sequence that leads to formation of a fibrous atheroma. **(Fig.2.)**

Plasma lipid levels are important factors in atherosclerosis. Regulation of cholesterol levels is a complicated process, involving cholesterol uptake, biosynthesis, transport, metabolism, and secretion. The concentration of plasma cholesterol depends on the integrated balance of the endogenous and exogenous pathways of cholesterol metabolism. Plasma cholesterol concentrations are maintained by biosynthesis through the endogenous pathway and absorption of dietary and biliary cholesterol through the exogenous pathway. In the endogenous pathway, cholesterol is synthesized by the liver and extrahepatic tissues and secreted into plasma, whereas the intestine is the primary site of the exogenous pathway of dietary cholesterol uptake. Alteration of either pathway will affect the concentration of plasma cholesterol

1. Lipid metabolism and its key enzymes

There are three main pathways responsible for the generation and transport of lipids within the body. These pathways include the exogenous pathway, the endogenous pathway, and the pathway of reverse cholesterol transport. (Tailleux et al 2002)

1.1. Exogenous pathway

After a meal, intestinal cells absorb fatty acids and cholesterol, esterifies them into triglyceride (TG) and cholesteryl ester, and incorporates them into the core of chylomicrons. Triglyceride greatly predominates over cholesterol ester in the

chylomicron core. The chylomicrons are secreted into plasma, where apolipoprotein (apo) C-II on the chylomicron surface activates endothelial-bound lipoprotein lipase (LPL). LPL in turn hydrolyzes the chylomicron's core triglyceride and releases free fatty acids, which are taken up by adipose tissue for storage and by muscle for energy. During lipolysis, the chylomicron decreases in size, and some surface components are transferred to the high-density lipoprotein (HDL); the remaining molecule is the chylomicron remnant particle. This chylomicron remnant next acquires apo E from HDL and is subsequently taken up by the liver after binding to sites that recognize apo E. It is then degraded, thereby delivering dietary cholesterol to the liver. (Tailleux et al 2002, **Fig.3.**)

1.2. Endogenous pathway

The endogenous pathway involves the liver synthesizing lipoproteins. TG and cholesterol ester are generated by the liver and packaged into very low density lipoprotein (VLDL) particles and then released into the circulation. VLDL is then processed by LPL in tissues to release fatty acids and glycerol. The fatty acids are taken up by muscle cells for energy or by the adipose cells for storage. Once processed by LPL, the VLDL becomes a VLDL remnant. The majority of the VLDL remnants are taken up by the liver via the LDL receptor, and the remaining remnant particles become intermediate-density lipoprotein (IDL), a smaller, denser lipoprotein than VLDL. The fate of some of the IDL particles requires them to be reabsorbed by the liver (again by the LDL receptor); however, other IDL particles are hydrolyzed in the liver by hepatic-triglyceride lipase to form LDL, a smaller, denser particle than IDL (**Fig.3.**).

LDL is the main carrier of circulating cholesterol within the body, used by extra-hepatic cells for cell membrane and steroid hormone synthesis. Much of the LDL particles are taken up by LDL receptors in the liver; the remaining LDL is removed by way of scavenger pathways at the cellular level. As LDL is taken up by receptors, free cholesterol is released and accumulates within the cells. LDL receptor activity and uptake of LDL regulate plasma LDL concentration by several mechanisms, including decreasing the synthesis of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (which controls the rate of cholesterol synthesis), suppressing the synthesis of new LDL receptors in the cells, and activating the enzyme, acyl-coenzyme A

cholesterol acyltransferase, which esterifies free cholesterol into cholesterol ester, storing cholesterol in the cell. (Hachem et al 2006)

The liver secretes triglyceride-rich VLDL into plasma, where they too acquire apo C-II from HDL. As with chylomicrons, VLDL interacts with LPL on the capillary endothelium, and the core triglyceride is hydrolyzed to provide fatty acids to adipose and muscle tissues. About half of the catabolized VLDL remnants (IDL density) are taken up by hepatic receptors that bind to apo E for degradation; the other half — apo B-100 particles, depleted of triglyceride relative to cholesteryl ester — are converted by the liver to cholesteryl ester-rich LDL. As IDL is converted to LDL, apo E becomes detached, leaving only one apolipoprotein, apo B-100. Each particle in this cascade from VLDL to LDL contains one molecule of apo B-100.

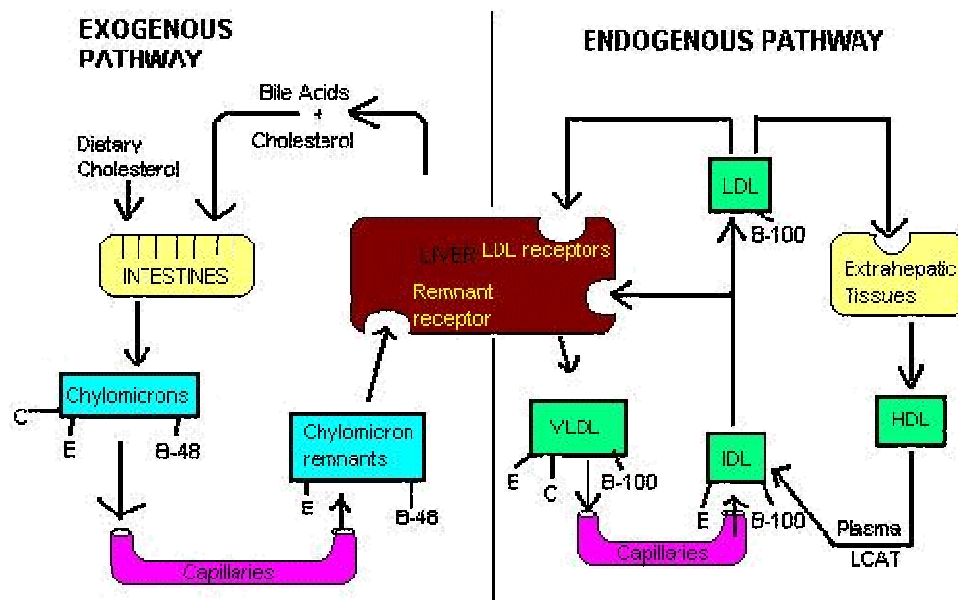


Fig. 3. Lipid metabolism

Tailleux A, et al. Atherosclerosis. 2002 Sep;164(1):1-13

In the metabolism of both chylomicrons and VLDL, apo C-II permits the hydrolysis of triglyceride by lipoprotein lipase, and apo E prompts hepatic uptake of remnants. A major difference in the metabolism of these particles is that chylomicrons contain a truncated form of apo B (i.e., apo B-48), whereas VLDL contains the complete form (i.e., apo B-100). Another difference is that chylomicron remnants are

degraded after they are absorbed by the liver, whereas many of the VLDL remnants are most likely processed in the hepatic sinusoids to become LDL. (Tailleux et al 2002)

1.3. Cholesteryl ester transfer protein (CETP)

Cholesteryl ester transfer protein (CETP) is a glycoprotein that is secreted mainly by the liver. CETP circulates in the plasma bound to lipoproteins, mainly to HDLs. Two principal actions of CETP have been identified. The primary action of CETP is to mediate the transfer of cholesteryl esters from HDL to VLDL and LDL in exchange for triglyceride. CETP also promotes the transformation of HDL2 to HDL3, an action that could promote reverse cholesterol transport. CETP inhibition, on the other hand, results in an increase in HDL by markedly delaying catabolism of apoA-I and A-II. This action also can increase reverse cholesterol transport. This overlap of the potential effects of CETP and CETP inhibition has served to confound an understanding of potential therapeutic mechanisms in atherosclerosis. (Klerkx et al 2006)

The effect of CETP expression on atherosclerosis in mice is highly variable and dependent on the model used. In general, it is anti-atherogenic in models that have relatively intact clearance of apoB-lipoproteins and pro-atherogenic in models that have markedly impaired apoB-lipoprotein turnover (Rader 2009). Previous studies of CETP inhibition in rabbits have consistently shown a reduction in atherosclerosis (Rader 2004). However, a large clinical outcome trial of the CETP inhibitor torcetrapib was terminated early due to significantly increased cardiovascular events and total mortality, despite effective elevation of HDL-C levels (Barter 2007). Complicating the interpretation of these studies, however, is the fact that torcetrapib can raise blood pressure through direct effects on adrenal steroidogenesis. Therefore, the effect of CETP inhibition on atherosclerosis in humans remains unresolved (Rader 2009).

1.4. Lecithin-cholesterol acyltransferase (LCAT)

Lecithin-cholesterol acyltransferase is a plasma enzyme catalyzing the transfer of a fatty acid from the 2-position of lecithin to the 3-beta-OH group of the free cholesterol, to produce cholesteryl ester and lysolecithin. LCAT is responsible for the generation of the majority of the cholesterol esters present in human plasma. The conversion of

cholesterol to cholesterol esters is necessary to facilitate reverse cholesterol transport, the process which removes excess cholesterol and phospholipids from peripheral cells and delivers them to the liver for disposal. The LCAT reaction occurs primarily on the surface of high-density lipoproteins.

The relationship of LCAT to atherosclerosis is complex. In humans, LCAT deficiency is not obviously associated with increased risk of atherosclerosis, although LCAT clearly has an important role in HDL metabolism suggesting that LCAT has an antiatherogenic effect, especially in the presence of CETP (Jin W 2002).

2. The protective role of HDL in atherogenesis

The association between low levels of high-density lipoprotein cholesterol (HDL-C) and an increased risk for cardiovascular disease has been well established. Experimental, epidemiologic, clinical, and therapeutic intervention investigations have shown that there is an inverse relationship between HDL-C and atherosclerotic disease. The World Health Organization analysed data from 19 countries and showed an inverse correlation between HDL values and mortality due to cardiovascular disease. (Simons LD et al 1986)

Combined analysis of the Framingham and MRFIT (Multiple Risk Factor Intervention Trial) control groups revealed that, after the adjustment for other risk factors, for each 1 mg/dl reduction in HDL-C, the risk for CHD increases 3% in women and 2% in men. (Gordon et al 1989)

Based on meta-analysis of these data, the HDL particle has recently come into focus of intensive research.

2.1. The structure and composition of HDL

High-density lipoproteins are a fraction of serum lipoproteins characterized by similar molecule density ($1.063 < d < 1.21$ g/ml) and size (5–17 nm in diameter). As a result of qualitative and quantitative differences in lipid, protein and enzyme content HDL particles are multi-shaped molecules with varying density, fluidity, charge, and antigenicity. HDL particles consist of 50% apoproteins (AI in larger quantity, AII, CI, CII, CIII, E, and J), 20% of free cholesterol (FC) and esterified cholesterol (CE), 15%

of phospholipids (PL), and 5% of triglycerides. The most frequently used method of lipoprotein isolation — the isopycnic ultracentrifugation — separates two major HDL fractions—HDL₂ ($d=1.063\text{--}1.0125$ g/ml) and HDL₃ ($d=1.125\text{--}1.250$ g/ml). Most of the HDL particles have a globular shape. Unesterified cholesterol distributes between the surface and the core of HDL particles. Charged lipids, phospholipids, and proteins are found primarily in outer parts of the lipoproteins. The most abundant protein of HDL is apolipoprotein A-I. Apo A-II, apo A-IV, apo-C's, apo E, and apo J are found in lower amounts. Some proteins associated with HDL have enzymatic activity. The best-known are the lecithin-cholesterol acyltransferase, the cholesterol ester transfer protein and the phospholipid transfer protein (PLTP), the platelet activating factor acetylhydrolase (PAFAH), and the paraoxonase. Nonpolar elements of HDL such as triglycerides and esterified cholesterol are localized in the center of the lipoprotein molecule. (Forti et al 2006)

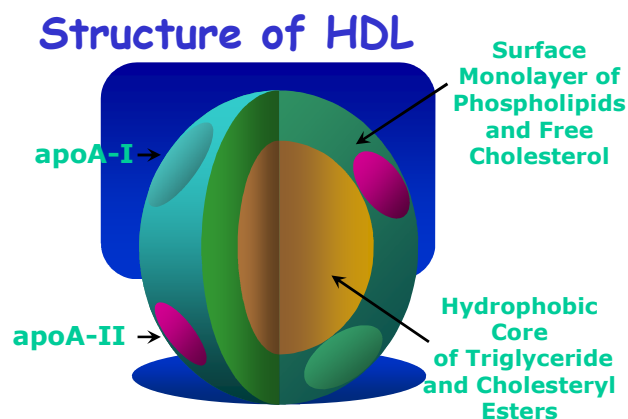


Fig.4. Structure of HDL
www.lipidsonline.org

Several steps are involved in the formation of HDL. These steps can be called the maturation of HDL (**Fig. 5.**).

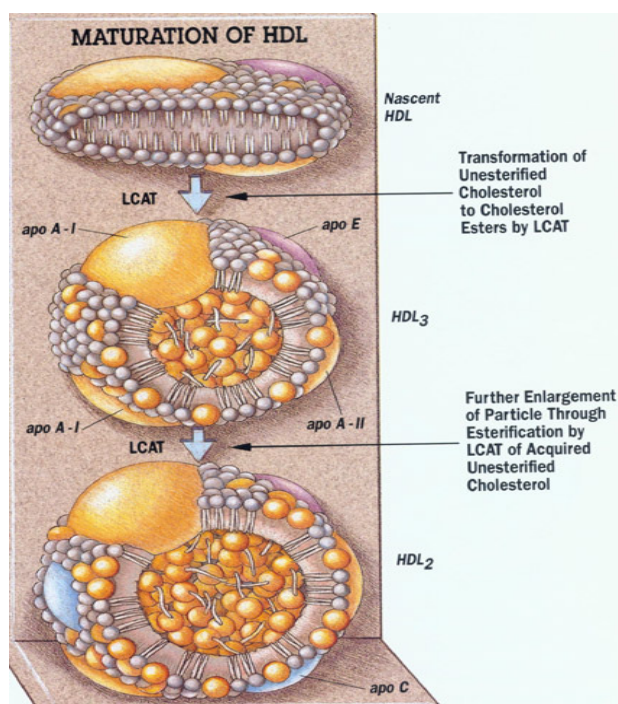


Fig.5. Maturation of HDL

Grundy, S.M.: *Atlas of lipid disorders, unit 1.* Gower Medical Publishing, New York, 1990.

The liver and gut secrete precursor particles called nascent HDL that consists largely of apo A's and phospholipids. Newly synthesized HDL molecules lack the central nonpolar core and exhibit a disc-like bilayer structure. These disc shaped particles circulate in the plasma and acquire unesterified cholesterol that becomes esterified by receiving a fatty acid from lecithin. This reaction is catalyzed by the LCAT. Cholesterol esters enter the core of the lipoprotein to produce a spherical particle called HDL₃. This lipoprotein contains both apo A-I and apo A-II. HDL₃ can acquire more cholesterol ester to become HDL₂. The latter contains more apo A-I than apo A-II, but it also has small quantities of apo C's and apo E's. HDL₃ and HDL₂ undergo interconversions, which is called the HDL cycle (**Fig.6.**).

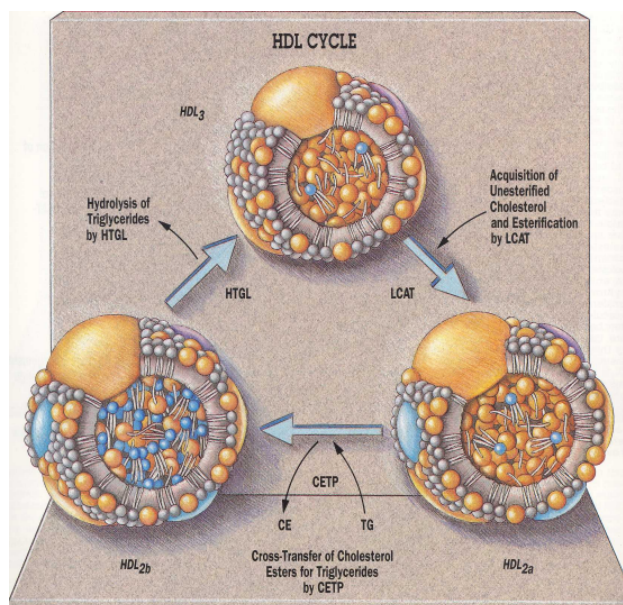


Fig. 6. HDL cycle

Grundy, S.M.: *Atlas of lipid disorders, unit 1*. Gower Medical Publishing, New York, 1990.

The first product of further enrichment of HDL₃ with cholesterol esters is HDL_{2a}. Some of the cholesterol ester in HDL_{2a} is transferred to VLDL in exchange for triglycerides; this exchange is mediated by cholesterol-ester transport protein (CETP). Enrichment of HDL₂ with triglyceride leads to HDL_{2b}, the triglyceride of which are then hydrolyzed by hepatic triglyceride lipase (HTGL) converting HDL_{2b} back to HDL₃. These processes cause that the HDL particles in human plasma are heterogeneous (**Fig.7**). They vary in particle shape, in apolipoprotein composition, lipid composition, and size (Mineo et al 2006). The clinical relevance of circulating levels of HDL subfractions to atherogenesis and cardiovascular diseases has not been fully explored yet.

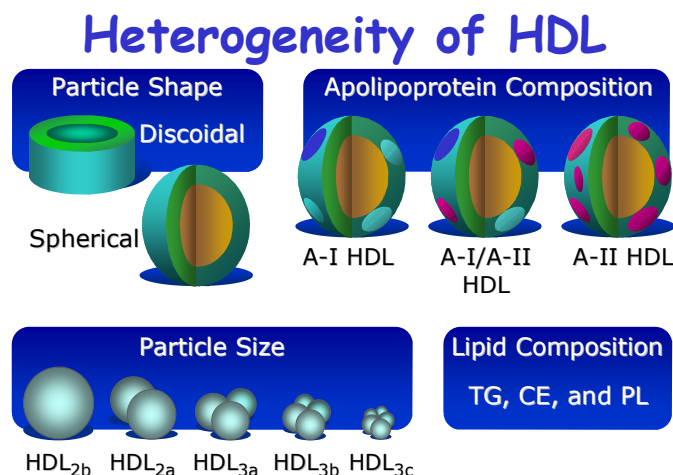


Fig. 7 . HDL subpopulations
www.lipidsonline.org

The HDL cycle plays a role in reverse cholesterol transport, returning cholesterol from peripheral tissues to the liver for excretion. HDL apparently acquires unesterified cholesterol from peripheral tissues and esterifies it. The resulting cholesterol ester can be returned to the liver either by hepatic uptake of HDL, or indirectly transfers to VLDL.

2.2. Reverse Cholesterol Transport

Promotion of cholesterol efflux from macrophages and its return to the liver, bile, and feces, completing the pathway of “reverse cholesterol transport” (RCT), is thought to be one of the most important mechanisms by which HDL protects against atherosclerosis.

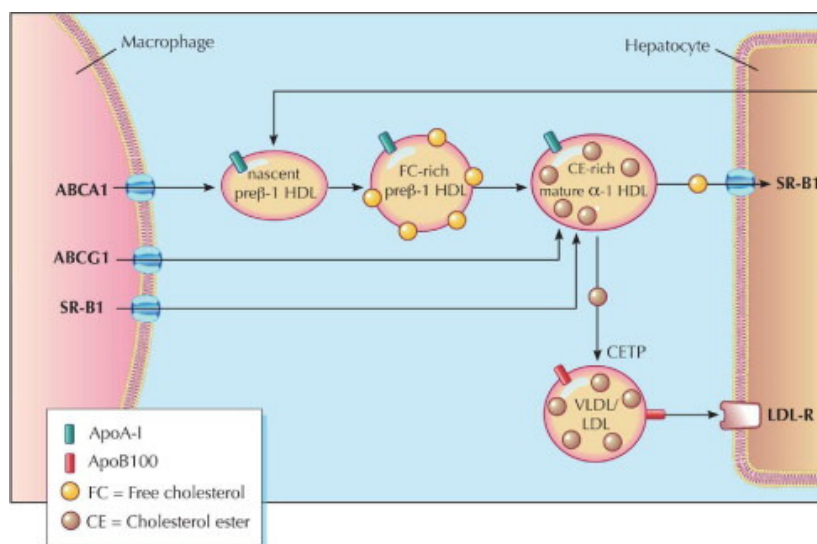


Fig. 8. Reverse Cholesterol Transport

deGoma EM, deGoma RL, Rader DJ; J Am Coll Cardiol. 2008 Jun 10;51(23)

HDL facilitates the process known as reverse cholesterol transport, in which cholesterol from the peripheral tissues effluxes to HDL and returns to the liver in order to get excreted into bile (Ballantyne et al. 2003). The first step of HDL formation is the secretion of a HDL precursor, called nascent HDL (**Fig.8**). Discoidal HDLs are small particles consisting of apoA1, A2 and possibly apoE embedded in a lipid monolayer constituted of phospholipids and free cholesterol (Segrest et al. 2000). Nascent HDL is a good acceptor of unesterified cholesterol. Transformation of unesterified cholesterol to cholesterol ester is catalyzed by the lecithin:cholesterol acyltransferase. This process leads to the formation of a lipid core, transforming the nascent HDL to a small spherical particle, HDL3, which continues to acquire more unesterified cholesterol. Further enlargement of the lipoprotein particle gives rise to a particle called HDL2a. CETP is assumed to mediate the heteroexchange of triglycerides and cholesterol esters primarily between HDL2a and the apoB-containing lipoproteins. Such a neutral lipid exchange results in the formation of HDL2b, which subsequently becomes enriched in triglyceride and deficient in cholesterol ester. HDL2b is then transformed back into HDL3 by hydrolysis of triglycerides and phospholipids by the action of hepatic lipase (Tall et al. 1984) (**Fig.8**).

2.3. Predictive ability of HDL subfractions for CHD risk

Data regarding the predictive ability of HDL subfractions for CHD risk are not conclusive, although the concept that larger HDL particles may be associated with greater protection against atherosclerosis has been widely accepted in the last decades.

Using ultracentrifugation methods, HDL fraction can be divided into HDL2 and HDL3 subfractions (Movva 2008). In the Kuopio study HDL2 level showed an inverse association with the risk of acute myocardial infarction in randomly selected asymptomatic subjects (Salonen 1991). Results from the Québec Cardiovascular Study suggested that the HDL2 subfraction may be more closely related to the development of CHD than the HDL3 subfraction. They noted that the measurement of HDL subfractions did not appear to provide any additional information on the risk of CHD than HDL cholesterol alone (Lamarche 1997). Both HDL3 and HDL2 levels were associated with a substantially decreased risk of myocardial infarction in the Physicians' Health Study, but the HDL3 level was the strongest predictor (Stampfer 1991). Both HDL2 and HDL3 cholesterol were inversely associated with the incidence of CHD in the Caerphilly and Speedwell prospective studies (Sweetnam 1994) and in the 9-year follow-up of the Caerphilly study (Yu 2003). In the latter study HDL3 cholesterol was an independent predictor of incident CHD and may be more closely related to the development of CHD than HDL2 cholesterol.

HDL subfractions can be separated based on size by using nondenaturation polyacrylamide gradient gel electrophoresis (NGGE). A recent study investigated the relationship of HDL subclasses determined by NGGE with the Framingham risk score in middle-aged asymptomatic individuals. This study could not prove any significant association between the calculated risk and HDL subfractions, although they found a notable incidence of small size HDL phenotype in subjects categorized as low-risk (Vekic 2007).

Separation of HDL by 2-dimensional electrophoresis incorporates size-based and charge-based separations and results in pre- β -1, pre- β -2, and pre- α subpopulations in addition to α -migrating HDL subpopulations (Movva 2008). A strong negative correlation has been found between large α -1 HDL particles and CHD (Asztalos 2003).

In male participants of the Framingham Offspring Study CHD cases had higher pre- β -1 and α -3 particle and lower α -1, pre- α -3, and pre- α -1 particle levels than either all or HDL cholesterol-matched controls. α -1 and pre- α -3 levels had an inverse association, whereas α -3 and pre- α -1 particle levels had a positive association with CHD prevalence (Asztalos 2004). An altered HDL subpopulation profile marked with low α -1 and α -2 levels and a high α -3 level in coronary heart disease patients indicated an elevated risk for new cardiovascular events in the patients of the Veterans Affairs HDL Intervention Trial (Asztalos 2005).

Large and small HDL particle concentrations quantified by nuclear magnetic resonance (NMR) spectroscopy were inversely associated with progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial (Rosenson 2002). However, in the post-hoc analysis of the IDEAL (Incremental Decrease in End Points through Aggressive Lipid Lowering) and the EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk case-control study, the highest HDL particle size assessed by NMR was found to be positively associated with cardiovascular disease events (van der Steeg 2008).

Therefore, the relative value of the HDL subfractions as predictors of cardiovascular risk is still unresolved.

2.4. HDL Functions - Other Than Reverse Cholesterol Transport

High-density lipoprotein exerts a number of potentially antiatherogenic effects independent of cholesterol efflux and centripetal transport, including inhibiting lipid oxidation, impairing leukocyte adhesion and monocyte activation, promoting nitric oxide (NO) production and flow-induced vasodilation, preventing endothelial cell damage and death, and inhibiting activation of platelets and the coagulation cascade (Mineo et al 2006) (**Fig.9.**).

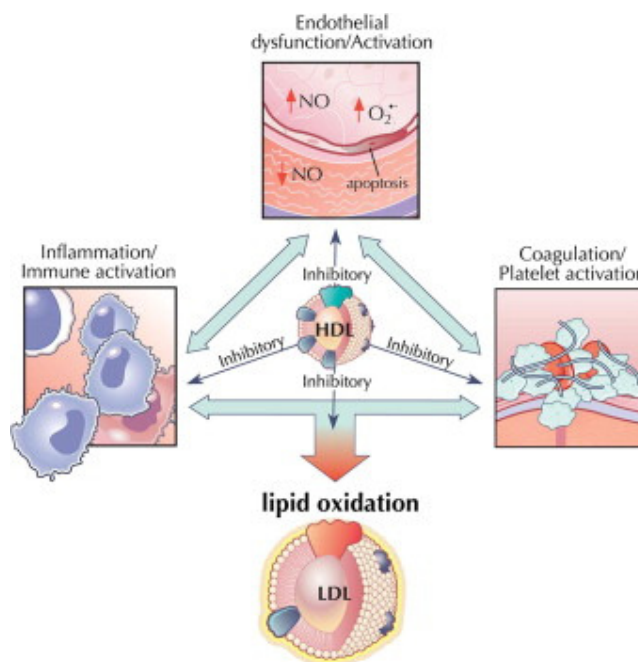


Fig. 9. HDL Functions - other than reverse cholesterol transport

deGoma EM, deGoma RL, Rader DJ; J Am Coll Cardiol. 2008 Jun 10;51(23)

2.4.1. Antioxidant properties of HDL

HDL is effective in preventing the *in vitro* oxidative modification of LDL both by transition metal ions and by cells in tissue culture. This is the case for a wide range of indices of lipid peroxidation, including thiobarbituric acid reactive substances (TBARs), total lipid peroxides, change in electrophoretic mobility typical of oxidized LDL or change in the uptake of oxidized LDL by macrophages. This effect of HDL in decreasing LDL oxidation is maintained for longer than that of antioxidant vitamins and could thus be more protective. The most likely mechanism by which HDL diminished lipid peroxide accumulation was an enzymatic hydrolysis of phospholipid hydroperoxides (Mackness et al 1993).

There are several proteins present in human HDL that possess enzymatic activity:

Paraoxonase1 (PON1)

Lecithin:cholesterol acyltransferase (LCAT)

Platelet activating-factor- acyl-hydrolase (PAFAH)

Protease (elastase-like)

Phospholipase D

Albumin

Apolipoprotein A1.

Durrington et al. have demonstrated that human PON1 was principally responsible for the breakdown of lipid peroxides before they could accumulate on LDL. Purified PON1 was highly effective in preventing lipid peroxidation of LDL (Durrington et al. 2001) (**Fig. 10**).

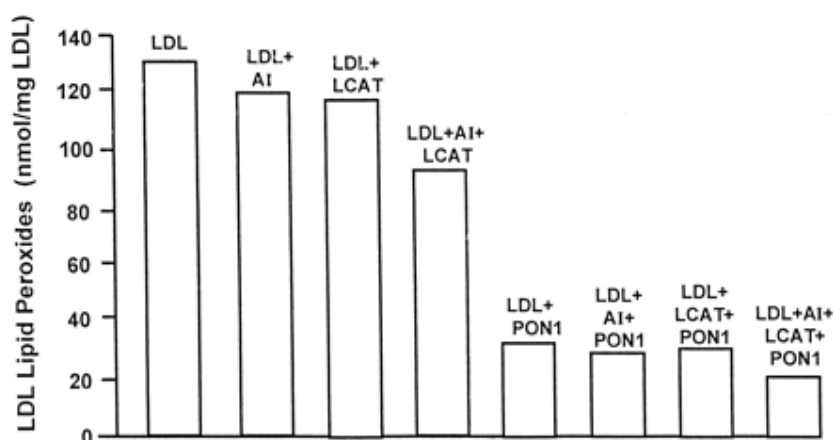


Fig. 10 . Lipid peroxide accumulation on LDL incubated under oxidizing conditions alone and in the presence of apoA-I (AI), LCAT, PON1, and combinations of these.

Durrington PN, Mackness B, Mackness MI. Arterioscler Thromb Vasc Biol. 2001 Apr;21(4):473-80.

PON1 was substantially more effective than LCAT or apoA-I in protecting LDL against oxidation, although the combination of all three substances did slightly enhance the effect of PON1 alone. Experiments with inhibitors of PON1 also suggest that it is responsible for the antioxidant effect of HDL (Aviram et al 1998).

PON1 is located in a subfraction of HDL that contains apoA-I and clusterin (apoJ). (Mackness et al 1981, Kelso et al 1994, Blatter et al 1993) This subfraction of HDL may function to protect cell membranes generally against lipid peroxidation and

other toxic effects. (Mackness et al 1995). Clusterin has likewise been proposed as a protein protecting cell membranes. (Jordan-Starck et al 1992) HDL is the most abundant protein in the tissue fluid and, indeed, the only lipoprotein in the central nervous system. It is unlikely that its antioxidant function has evolved to protect humans against atheroma, a disease that appears to have been prevalent for less than a century. (Herrick et al 1912) Therefore, its antioxidant capacity is probably part of a much older protective role, and LDL shares in this protection because of its resemblance to a cell membrane. Although PON1 was discovered as the result of its ability to hydrolyze xenobiotic toxins, there are natural organophosphate toxins (La Du et al 1996) and numerous other exogenous and endogenous esters, such as homocysteine thiolactone, (Jakubowski et al 2000) other lactones, and cyclic carbonates, (Biggadike et al 2000, Billecke et al 2000) that can be detoxified by catalyzing their hydrolysis. (La Du et al 1996)

3. The paraoxonase enzyme family

The paraoxonase gene family has three known members, PON1, PON2 and PON3, located on the long arm of chromosome 7 between q21.3 and q22.1 in humans. (Mackness et al 2002). The three PON genes, PON1, PON2, and PON3, are highly conserved in mammals, suggesting an important physiological roles. PON-like proteins can be found in all animal species, and even in fungi and bacteria. The genes share considerable structural similarity and appear to have been arisen by gene duplication from a common evolutionary precursor. PON1 and PON3 are expressed in the liver and excreted in the blood where they are associated with the HDL particle. PON2 is not present in blood, but is expressed widely in a number of tissues, including the liver, lungs, brain and heart. (Mackness et al 2002). PON1 has been studied for many years following its discovery because of the capacity of human serum to hydrolyse xenobiotics was due to its presence. More recently, attempts have been made to identify its physiological substrates and to explore its relationship with atherosclerosis. PON2 and PON3 have only recently become the subject of investigation and are less well understood than PON1.

3.1 Human paraoxonase1

3.1.1 Gene and protein product

Human serum paraoxonase (PON1, arylalkylphosphatase, EC 3.1.8.1) is an HDL-associated enzyme belonging to a family of calcium-dependent hydrolases. PON1 is a 45 kDa glycoprotein, composed of 354 amino acids, synthesized in the liver and secreted into the serum. PON1 is found in the HDL2 species of HDL in a particle enriched in triacylglycerols. A large proportion of PON1 is associated with HDL containing apoAI, although particles containing apoAI, apoAII and PON1 do exist. There is also a subpopulation of HDL containing PON1 that is associated with apoJ or clusterin. Essentially, PON1 tends to bind to larger sized species of HDL (Deakin and James 2004). Recently it has been shown that PON1 can be associated with VLDL in the human serum, where triglycerides correlated independently with variations in serum mass and activity of the enzyme. VLDL-associated PON1 exerted an anti-oxidative effect, which may be of physiological benefit (Deakin et al 2005). Chylomicrons may also contain PON1, where PON1 has been shown to exhibit a triacylglycerol lipase-like activity. PON1 attenuates the postprandial oxidative stress response, and this could have resulted from PON1 lipase-like activity on chylomicron triacylglycerols (Fuhrman et al 2006).

The enzyme has two calcium-binding sites: the higher affinity calcium is required for the structural integrity, whereas the lower affinity calcium is involved in the catalytic activity (Fig.11.). The activity of PON1 shows great inter-ethnic variability. Furthermore, serum levels of PON1 activity vary widely among individuals; however, activity remains relatively constant for a given individual (Davies et al 1996). There is a wide variation (up to 13-fold) in PON1 serum concentration and activity between individuals even within genotype groups (Richter and Furlong 1999).

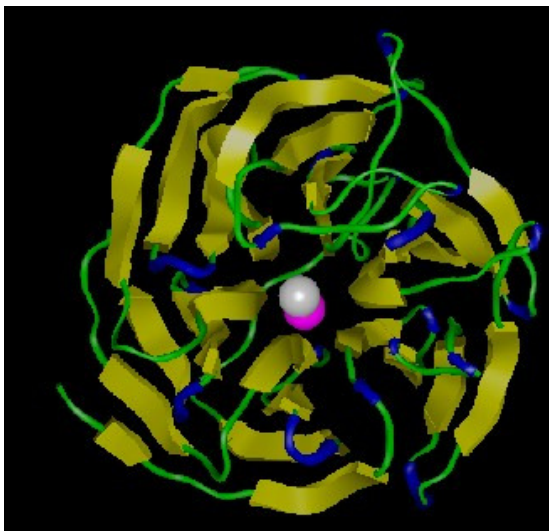


Fig. 11. Overall structure of PON1
Harel et al., Nat Struct Mol Biol. 2004, 11(5):412-9
 2004

3.1.2 Substrates

PON1 catalyzes the hydrolysis of multiple substrates: lactones, thiolactones, carbonates, esters, and phosphotriesters, including paraoxon, from which its name is derived. The enzyme has been studied mainly for its ability to break down pesticides and nerve gases, such as sarin. The hydrolysis products of paraoxon are relatively nontoxic, in contrast to paraoxon itself, a potent inhibitor of the cholinesterases that break down the neurotransmitter acetylcholine (Heinecke 1998). Although PON1 catalyzes the hydrolysis of multiple substrates it has been shown that PON1 and the other PONs are in fact lactonases, catalyzing both the hydrolysis and formation of a variety of lactones (Draganov et al 2005). Studies on such substrates may shed further light on different mechanisms by which paraoxonases beneficially influence atherosclerosis, as well as defining possible roles in limiting bacterial infection and in innate immunity (James 2006).

3.1.3 Antiatherogenic function

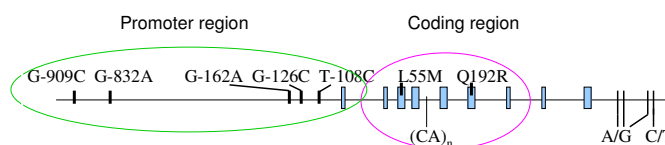
Although its physiological functions and endogenous substrates are unknown, there is ample evidence linking PON1 with the prevention of atherosclerosis. Serum PON1 levels appear to be inversely related to the risk of coronary heart disease

(Mackness et al 2001), and PON1-knockout mice are susceptible to atherosclerosis (Shih et al 1998). It has been suggested that HDL-bound PON1 could protect against oxidative stress by hydrolyzing oxidized lipids in lipoproteins and in atherosclerotic lesions (Gaidukov et al 2006). Serum PON1 activity and serum HDL susceptibility to oxidation are inversely related (Aviram et al 1998). Moreover, oxidatively modified LDL is more immunogenic than its native counterpart and induces the production of heterogenous population of anti-oxLDL autoantibodies (Binder et al 2006).

3.1.4 Significance of PON1 polymorphisms

PON1 gene is localized at q21-q22 on the long arm of chromosome 7 (Humbert et al 1993) It is a moderate size gene with 25.8 kb downstream from the initiation codon encoding the 355 amino acids. It contains five common single nucleotide polymorphisms (SNPs), three are located in the promoter (-909G > C, -162A > G, -108C > T) and two are in the coding region (L55M, Q192R) with incomplete linkage disequilibrium (Chen et al 2003) (**Fig. 12.**). Promoter variants may affect the level of expression by more than 2-fold. PON1 gene promoter polymorphisms account for approximately 25% of variation in serum PON1 concentration (Brophy et al 2001). Q192R affects the relative rate of hydrolysis of certain organophosphate substrates, such as paraoxon, compared with phenylacetate by as much as an order of magnitude, but has only a small effect on the relative rates of hydrolysis of chlorpyrifos oxon and phenylacetate (Davies et al 1996). Recently it has been shown that the PON1-192Q binds HDL with a 3-fold lower affinity than the R isozyme and consequently exhibits significantly reduced stability, lipolactonase activity, and macrophage cholesterol efflux. The lower affinity and stability of the 192Q versus the 192R isozyme in sera of individuals belonging to the corresponding genotypes has been also observed (Gaidukov et al 2006). The L55M polymorphism may affect PON1 protein stability (Leviev et al 2001), and contributes significantly to plasma enzymatic activity. The PON1-192 polymorphism has the most significant impact on enzyme activity.

PON1 polymorphisms



- 5 promoter region polymorphisms
- 2 coding region polymorphisms

Fig. 12. PON1 polymorphisms

Recent investigations have suggested that the hydrolytic activity towards lactones (cyclic esters) is the native activity of PON1: structure-activity studies show that lactones are PON1's preferred substrate for hydrolysis (Khersonsky and Tawfik 2005). In addition, all members of the PON family have lactonase activity, implying that this activity has been conserved throughout the evolution of the enzyme. The capacity of blood to hydrolyse paraoxon (paraoxonase activity) is often used as a marker for the PON1 enzyme activity. This enzyme activity reflects the combined effects of the 192Q>R polymorphism and the variation in concentration of the PON1 enzyme. In addition to the paraoxonase activity, the PON1 concentration can be measured directly in serum with an enzyme-linked immunosorbent assay (ELISA) (Oka et al 2000). Otherwise, because PON1 esterase activity is not polymorphic (i.e. influenced by the 192Q>R polymorphism), the PON1 concentration can be estimated by measuring phenylacetate hydrolysis (arylesterase activity) (Eckerson et al 1983).

3.1.5 The role of PON1 in cardiovascular diseases

There have been many case-control studies to test the hypothesis that the 192R allele of the PON1 gene is associated with coronary heart disease. A meta-analysis of these trials found that the PON1-192R allele was significantly related to the presence of CHD, but there was evidence of publication bias (Mackness et al 2001). These data suggest that the link between PON1 genetic polymorphisms and CHD is, at best, weak.

The PON1 polymorphisms should be added to the list of other genes for which there is a significant evidence for contributions to common forms of CHD. However, a number of other genetic variations are very likely to affect the risk of CHD, including genes of scavenger receptors, hepatic lipase, methylene-tetrahydrofolate reductase, and the two other paraoxonases (Heinecke 1998).

Some of the genetic case-control studies failed to test the hypothesis that serum PON1 activity protects against CHD, because the 192 polymorphism accounts for only a small part of the 40-fold individual variation in serum PON1 activity (Mackness et al 2003). Also, a variety of studies have suggested that low paraoxonase levels are associated with CHD and atherosclerosis (Jarvik et al 2003, Mackness et al 2001). In fact, the results of earlier studies have suggested that the ratio of clusterin to PON1 may be more accurate predictor of CHD than the ratio of total cholesterol to HDL-C (Navab et al 1997). In another study, the ratio of PON1 to either clusterin, apoAI, or HDL-C largely reflected to the low PON1 in the CHD population. It was also shown that PON1 activities toward paraoxon and PON1 concentrations are lower in subjects with CHD than in control subjects regardless of the PON1 genotype. These results suggested that the quality of the PON1 enzyme is a more important factor in CHD (Mackness et al 2001). The fact, that the majority of published studies do not take into account serum PON1 levels may responsible for the lack of conclusive epidemiological evidence for a link between PON1 and CHD.

There is a substantial 40-fold, interindividual variation in PON1 activity, which is independent of either the 55 or 192 polymorphisms. This may be due to acquired factors acting either on the composition of the lipid environment of HDL, in which PON1 operates, or on the promoter region of the PON1 gene or in some manner as yet unidentified. When PON1 activity is measured directly in patients with CHD, it is about half of that of disease-free controls (McElveen et al 1986, Ayub et al 1999 and Mackness et al 2001). This appears to be the case even within a few hours of the onset of cardiac ischemic chest pain in myocardial infarction, suggesting that low serum PON1 activity may have preceded the event (Ayub et al 1999). Low serum PON1 activity independent of genotype has been reported with several diseases, which are known to be associated with CHD, including clinical and experimental diabetes mellitus (Ikeda et al 1998, Mackness et al 1991, Abbott et al 1995, Mackness et al 1998b and

Patel et al 1990), hypercholesterolemia (Mackness et al 1991) and renal failure (Hasselwander et al 1998).

3.1.6 The role of PON1 in atherosclerosis

To date, the role of PON1 *in vivo* is unclear, but in general, PON1 is thought to attenuate the oxidation of LDL. This hypothesis was based on *in vitro* findings, showing that purified PON1 inhibited the accumulation of lipid peroxides in LDL. In the arterial wall, the oxidised LDL particle is recognised by oxLDL specific receptors on the macrophage and taken up into the cell. Since there is no negative feedback mechanism for this uptake, this process eventually leads to an overload of lipids in the macrophage, which causes the lipid-laden macrophages to aggregate and form a fatty streak that is characteristic of atherosclerosis (Lusis 2000). The oxidation of LDL is the key step in the pathophysiology of atherosclerosis and the onset of cardiovascular disease, and therefore, it is not surprising that PON1 has been the subject of increasing scientific interest since its supposed role in the oxidation of LDL.

There are some evidences that PON1 plays a central role in atherogenesis:

3.1.6.1 Animal studies

HDL isolated from the blood of PON1 knockout mice or from avian species, which naturally lack PON1, has at best, no effect on LDL-oxidation and at worst promotes LDL-oxidation (Shih et al 1998, Mackness et al 1998a).

On the contrary, HDL isolated from mice overexpressing human PON1 completely abolishes LDL oxidation (Tward et al 2002). Oda et al. found that over-expression of PON1 inhibited lipid hydroperoxide formation on HDL and protected HDL integrity and function (Oda et al 2002). Van Lenten et al. have reported that oxidised phospholipids found in mildly oxidised LDL act acutely to decrease the expression of PON1 and increase that of apo J partly through the inflammatory cytokine, IL-6 (Van Lenten et al 2001).

Finally, and perhaps the strongest evidence that PON1 plays a role in atherogenesis, PON1 deficient mice are more prone to develop atherosclerosis than wild-type mice, when fed a high-fat/high-cholesterol diet (Shih D et al 1998).

3.1.7 Mechanisms of the anti-atherogenic effects of PON1

On one hand, PON1 protects HDL and LDL against oxidation. *In vitro* supplementation of human HDL or purified PON1 significantly inhibits copper-induced lipoprotein oxidation in a concentration-dependent manner; furthermore, PON1-specific inhibitors enhance HDL oxidation (Aviram et al 1998). HDL-associated and purified PON1 protected human LDL from oxidation. On the other hand, PON1 can hydrolyze oxidized lipids in oxidatively modified LDL. Moreover, PON1 hydrolyzes and reduces lipid peroxides not only in LDL particles but also in human coronary and carotid lesions by an esterase and peroxidase activity (Aviram 1999).

3.2. Factors that can influence the activity of PON1

In addition to genetic polymorphisms, PON1 levels can be modified by acquired factors such as diet, lifestyle and pharmacological treatment.

3.2.1. Diet

Both in animal and human studies, pro-atherogenic diet and meals rich in used cooking fat with high content of oxidized lipids caused a significant fall in PON1 activity and mass, which correlated with the reduction in HDL-cholesterol. In contrast, oleic acid from olive oil and argan oil are associated with increased enzyme activity (Cherki et al 2006). Dietary antioxidants (i.e., quercetin and glabridin), pomegranate juice and red wine, rich in polyphenols and other antioxidants could raise PON1 activity. Effects of antioxidant vitamins C and E are debated. Moderate alcohol consumption increased both PON1 activity and mass. Interestingly, there was no difference between red wine, beer or spirits (Deakin and James 2004).

3.2.2. Lifestyle

PON1 activity and concentration were reduced in smokers compared with non-smokers, and the influence of smoking on PON1 was reversible (James et al. 2000, Senti et al 2003). PON1 activity levels were significantly higher in physically active subjects than in those who were inactive. Interestingly, R carriers showed a significant decreasing trend in triglyceride levels and in log-triglyceride-to-HDL-cholesterol ratio and a significant increasing trend in HDL-cholesterol concentration with the amount of physical activity. The beneficial association of the amount of physical activity and lipid traits found in men with the R allele suggests that this population subgroup needs to be physically active to achieve a favourable lipoprotein phenotype similar to that observed in QQ homozygous men (Senti et al 2000). In another study, well trained amateur athletes participating in an ultra-distance triathlon in comparison with healthy sedentary controls have been investigated. After classification by paraoxonase phenotype, only sportsmen belonging to the QR phenotype showed higher HDL susceptibility to in vitro oxidation (Brites et al 2006).

3.2.3. Pharmacological treatment

In the last decades various therapeutic strategies that target lowering of LDL-C or augmentation of HDL-C have been employed to prevent atherogenesis (Saini et al 2005). Among these strategies, fibrates and statins are the clinically most important and widely used agents. Primary and secondary prevention trials with lipid lowering agents in a wide variety of populations have demonstrated that lowering plasma LDL-C levels retards the progression of atherogenesis and reduces the risk of coronary events.

3.2.3.1. Statins

HMG-CoA reductase is the rate-limiting enzyme for cholesterol formation in the liver and other tissues. Statins inhibit this enzyme and are beneficial in the primary and secondary prevention of cardiovascular diseases. The HMG-CoA reductase inhibitors (statins) were developed to compete with HMG-CoA for binding at the catalytic site of HMG-CoA reductase and thereby reduce the synthesis of mevalonate. By inhibiting

HMG-CoA reductase, statins reduce the hepatocyte cholesterol content, therefore stimulate the expression of LDL receptors, and ultimately enhance removal of LDL-C from the circulation (**Fig.13.**). The plasma cholesterol lowering effect of these drugs results mainly from the enhanced receptor-mediated uptake of LDL in the liver via the upregulation of LDL-receptor (LDL-R).

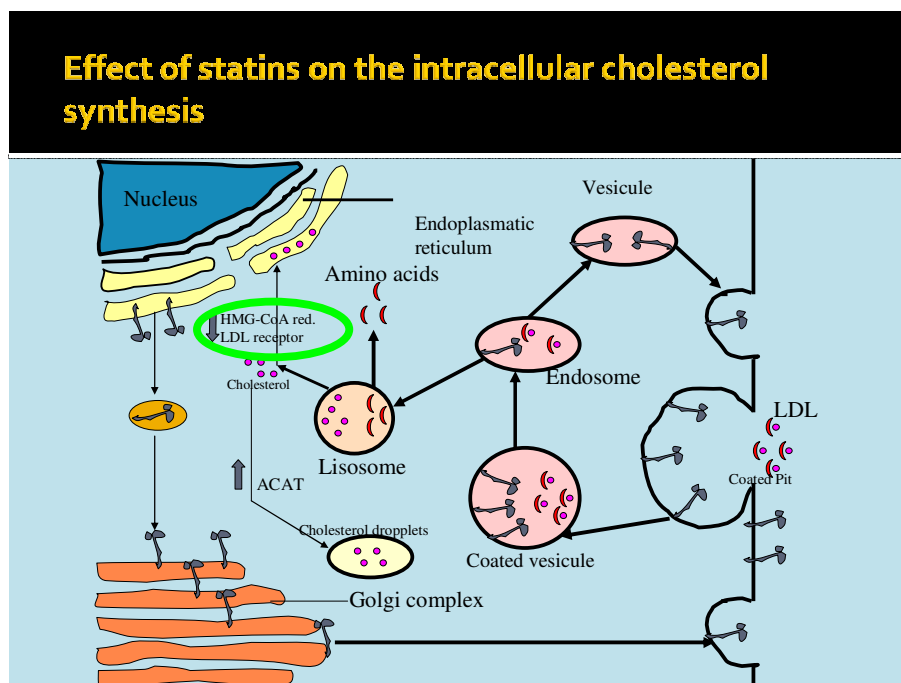


Fig.13. Effect of statins on the intracellular cholesterol synthesis

The newer statins, such as atorvastatin and rosuvastatin are more effective and are able to reduce plasma cholesterol by up to 60% (Kleemann et al 2005). The efficacy of HMG-CoA reductase inhibitors in lowering serum cholesterol levels is well documented. Since their discovery, statins are considered as one of the most effective classes of drugs for reducing LDL-C and total cholesterol.

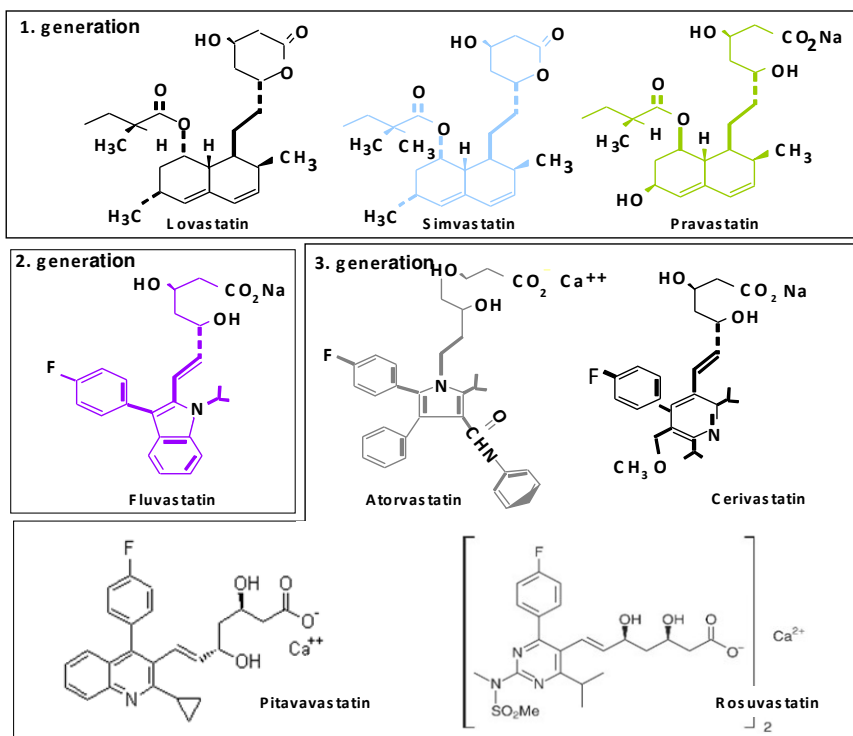


Fig.14. Chemical structure of statins

There are three generations of statins, as indicated in **Fig.14**. Statins differ in their absorption, plasma protein binding, excretion, and water solubility and exhibit variable dose-related efficacy in reducing LDL-C. They are metabolized by different isoforms of the cytochrome P450 system (CYP), as shown on **Table A**.

Table A

Comparative Efficacy and Pharmacology of the 6 Currently Available Statins

Drug	TC, %	LDL-C, %	HDL-C, %	TG, %	Tablets, mg	Metabolism	Binding, %	T1/2, h	Hydrophilic
Atorvastatin	25–45	26–60	5–13	17–53	10, 20, 40, 80	CYP3A4	98	13–30	No
Fluvastatin	16–27	22–36	3–11	12–25	20, 40, 80	CYP2C9	98	0.5–3.0	No
Lovastatin	16–34	21–42	2–10	6–27	10, 20, 40	CYP3A4	>95	2–4	No
Pravastatin	16–25	22–34	2–12	15–24	10, 20, 40, 80	Sulfation	43–67	2–3	Yes
Rosuvastatin	33–46	45–63	8–14	10–35	5, 10, 20, 40	CYP2C9	88	19	Yes
Simvastatin	19–36	26–47	8–16	12–34	5, 10, 20, 40, 80	CYP3A4	95–98	1–3	No

TC indicates total cholesterol; TG, triglycerides; and T1/2, half-life.

Vaughan and Gotto, *Circulation*. 2004;110:886-892.)

Pleiotropic effects of statins

The overall benefits observed with statins appear to be greater than what might be expected from reducing lipid levels alone, suggesting effects beyond cholesterol lowering. Indeed, recent studies indicate that some of these cholesterol-independent or “pleiotropic” effects of statins involve improving endothelial function, enhancing the stability of atherosclerotic plaques, decreasing oxidative stress and inflammation, and inhibiting the thrombogenic response. Furthermore, statins have beneficial extrahepatic effects on the immune system, central nervous system and bone tissue. Many of these pleiotropic effects are mediated by the inhibition of isoprenoid synthesis, which produce lipid attachments for intracellular signaling molecules. Statins and their metabolites may have an antioxidant potential (Aviram and Rosenblat 2005).

Only a few studies have shown the ability of statins to reduce the levels of circulating oxidized LDL or other measures of LDL oxidation, such as circulating conjugated dienes or malondialdehyde (MDA). The pioneer work of Aviram et al. in an *in vitro* study using several oxidation systems on isolated lipoproteins demonstrated that oxidized metabolites of atorvastatin (5-50 μ M) but not the parent compound itself exert inhibitory effect on lipoprotein oxidation (Aviram et al 1998). Several *in vitro* studies proved that statins show a dose-dependent decreasing effect on free radical production and this effect can be reversed by mevalonate (Giroux et al 1993). The majority of these studies were performed on animal models and their results are contradictory. Fuhrman et al. studied the effect of one-month atorvastatin therapy on isolated monocytes of hypercholesterolemic patients. Atorvastatin was found to increase serum paraoxonase activity, and reduce free radical-induced lipid peroxidation and scavenger receptor expression (Fuhrman et al 2002).

The question is how statins can affect paraoxonase activity and serum paraoxonase enzyme concentrations. What is the effect of statins on HDL level and paraoxonase activity?

Effect of statins on PON1 activity – clinical studies

All of the previous studies demonstrated a significant LDL-C lowering effect of statins. In each study the HDL-C level was increased. Only Kural and coworkers demonstrated that 10 mg atorvastatin increased significantly the HDL-C level unlike the other studies which could not demonstrate significant HDL-C elevation in similar doses (Kural et al. 2004). Tomás et al. proved that a 4-month simvastatin treatment could increase PON1 activity, thereby improving the antiatherogenic effect of the drug (Tomás et al. 2000). However, Balogh et al. found a non-significant decrease in PON1 activity after one month of simvastatin treatment in patients with types IIa and IIb hyperlipidemia (Balogh et al. 2001). Paragh et al. investigated the effects of a 3-month treatment with simvastatin and atorvastatin on PON1 activity in hyperlipidemic patients. They found that a short-term administration of simvastatin did not influence the activity of PON1, while atorvastatin significantly increased it (Paragh et al. 2004). Tsimihodimos et al. studied the effect of atorvastatin therapy on the activities of serum PAFAH and PON1 in patients with types IIa and IIb dyslipidemia. They found that atorvastatin did not affect PON1 activities either towards paraoxon or phenylacetate in both patient groups (Tsimihodimos et al 2002). Harangi et al. found that atorvastatin was able to improve serum PON1 activity and PON1/HDL ratio in type IIa hyperlipidemic patients, while the serum PON1 concentration was not significantly decreased after atorvastatin therapy (Harangi et al. 2004). Kassai et al. reported a significant increase in PON1 activity after 3 months of atorvastatin administration in patients with dyslipidemia of types IIa and IIb (Kassai et al. 2007). The paraoxonase activity changes were between 12.3-37.9%. The reasons for these differences could be attributed to the diverse study populations, different types of hyperlipoproteinemia and various types of statins and their dosages. To assess whether the observed rise in paraoxonase activity was due to HDL elevation, the PON/HDL ratio was calculated. The effect of statins on paraoxonase activity seems to be independent of HDL elevation. Kural et al. showed that PON1 activity in serum and isolated HDL was significantly increased after atorvastatin treatment in dyslipidemic patients (Kural et al. 2004). A recent study on 134 patients with familial hypercholesterinemia demonstrated that PON1 modifies the HDL-C increment during statin therapy (van Himbergen et al. 2005)

The effect of statin treatment on PON1 – genetic studies

There is incomplete data on the response of lipid parameters and PON1 activity to statin treatment in patients with different genotypes. Only a few studies focused on the possible modulatory role of PON1 genotypes in statin treatment. Tomás et al. investigated the effect of 4 months of simvastatin therapy on serum lipid parameters and PON1 activity in familial hypercholesterolemic patients; however, they could not find differences in the therapeutic response of PON1 activity between various genotype groups (Tomás et al. 2000). Malin et al. found that PON1-192 and 55 genotypes were significant determinants of HDL-C concentration change in hypercholesterolemic male subjects who underwent 6 months of pravastatin treatment (Malin et al. 2001). Christidis et al. evaluated the data of more than two hundred hypercholesterolemic patients treated with low dose fluvastatin for 6 months. They concluded that PON1-192 and 55 genotypes did not influence the effect of fluvastatin on lipid parameters and PON1 activity, although in the R allele carriers the fluvastatin treatment lowered the triglyceride levels more effectively, but the difference was not significant (Christidis et al. 2007).

Recently, Deakin et al. found a pharmacogenetic correlation between PON1 gene promoter polymorphism C-107T and simvastatin in HepG2 cells. Hypercholesterolemic patients homozygous for the C allele showed a significant increase in serum PON1 activity and in the mass of paraoxonase during treatment with simvastatin, whereas patients homozygous for the T allele showed no increase. They concluded that patients with the C allele are likely to derive greater benefit from statin therapy (Deakin et al. 2007). Some of our previous observations also suggested that PON1 phenotype estimating the PON1-192 genotype might modulate the effect of statins on PON1 activity (unpublished data).

Summarizing these previous results:

- It has been suggested that the human paraoxonase-1 (PON1) genotype is an important determinant of the therapeutic response given to statin treatment.
- We also know that the PON1 activity status is a better predictor of CHD risk than any of the known PON1 genotypes.

II. OBJECTIVES

Our goal was to answer these previously uninvestigated, but clinically still relevant questions:

1. How statins influence PON1 activity in humans and what is the putative mechanism of the effect of statins on the PON1 activity?
2. How PON1 phenotype might influence the effect of different statins (simvastatin, atorvastatin and extended-release fluvastatin) on the serum lipid parameters, especially on the HDL-C levels?
3. Does the PON1 phenotype have an impact on the paraoxonase-activating and lipid-lowering effect of different types of statins?
4. Is any clinically important difference between the effect of the three statins on PON1 activities due to their structural and pharmacokinetic differences?
5. How can the atorvastatin treatment influence the LDL particle size and HDL subfraction profile?
6. What is the role of the enzymes involving HDL remodelling (LCAT, CETP) in the PON1 activity changes?
7. Is any correlation between the changes of HDL composition pattern and the activity of the antioxidant PON1, LCAT and CETP?

III. PATIENTS AND METHODS

Study population

The study was carried out in accordance with the Helsinki Declaration (2000) of the World Medical Association and approved by the Ethics Committee of the University of Debrecen Medical and Health Science Center. Subjects participated in the study after their written informed consent was obtained, explaining the nature and the purpose of the study.

Subjects between 21 and 70 years of age, nonsmokers, with previously untreated type IIb hyperlipidemia were enrolled in this study. Exclusion criteria were the presence of a significant endocrine disorder, active liver disease or hepatic dysfunction defined as alanine aminotransferase or aspartate aminotransferase greater than 1.5 times of the upper limit of normal range, renal disease, malignancy, diabetes mellitus, coronary artery disease, blood pressure above 160/100 mm Hg, cerebral vascular disease, smoking, triglyceride level exceeding 4.5 mmol/l, alcoholism, drug dependence, infectious disease, significant inflammation, pregnancy or lactation, anticoagulant, lipid lowering, glucocorticoid, oral contraceptive or sex hormone replacement medication.

After six weeks on the National Cholesterol Education Program (NCEP) step 1 diet, patients were randomly divided into three groups: the first group received 10 mg atorvastatin/day (ATO), the second group was treated with 10 or 20 mg simvastatin/day (SIM), and the third group was administered with 80 mg extended-release fluvastatin/day (FLU) for three months. Atorvastatin and simvastatin were given at commonly used low doses. In the case of fluvastatin, an extended release form was used, which was available only in an 80 mg daily dose.

Blood sampling

After 12 hours of fasting, 10 ml venous blood sample was taken from the cubital vein between 7.30 and 8.00AM. The lipid parameters were determined from the fresh serum. The sera for paraoxonase and arylesterase activity measurements were kept at -70°C before analysis. LCAT, CETP and HDL subfraction analysis were carried out from frozen serum samples.

Lipid measurements

Lipid parameters were determined in the central laboratory (Dept. of Clinical Biochemistry and Molecular Pathology) of the University of Debrecen Medical and Health Science Center. Serum cholesterol and triglyceride levels were measured by using enzymatic, colorimetric tests (GPO-PAP, Modular P-800 Analyzer, Roche/Hitachi), while HDL-C was assessed by homogenous, enzymatic, colorimetric assay (Roche HDL-C plus 3rd generation). The LDL-C fraction was calculated indirectly using the Friedewald equation. Apolipoprotein examination was performed by immuno-turbidimetric assay Tina-Quant APO A (Version 2, Roche), Tina-Quant APO B (Version 2, Roche).

Analysis of paraoxonase activity

PON1 activity was measured as previously described (Paragh et al. 1998). Briefly, we set up the following enzymatic reaction using paraoxon (O,O-diethyl-O-p-nitrophenylphosphate, Sigma) as substrate and the generation of 4-nitrophenol was followed spectrophotometrically: 50 μ l serum was dissolved in 1 ml Tris/HCl buffer (100 mmol/l, pH=8.0) containing 2 mmol/l CaCl_2 and 5.5 mmol/l paraoxon. We measured the absorbance at 412 nm (25 °C), using a Hewlett-Packard 8453 UV-visible spectrophotometer. Enzyme activity was calculated using the molar extinction coefficient 17100 $\text{M}^{-1}\text{cm}^{-1}$. One unit of PON1 activity was defined as 1 nmol of 4-nitrophenol formed per minute under the assay conditions mentioned above.

Arylesterase assay

Arylesterase activity was measured spectrophotometrically as previously described (Paragh et al. 1998). The assay contained 1 mM phenylacetate (Sigma) in 20 mM Tris-HCl, pH=8.0. The reaction was started by the addition of the serum, and then the absorbance was monitored at 270 nm. Blanks were included to correct for the spontaneous hydrolysis of phenylacetate. Enzyme activity was calculated using the molar extinction coefficient 1310 $\text{M}^{-1}\text{cm}^{-1}$. Arylesterase activity was expressed in U/ml; where 1 U was defined as 1 μ mol phenylacetate hydrolyzed per minute.

Paraoxonase phenotyping

The phenotype distribution of PON1 was determined by the dual substrate method (La Du and Eckerson, 1984). The genetic polymorphism at codon 192 Q->R is responsible for the presence of two isotypes: A (low activity) and B (high activity). The ratio of the hydrolysis of paraoxon in the presence of 1 M NaCl (salt-stimulated PON1 activity) to the hydrolysis of phenylacetate (arylesterase activity) was used to assign individuals to one of the three possible (AA, AB, BB) phenotypes. Cut-off values between phenotypes were as follows: type AA, ratio < 3.0; type AB, ratio 3.0-7.0; and type BB, ratio > 7.0. AA represents low; AB intermediate; and BB high enzyme activity.

Measuring the LCAT activity

We determined the LCAT activity by a commercially available kit (Roar Biomedical Inc.). Plasma was incubated with a fluorescent substrate, and the fluorescence intensity of the intact substrate was measured at 470 nm by a Hitachi F-4500 Fluorescence Spectrophotometer. A monomer was formed as the substrate was hydrolyzed by LCAT. This was detectable at 390 nm. The LCAT activity was calculated by measuring the change in 470/390 nm emission intensity. The intra-assay and inter-assay coefficients of variation were less than 5%.

Measuring the CETP activity

CETP activity was measured by a CETP Activity kit (Roar Biomedical Inc.). The kit contains donor (synthetic phospholipids and cholesteryl ester) and acceptor (VLDL) particles. A 6 μ L plasma and a 20 μ L donor/acceptor mixture were added to a 1 mL buffer (150 mM NaCl, 10 mM Tris and 2 mM EDTA, pH 7.4). The cholesterol ester transfer from the donor to the acceptor molecule mediated by the CETP was quantified by measuring the increased fluorescence intensity using a Hitachi F-4500 Fluorescence Spectrophotometer. The excitation was conducted at 465 nm and the emission at 535 nm. The intra-assay and inter-assay coefficients of variation were less than 3%.

Quantification of oxidized LDL (oxLDL)

The oxLDL was quantified by sandwich ELISA. We measured antibodies against oxLDL with the Wak-Chem-Med (Berlin, Germany) kit. In this test, plasma

oxLDL reacted with mouse monoclonal antibody. After washing, the antibody conjugated with peroxidase against apolipoprotein B recognized the oxLDL fixed to the solid fraction. The fixed conjugation was detected by the tetramethylbenzidine reaction and measured spectrophotometrically. Intra- and inter-assay coefficients of variance (CV) were 5.4% and 8.3%, respectively.

Determination of the ratios of HDL subclasses

HDL size phenotypes were measured as previously described (Blanche 1981 , Singh et al. 1995). Lipoproteins in plasma were separated based on their size, using non-denaturing 3% to 30% polyacrylamide gradient gels. Gels were stained with bromophenol-blue (Sigma, St Louis, Mo) and scanned with a laser densitometer (LKB-Ultroscan LX, LKB Instruments Inc, Paramus, NJ). Molecular weights were determined using thyroglobulin, apoferritin, lactate dehydrogenase, and bovine serum albumin as standards (High Molecular Weight Electrophoresis Calibration Kit, Pharmacia). HDL absorbance profiles were analyzed by fitting curves representing the generally accepted HDL subclasses, as suggested previously. Curves were fit to HDL3 (<8.8 nm), HDL2a (8.8 to 9.8 nm), and HDL2b (≥ 9.8 nm).

Determination of LDL diameter

The LDL peak diameter was determined from whole plasma by lipid-stained, non-denaturing gel electrophoresis using 3% to 16% polyacrylamide gradient gels as previously described (Campos et al. 1992). Gels were stained for lipids with Sudan black (Sigma, St Louis, Mo) and scanned with a laser densitometer (LKB-Ultroscan LX, LKB Instruments Inc, Paramus, NJ). Size of LDL was estimated from calibration curves that were constructed using latex beads (Bangs Laboratories, Fishers, IN, USA) and high molecular-weight standards (Pharmacia Biotech, St. Albans, England) as previously described (Singh et al. 1995). The between-run coefficient of variation was 0.95% for the internal control sample included in every gel, and 0.54% for 20 blinded duplicate samples interspersed throughout the samples in the study.

Statistical analysis

The SAS for Windows 6.12 (SAS Institute Inc.) software was used for the statistical analysis. Normality of distribution of data was tested by Kolmogorov-Smirnov test. Non-normally distributed parameters were transformed logarithmically to correct their skewed distributions. Data were expressed as means \pm S.D. in case of normal distribution; and as medians and 95% confidence intervals for medians, in case of non-normal distribution. To evaluate the effect of treatment, we analyzed changes with repeated measures of ANOVA and with post hoc Newman–Keuls critical ranges test. Correlations between continuous variables were assessed by calculation of linear regression using Pearson's test. The value of $P < 0.05$ was considered statistically significant.

IV. RESULTS

IV.1. Paraoxonase phenotype modifies the effect of statins

Demographic data, laboratory parameters and characteristics of the study population are shown in **Table 1**.

IV.1.1. Lipid parameters

The initial cholesterol and LDL-C levels were significantly higher in the FLU group compared to the ATO group (7.61 ± 0.98 mmol/l vs. 6.98 ± 0.90 mmol/l, 5.17 ± 0.78 mmol/l vs. 4.70 ± 0.81 mmol/l, respectively, $p < 0.05$). We could not find significant differences between the FLU, ATO, and SIM groups in the other lipid parameters.

The statin treatment significantly decreased the total cholesterol and the apoB levels in the whole study population and in the three statin groups. Significantly decreased triglyceride levels were found in the whole study population and in the ATO group. The LDL-C level significantly decreased in the whole study population and in the ATO and FLU groups. The HDL-C levels did not change significantly. A significant increase in the apoA1 levels was found in the SIM group. The LDL/ApoB ratio significantly decreased in the whole study population and in the ATO and FLU groups (**Table 1**).

IV.1.2. Paraoxonase and arylesterase activities

The paraoxonase activity significantly increased in the whole study population and in all three statin groups. There was a significant increase in the arylesterase activity in the whole study population and in the SIM and FLU groups (**Table 1.**)

Table 1. Characteristics and laboratory parameters of the study population. Values are mean \pm SD.

	All statins			ATO			SIM			FLU		
N	164			61			46			57		
Age (ys)	58.2 \pm 9			58.6 \pm 8.6			54.8 \pm 8.9			59.6 \pm 9.5		
Gender (M/F)	79/85			29/32			24/22			26/31		
BMI (kg/m²)	26.9 \pm 3.2			26.9 \pm 2.8			25.7 \pm 3.7			28.2 \pm 3.3		
	Before	After	%	Before	After	%	Before	After	%	Before	After	%
Cholesterol (mmol/l)	7.20 \pm 1.27	5.61 \pm 1.5*	-24	6.98 \pm 0.90	4.54 \pm 0.90*	-39	7.19 \pm 1.62	6.68 \pm 1.49#	-5.06	7.61 \pm 0.98	5.39 \pm 1.25*	-29
Triglyceride (mmol/l) (median)	1.93	1.6#	-15.8	1.74	1.31*	-28.6	1.98	1.97	-8.7	2.12	1.92	-5.4
(95% CI)	(1.77-2.08)	(1.42-1.78)	(-22- -9.5)	(1.48-2)	(1.17-1.44)	(-36- -20)	(1.83- 2.13)	(1.55-2.39)	(-17- -0.2)	(1.64-2.59)	(1.59-2.25)	(-17- -6)
HDL-c (mmol/l)	1.28 \pm 0.28	1.32 \pm 0.31	5.5	1.31 \pm 0.24	1.37 \pm 0.28	6.2	1.20 \pm 0.29	1.25 \pm 0.33	-6.0	1.24 \pm 0.25	1.30 \pm 0.31	5.4
LDL-c (mmol/l)	4.83 \pm 1.19	3.43 \pm 1.39*	-31	4.70 \pm 0.81	2.63 \pm 0.74*	-43	4.79 \pm 1.64	4.15 \pm 1.45	-13.4	5.17 \pm 0.78	2.95 \pm 0.97*	-43
apoA1 (g/l)	1.61 \pm 0.28	1.64 \pm 0.27	3.3	1.65 \pm 0.25	1.60 \pm 0.21	-2.1	1.62 \pm 0.33	1.73 \pm 0.29**	9.45	1.54 \pm 0.19	1.52 \pm 0.23	-1.0
apoB (g/l)	1.59 \pm 0.49	1.21 \pm 0.39*	-22	1.56 \pm 0.32	0.95 \pm 0.23*	-42	1.68 \pm 0.76	1.43 \pm 0.38**	-14.9	1.45 \pm 0.27	1.05 \pm 0.26*	-26.7
LDL/apoB	3.03 \pm 0.83	2.83 \pm 0.73*	-9.2	3.01 \pm 0.62	2.77 \pm 0.52*	-8.0	2.85 \pm 0.95	2.90 \pm 0.89	1.7	3.56 \pm 0.69	2.80 \pm 0.57*	-21.4
Paraoxonase act. (U/l)(median)	113	124*	14	113	128*	13.1	109	122*	11	113	121*	15.3
(95% CI)	(107-118)	(117-131)	(12-16)	(100-127)	(117-140)	(10-16)	(99-119)	(105-139)	(7-14)	(102-122)	(110-132)	(12.2-18.4)
Arylesterase act. (U/l) (median)	89	93 #	6.6	88	90	7.8	91	96#	6.9	90	95*	8.8
(95% CI)	(87-90)	(90-96)	(4.8-8.4)	(83-93)	(83-97)	(0.7-16)	(85-96)	(89-103)	(4-9.9)	(84-95)	(90-100)	(4-13.6)

*p<0.001, **p<0.01, #p<0.05 Students' paired *t*-test was applied to 'before' and 'after'-treatment parameters

ATO: atorvastatin, SIM: simvastatin, FLU: fluvastatin

Table 2. Paraoxonase phenotype distribution and allelic frequencies in the whole study population and in the statin groups

	All statins (n=164)		ATO (n=61)		SIM (n=46)		FLU (n=57)	
	n	%	n	%	n	%	n	%
AA	104	63	38	62	29	63	37	65
AB	50	31	18	30	15	33	17	30
BB	10	6	5	8	2	4	3	5
A	258	78.7	94	77.1	73	79.3	91	79.8
B	70	21.3	28	22.9	19	20.7	23	20.2

ATO: atorvastatin, SIM: simvastatin, FLU: fluvastatin

Table 3. Relative changes in lipid parameters in the AA and in the AB+BB phenotype groups, expressed as percentage difference between the 'before' and 'after' -treatment mean values

	All statins		ATO		SIM		FLU	
	AA	AB+BB	AA	AB+BB	AA	AB+BB	AA	AB+BB
Δ chol (%)	-22.63	-24.86	-40.10	-38.79	-1.79	-9.42	-30.34	-26.72
Δ triglyceride (%)	-3.13	-24.11*	-17.07	-33.48	3.63	-17.80*	4.62	-17.56
Δ HDL-c (%)	4.25	9.56	5.73	6.80	2.94	13.11	4.38	7.50
Δ LDL-c (%)	-33.08	-28.89	-43.80	-41.41	-10.15	-11.39	-47.26	-34.20
Δ apoA1 (%)	2.03	5.20	-4.97	1.72	8.57	10.61	-0.49	-2.25
Δ apoB (%)	-18.66	-27.39	-34.83	-50.37*	-2.62	-10.44	-25.98	-28.30

*p<0.05

ATO: atorvastatin, SIM: simvastatin, FLU: fluvastatin

IV.1.3. Paraoxonase phenotype distribution

The allelic frequencies found in the pooled study population and in the three groups using the phenotypic determination are shown in **Table 2**. The allelic frequencies were in accordance with the results of our previous studies and the literature, following the Hardy-Weinberg equilibrium ($p < 0.05$). PON1 phenotype distribution of the three groups did not differ significantly ($p = 0.1005$).

IV.1.4. Responses of patients to statin treatment according their phenotypes

Because of the low number of patients with BB phenotypes the study population was divided into AA and AB+BB phenotype groups.

IV.1.5. Changes in lipid levels in the AA and in the AB+BB phenotype groups

There was no significant difference between the phenotypes in initial lipid levels (*data not shown*). The statin treatment decreased the triglyceride levels more effectively in the AB+BB group compared to the AA group in the whole study population (24.11 % vs. 3.13 %) and in the SIM group (17.80 % vs. 3.63 %). Although similar tendencies were found in the ATO and FLU groups, the differences between the different phenotype groups were not significant. The change in HDL-C level was consequently higher in the AB+BB phenotype group in the whole study population and in the three statin groups, but the differences between the two phenotype groups were not significant. The atorvastatin treatment was significantly more effective on apoB levels in the AB+BB phenotype compared to the AA phenotype group (50.37 % vs. 34.83 %) (**Table 3**).

IV.1.6. Changes in paraoxonase and arylesterase activities in the AA and in the AB+BB phenotype groups

In patients with AB+BB phenotype the statin treatment was significantly more effective on the paraoxonase activity in the whole study population and in all the three statin groups (**Fig.15A**). While in the AA phenotype group there was no significant change in the paraoxonase activity in the whole study population and in the ATO and SIM groups, in the AB+BB phenotype group a significant increase was found in all the studied statin groups. In the FLU group we could find a significant increase in both phenotype groups, however, in the AB+BB group the change was more prominent. Similar results were found when we examined the changes in arylesterase activity with the exception of atorvastatin (**Fig.15B**).

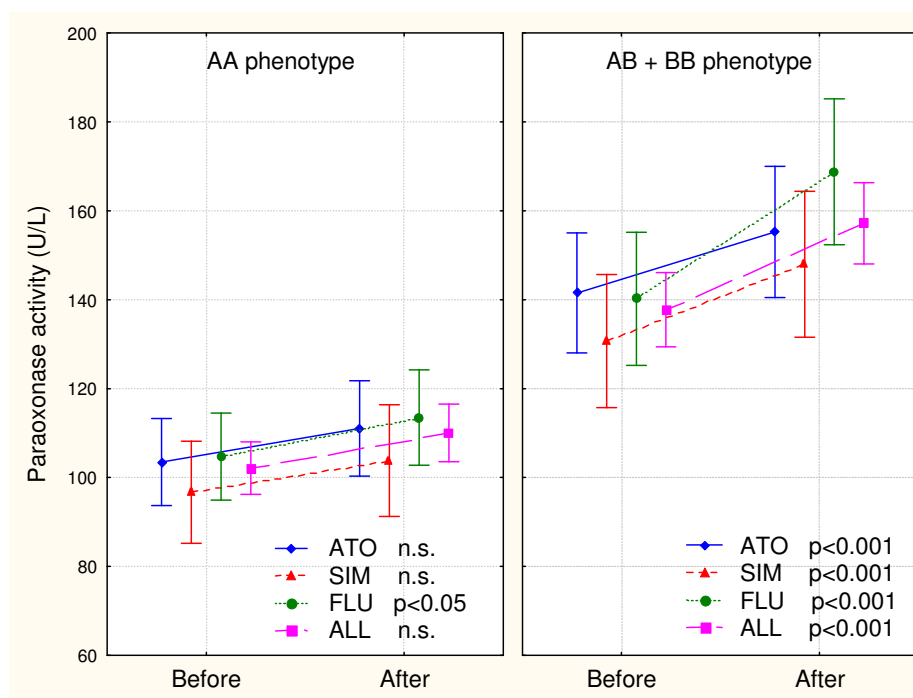
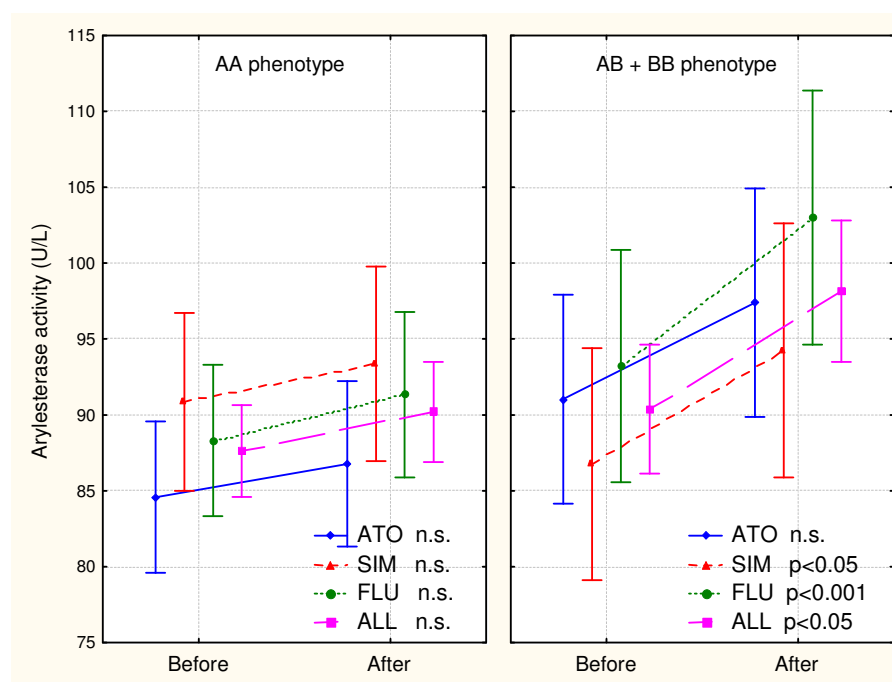
Fig. 15.A.**Fig.15.B**

Fig. 15. Responses of patients to statin treatment in the AA and in the AB+BB phenotype groups. **A:** paraoxonase activity; **B:** arylesterase activity. Results are means and vertical bars denote 0.95 confidence intervals. Repeated measures ANOVA with a post hoc Newman–Keuls test was carried out for comparisons between ‘before’ and ‘after’-treatment parameters.

IV.2 Atorvastatin effect on the distribution of HDL subfractions and human paraoxonase activity

Previous studies have investigated the effect of statin treatment on HDL and/or LDL subfractions in heterogeneous patient populations, but the results are contradictory [18-23]. In a previous study we found that atorvastatin treatment significantly increased PON1 and LCAT activities in hyperlipidemic patients, while CETP activity was significantly decreased [24].

Correlations between the CETP and LCAT activity and HDL subclasses after atorvastatin treatment have not yet been investigated.

Demographic data, laboratory parameters and characteristics of the study population are shown in **Table 4**.

Table 4. Characteristics and laboratory parameters of the study population. Values are mean \pm SD.

N	33		
Age (ys)	62.8 \pm 5.8		
Gender (M/F)	16/17		
BMI (kg/m²)	27.2 \pm 2.9		
	Before treatment	After treatment	Change (%)
Cholesterol (mmol/l)	6.68 \pm 0.51	4.57 \pm 0.78*	-31.6*
Triglyceride (mmol/l)	1.75 \pm 0.77	1.21 \pm 0.31*	-30.9*
HDL-c (mmol/l)	1.49 \pm 0.29	1.43 \pm 0.31	4.0
LDL-c (mmol/l)	4.39 \pm 0.51	2.66 \pm 0.54*	-39.4*
apoA1 (g/l)	1.65 \pm 0.24	1.65 \pm 0.21	0.0
apoB (g/l)	1.40 \pm 0.24	0.88 \pm 0.16*	-45.0*
LDL/apoB	3.32 \pm 0.63	2.94 \pm 0.40*	-11.4*
Lp(a) ()	217 \pm 266	190 \pm 241	-12.4
oxLDL (U/l)	60.5 \pm 15.9	32.7 \pm 9.4*	-46.0*
Paraoxonase activity (U/l)	120 \pm 84	126 \pm 41*	5.0*
Arylesterase activity (U/l)	81 \pm 37	93 \pm 18	14.8
LCAT (nmol/ml/h)	37 \pm 19	47 \pm 18**	27.0**
CETP (pmol/ml/h)	151 \pm 11	143 \pm 9*	-5.3*

*p<0.001, **p<0.01, #p<0.05 Students' paired *t*-test was applied to compare „before” with „after”-treatment parameters.

IV.2.1. Changes in the distribution of HDL subfractions

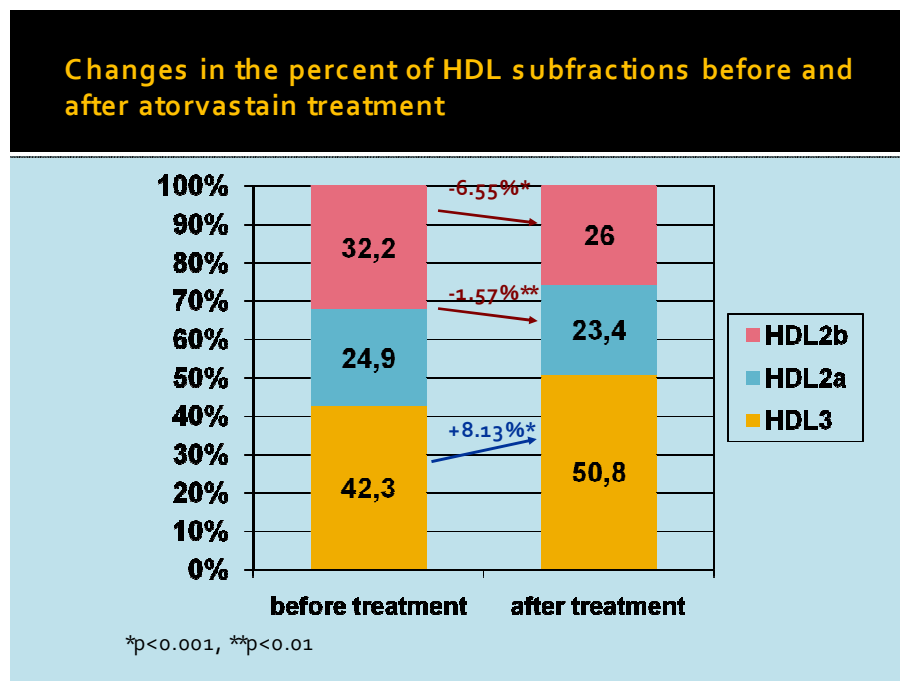
The atorvastatin treatment significantly increased the ratio of HDL3 by 8.13%, while the ratios of HDL2a and 2b were significantly decreased by 1.57% and 6.55%, respectively. The HDL2/HDL3 ratio was significantly decreased by 28.72% (**Table 5, Fig. 16**).

Table 5. LDL particle size and the distribution of HDL subfractions before and after atorvastatin treatment. Values are mean \pm SD.

	Before treatment	After treatment	Change (%)
LDL (nm)	27.0 \pm 0.85	27.9 \pm 1.0*	3.29*
HDL3 (%)	42.3 \pm 9.73	50.8 \pm 9.06*	8.13*
HDL2a (%)	24.9 \pm 5.11	23.4 \pm 5.0**	-1.57**
HDL2b (%)	32.2 \pm 8.76	26.0 \pm 9.81*	-6.55*
HDL2/HDL3	1.48 \pm 0.72	1.055 \pm 0.41*	-28.72*

*p<0.001, **p<0.01. Students' paired *t*-test was applied to compare „before” with „after”-treatment parameters.

Fig. 16.

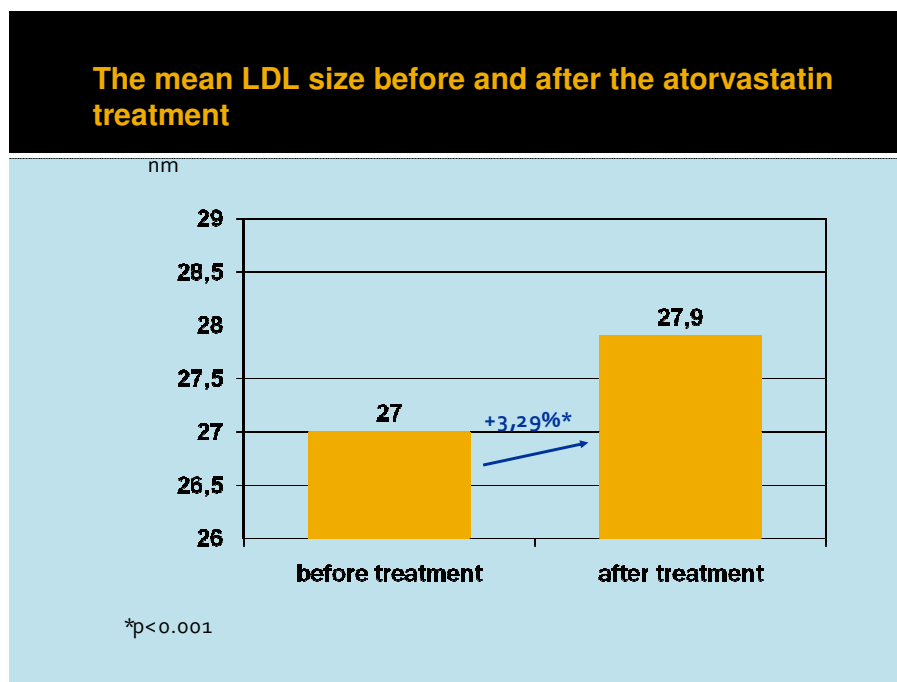


IV.2.2. Changes in the size of LDL

The mean LDL size significantly increased after the atorvastatin treatment, by 3.29% (Table 5, Fig. 17.). We could not find significant correlations between the size of LDL and the activities of PON1, LCAT and CETP when we pooled the results of

before and after atorvastatin treatment. The changes in LDL diameter did not correlate significantly with the changes in the activities of these enzymes.

Fig. 17.



IV.2.3. Correlation between HDL subclasses and CETP activity

A significant positive correlation was found between the CETP activity and the HDL2b ratio when we pooled the results of before and after atorvastatin treatment, while we found a significant negative correlation between CETP activity and HDL3 ratio. Similarly, there was a negative correlation between CETP activity and HDL2a ratio, the association being on the margin of statistical significance ($p=0.0503$) (**Fig 18. A, B and C**). There was a significant negative correlation between the change in CETP activity and the change in HDL2a ratio (**Fig. 19.A.** $r=-0.39$, $p<0.05$).

Fig.18.A. Correlation between CETP activity and ratio of HDL3

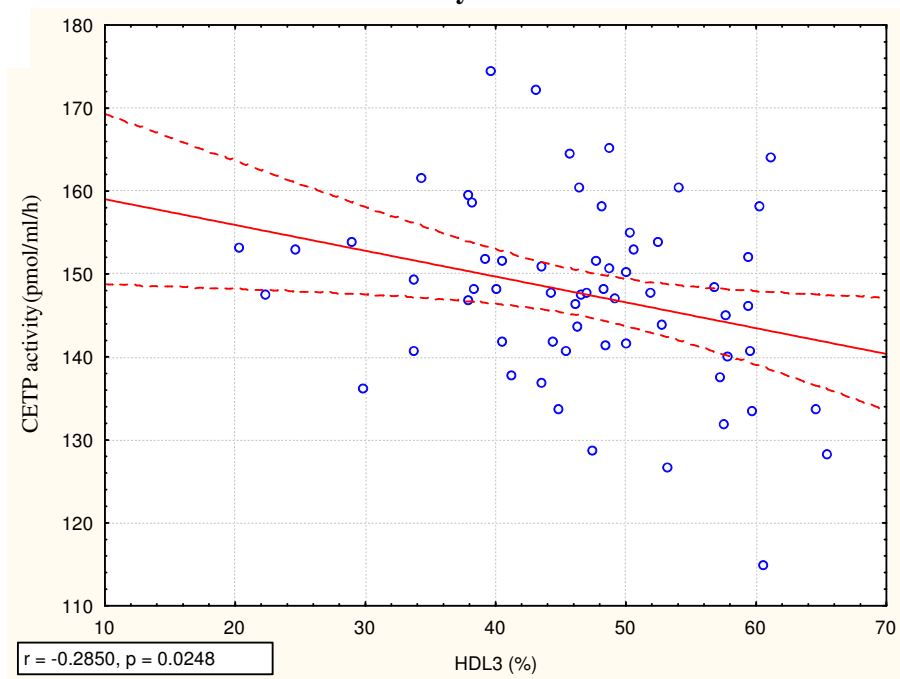


Fig.18.B. Correlation between CETP activity and ratio of HDL2b

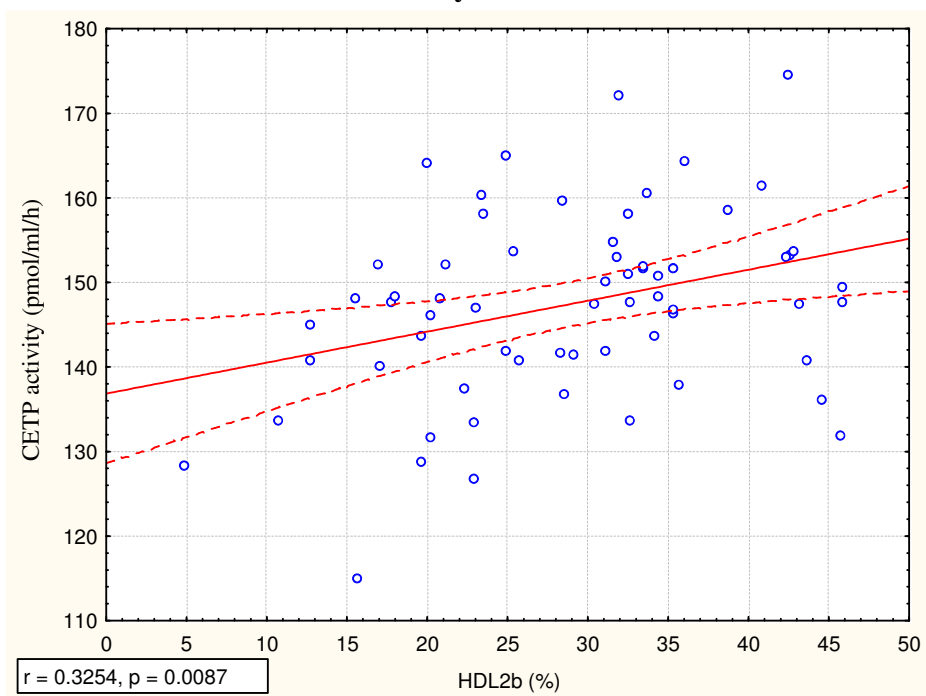


Fig. 18. C Correlation between CETP activity and ratio of HDL2a

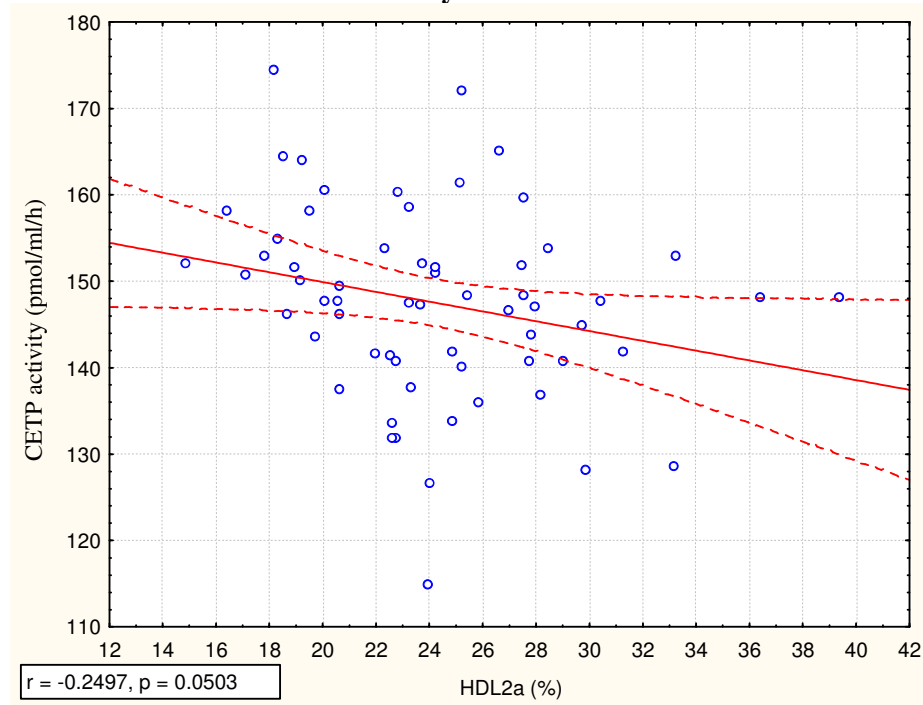
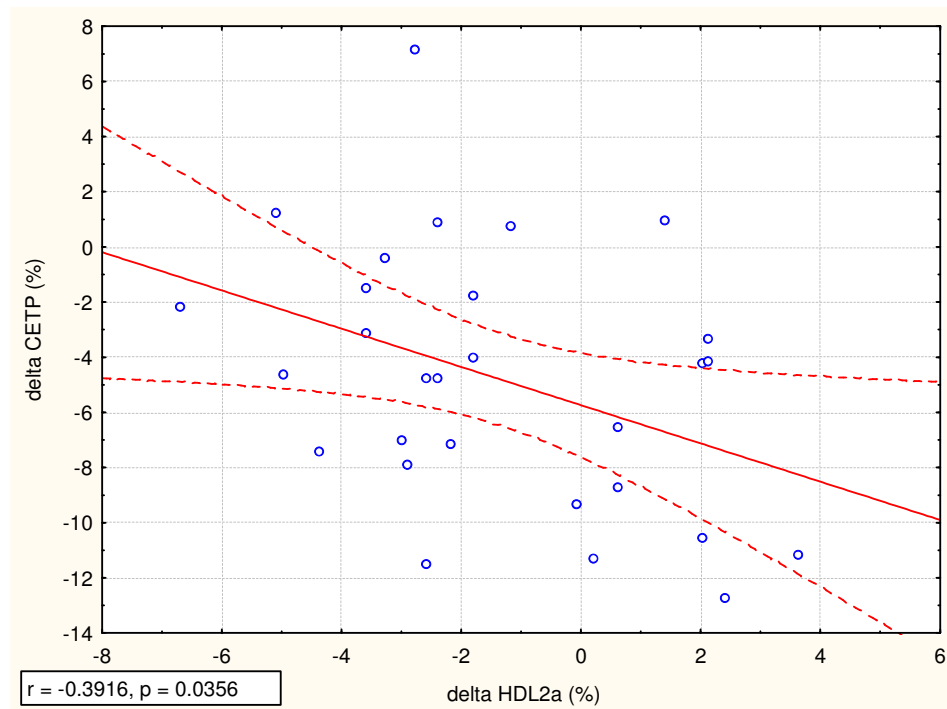


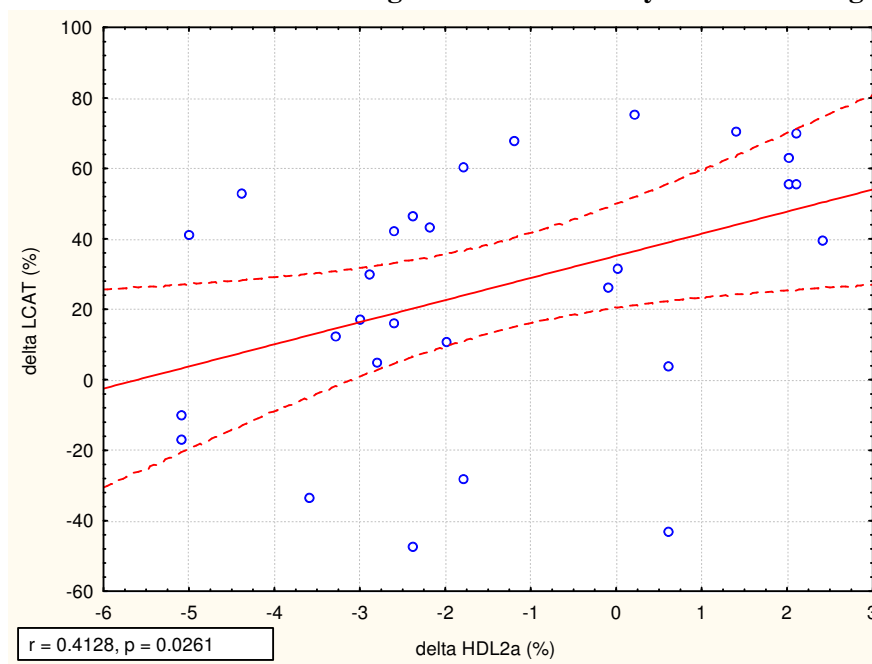
Fig.19.A. Correlation between the change in CETP activity and the change in HDL2a



IV.2.4. Correlation between HDL subclasses and LCAT activity

We did not find significant correlations between LCAT activity and HDL subclasses either before or after the atorvastatin treatment, or in the pooled data. However, there was a significant positive correlation between the change of LCAT activity and the change in HDL2a ratio (**Fig.19.B.**) ($r=0.41$, $p<0.05$). There was no significant correlation between the LCAT/CETP ratio and HDL subclasses.

Fig. 19.B. Correlation between the change in LCAT activity and the change in HDL2a



IV.2.5. Correlation between HDL subclasses and PON1 activity

We did not find significant correlations between PON1 activity and HDL subclasses either before or after the atorvastatin treatment, or in the pooled data (data not shown).

IV.2.6. PON1 phenotype distribution

The allelic frequencies found in the two groups using the phenotypic determination are shown in **Table 6**. The allelic frequencies were in accordance with the results of our previous studies and the literature, and following the Hardy-Weinberg equilibrium ($p<0.05$).

Table 6. Paraoxonase phenotype distribution and allelic frequencies in the study population

	n	%
AA	21	63.6
AB	8	24.3
BB	4	12.1
A	50	75.8
B	16	24.2

IV.2.7. HDL subclasses in subjects with different PON1 phenotypes

Since only a few subjects belonged to the BB phenotype, only phenotypes AA and AB+BB could be compared. No significant associations were found between PON1 activity and HDL2b ($p=0.76$), HDL2a ($p=0.20$) and HDL3 ($p=0.48$) in subjects with AB+BB phenotypes. Similarly, we could not find a significant correlation between PON1 activity and HDL2b ($p=0.57$), HDL2a ($p=0.12$) and HDL3 ($p=0.82$) in subjects with AA phenotypes.

V. DISCUSSION***V.1. Paraoxonase phenotype modifies the effect of statins***

In the last few years, several clinical studies examined the effect of statins on PON1 activity. In most of these studies simvastatin and atorvastatin were administrated showing that statins could increase PON1 activity and the PON1/HDL ratio (**Table 7.**)

Table 7 . Clinical studies on the effect of statins on human paraoxonase-I (PON1) activity

Author, year of publication	statin (dose)	patients (number)	treatment period	PON1 activity	PON1/HDL
				$\Delta\%$ (p)	$\Delta\%$ (p)
Tomás et al, 2000.	Simvastatin (20 mg/d)	familial hypercholesterolemia (64)	4 months	$\uparrow 12.3\%$ (p=0.005)	$\uparrow 6.1\%$ (p<0.05)
Balogh et al, 2001.	Simvastatin (20 mg/d)	IIa and IIb hyperlipidemia (112)	4 weeks	$\downarrow 8.65\%$ (n.s.)	$\downarrow 10.9\%$ (n.s.)
Tsimihodimos et al 2002.	Atorvastatin (20 mg/d)	IIa hyperlipidemia (40)	4 months	$\downarrow 0.5\%$ (n.s.)	$\uparrow 1.5\%$ (n.s.)
	„	IIb hyperlipidemia (36)	„	$\uparrow 3.8\%$ (n.s.)	$\downarrow 3.99\%$ (n.s.)
Deakin et al. 2003.	Simvastatin (10/20 mg/d)	hypercholesterolemia, CHD (21)	6.7 weeks	$\uparrow 22.8\%$ (p<0.01)	$\uparrow 12.8\%$ (p<0.05)
Kural et al. 2004.	Atorvastatin (10 mg/d)	mixed hyperlipidemia (40)	no data	$\uparrow 23.6\%$ (p<0.05)	$\uparrow 19.1\%$ (p<0.05)
Paragh et al. 2004.	Simvastatin (20 mg/d)	IIa and IIb hyperlipidemia (49)	8 weeks	$\uparrow 25.1\%$ (p<0.05)	$\uparrow 14.4\%$ (p<0.05)
	Atorvastatin (10 mg/d)	„	8 weeks	$\uparrow 3.6\%$ (n.s.)	$\uparrow 1.8\%$ (n.s.)
Harangi et al, 2004.	Atorvastatin (10 mg/d)	IIa hyperlipidemia (13)	6 months	$\uparrow 37.9\%$ (p<0.05)	$\uparrow 8.7\%$ (p<0.05)
Kassai et al, 2007.	Atorvastatin (20 mg/d)	IIa and IIb hyperlipidemia (33)	3 months	$\uparrow 21.2\%$ (p<0.001)	$\uparrow 30.7\%$ (p<0.01)
Bergheanu et al. 2007.	Atorvastatin (20/40/80mg/d)	CHD, low HDL men (34)	6 weeks	\uparrow (n.s.)	
	Rosuvastatin (10/20/40 mg/d)	„ (34)	„	\uparrow (p<0.05)	
Christidis et al. 2007.	Fluvastatin (40 mg/d)	hypercholesterolemia (202)	6 months	\uparrow	
Mirdamadi et al. 2008.	Atorvastatin (10 mg/d)	IIb hyperlipidemia (61)	3 months	$\uparrow 9.5\%$ (p<0.001)	$\uparrow 3.0\%$ (p<0.05)
	Simvastatin (10/20 mg/d)	„ (46)	„	$\uparrow 9.0\%$ (p<0.001)	$\uparrow 5.7\%$ (p<0.01)
	Fluvastatin (80 mg/d)	„ (57)	„	$\uparrow 12.6\%$ (p<0.001)	$\uparrow 9.5\%$ (p<0.01)

CHD: coronary heart disease

The putative mechanism of statin-effect on PON1 activity is intensively investigated. Deakin et al. examined the influence of simvastatin on PON1 gene expression in HepG2 cells. They found that simvastatin was able to modulate the *in vitro* expression of PON1 regulated by sterol regulatory element-binding protein-2 (SREBP-2) and increased the serum PON1 concentration and activity (Deakin et al. 2003). Data from this study suggest that PON1 gene belongs to the wide range of genes regulated by SREBPs. Ota et al. found that pitavastatin significantly increased the promoter activity in HEK293 cells after PON1 (587/-6) plasmid transfection. Because the PON1 gene promoter activity was also increased by atorvastatin and simvastatin, it was concluded that the transactivation was not specific to pitavastatin, but it is rather a general effect of statins. Their results also suggested that statin-effect on PON1 activity may have occurred through the mevalonic acid-derived farnesyl pyrophosphate pathway (Ota 2005). Recently Deakin et al. proved that patients who were homozygous for the -107C allele showed a significant increase in PON1 activity and concentration after 2 months of simvastatin treatment; while in the -107TT homozygotes there was no significant impact of statin treatment (Deakin et al. 2007). This study indicated that only a portion of the population could benefit from this pleiotropic effect of simvastatin. On the other hand, other PON1 polymorphisms may also exert a beneficial effect on the response to statin treatment, although the supporting findings are scanty.

Previously, Christidis et al. found that the PON1-192 and 55 genotypes did not influence the change in PON1 activity after 40 mg/d fluvastatin treatment (Christidis et al. 2007). However, they demonstrated that in the R allele carriers the increase in paraoxonase activity was larger compared to the QQ homozygotes, although the difference was not significant. Tomás et al. reported similar results with 20 mg/d simvastatin treatment (Tomás et al. 2000). Our results support the initial hypothesis that the PON1 phenotype has a modulatory effect on paraoxonase activity: we found that the change in paraoxonase activity after statin treatment was significantly higher in the AB+BB phenotype group. The difference between the two phenotype groups was significant in each statin groups, nevertheless PON1 activity presents a great inter-individual variability (La Du et al. 1993). The effect of statins were similar on arylesterase activities, however, the difference between the two phenotype groups was not significant in the atorvastatin-treated group. This latter result suggests that there can be differences in the effect of phenotype using different type of statins. Each statin has different pharmacokinetic properties, bioavailability and hydrophilicity. These

individual properties, like affinity to cytochrome P450 isoenzymes may influence the effect of statins on PON1 activity (Bellosta et al. 2004). Diverse study populations, different types of hyperlipoproteinemia and various types of statin dosages could contribute to the discrepancies in the literature. Additionally, Vincent-Viry et al. reported 7.2% discordance between the results of genotyping and phenotyping in healthy French subjects (Vincent-Viry et al. 2003). It must be noted that Jarvik *et al.* demonstrated that PON1 phenotype is a better predictor of vascular disease than PON1-55 or PON1-192 genotypes (Jarvik 2000).

Malin *et al.* reported firstly that PON1 genotype could modify statin-effect on lipid parameters. They found that pravastatin increased the apoA1 concentration and tended to increase the HDL-c concentration in R allele carriers but not in QQ homozygotes. Although the change in total cholesterol and HDL-c levels was higher in the AB+BB phenotype group, the study was not powered to detect this difference at a statistically significant level (Malin 2001). On the other hand, Turban *et al.* found that the Q192R variants of PON1 are not associated with plasma lipid levels and response to treatment with 40mg/d fluvastatin (Turban 2001). The results of Christidis *et al.* showed that in R allele carriers the decrease in triglyceride levels was larger compared to the QQ homozygotes, although the difference was not significant (Christidis 2007). We also found association between PON1 phenotype and response of triglyceride levels to simvastatin treatment.

Although the exact mechanism is not clarified, in a previous study we found that in diabetic patients there was a significant positive correlation between paraoxonase and lipoprotein lipase (LPL) activities. Moreover, the PON1 paraoxonase activity of the AA phenotype group did not correlate with LPL activity, while in the AB+BB phenotype group there was a significantly positive correlation between paraoxonase and LPL activity (Kalmár 2005). It is also known that the activity of LPL inversely correlated with triglyceride concentration. Presumably, the activity of LPL may influence the HDL remodeling, and consequently, modifies the activity of the HDL-associated PON1. Based on these data, higher paraoxonase activity (AB+BB phenotypes) means higher LPL activity, and consequently, lower triglyceride level (Kalmár 2005).

According to our results there was no significant difference between phenotypes in initial lipid levels. Similarly to Malin *et al.*, we also found that statins were more effective on HDL-c in the AB+BB phenotype group, which represent the R allele

carriers, although the difference between the two phenotype groups was not significant (Malin 2001). Tomás *et al.* showed that the simvastatin treatment tended to be more effective on total cholesterol, LDL-c, triglyceride and apoB levels in the R carriers, but the differences were not significant (Tomás 2000). We also found that changes in total cholesterol levels tended to be higher in the AB+BB phenotype group.

Interestingly, in the SIM group the changes in cholesterol, LDL-c and HDL-c levels were less than they were expected comparing to previous larger clinical studies (Pedersen 1998, Law 2003). On the contrary, we found a remarkable decrease in cholesterol and LDL-c levels in the FLU group. It is known that the efficacy of statin treatment is influenced by the initial lipid levels (Winkler 2002); the initial cholesterol and LDL-c levels were higher in the FLU group, although we could not find significant difference between the FLU and SIM groups. On the other hand, in our study we used low dose simvastatin and high dose extended-release fluvastatin therapy.

It must be noted that there are some limitations of the study. The relatively small sample size clearly reduces the power of the study. Presumably, the increase in the size of the study population would result in a significant association between PON1 phenotypes and statin effect on cholesterol and HDL-c levels. Data on PON1 genotypes strengthen the hypothesis that the PON1 phenotype modifies the effect of statins on lipid parameters.

The PON1 phenotype may be a predictive factor for the effectivity of statin treatment on paraoxonase activity and serum lipid parameters. Different types of statins may exert different effects on these parameters. Therefore determination of PON1 phenotype can be recommended in future studies on hyperlipidemic patients before statin treatment. Further studies are needed to clarify the role of PON1 genotype and phenotype in the modification of statin treatment.

V.2. Atorvastatin effect on HDL and LDL subfractions

Data regarding the predictive ability of HDL subfractions for CHD risk are not conclusive, although the concept that larger HDL particles may be associated with greater protection against atherosclerosis has been widely accepted in the last decades. The metabolic and clinical significance of HDL subclasses is still not fully understood. In the past, it was widely accepted that the HDL2 subfraction was responsible for the beneficial health effects of high HDL-C (Stampfer et al 1991, Musliner 1988). Some other studies, however, have reported that HDL3, and not HDL2, is negatively associated with atherosclerosis (Levy 1984, Kempen 1987). Recently, Vekic et al. investigated the relationship of HDL subclasses with the Framingham risk score in middle-aged asymptomatic individuals. They could not prove any significant association between the calculated risk and HDL subfractions, although they found a notable incidence of small size HDL phenotypes (which included HDL3 particles) in subjects categorized as low-risk (Vekic et al 2007). Therefore, further studies will be necessary to evaluate the benefits of the increased HDL3 ratio after atorvastatin treatment found in our study. Similarly, in our results, increased HDL3 levels were found in hyperlipidemic patients after simvastatin and fluvastatin treatment using ultracentrifugal methods (Broyles et al. 1995, Mølgaard et al. 1999). It must be noted, however, that ultracentrifugal separation of lipoproteins makes small lipid-poor particles to be shed from larger particles. Therefore, this method may artificially increase the concentrations of HDL3 particles.

CETP is a glycoprotein that is physically associated with HDL particles. It facilitates the transport of CE from HDL to apolipoprotein B-containing lipoproteins. Simultaneously, an energy-neutral transfer of triglycerides in the opposite direction also occurs. Additionally, CETP is also a part of a remodeling cascade, required for the effective interaction of HDL with the scavenger receptor B1 (SR-B1) and selective uptake of cholesteryl esters by the liver (Collet et al. 1999). Furthermore, CETP may be involved in intracellular cholesterol metabolism. It was demonstrated that CETP inhibitors increased apoA-I synthesis (Shimoji et al. 2004). Previously, decreased CETP activity was found after atorvastatin treatment in different patient populations (Guerin et al. 2000, Singh et al 1995). In the present study, we also found similar results.

Lagrost et al. found that CETP could promote the formation of HDL3 particles at the expense of HDL2a (Lagrost et al. 1996) They reported a negative correlation between

CETP activity and HDL2a levels, and a positive correlation between CETP and HDL3b levels. Contrarily, Huesca-Gomez et al. found a negative correlation between CETP activity and HDL3b (Huesca-Gomez et al. 2004). We also found significant negative correlations between CETP activity and HDL3, and between CETP activity and HDL2a, while there was a significant positive correlation between CETP activity and HDL2b.

Huesca-Gomez et al. reported that in healthy individuals LCAT activity showed a significant negative correlation with large HDL2a, and in patients with higher LCAT/CETP ratio had a greater relative proportion of small HDL3 (Huesca-Gomez et al. 2004). In this study, we could not find a correlation between LCAT activity and the ratio of HDL subfractions. Moreover, we found a significant positive correlation between the change of LCAT activity and the change of HDL2a ratio after atorvastatin treatment. Further studies will be necessary to clarify the reasons for these conflicting results.

Only a few studies investigated the effect of statins on HDL subclasses, and the results are not concordant. Results of the clinical studies are summarized in **Table 8**. Unfortunately, different statins have been administrated in different doses for various patient populations. In addition, different assays have been used. Therefore the comparison of these results is strongly problematic. Yet, most of these studies demonstrated that statins increase the levels of the smaller HDL subfractions, but not all of them.

Table 8. Clinical studies on the effect of statins on HDL subfractions

Author (year of publication)	statin (dose)	patients (number)	changes in HDL subfractions	method
Franceschini et al. (1989)	Prava (40 mg/d)	CHD (18)	no significant change	rate zonal UC, polyacrylamide GGE
Guerin et al. (1995)	Prava (40 mg/d)	FH (9)	HDL2/HDL3↑ (n.s.)	GGE (Coomassie Brilliant Blue staining)
Broyles (1995)	Fluva (20 mg/d)	hypercholesterolemia (57)	↑HDL2 by 24%, ↑HDL3 by 3%	dextrane sulfate and magnesium precip.
Homma et al. (1995)	Simva (5/10 mg)	Ila and Iib hyperlip, FH (28)	↑HDL2 by 15.3%,no change in HDL3	UC and gradient PAGE
Linjen et al. (1996)	Prava (10/20/40 mg/d)	hypercholesterolemia (25)	no sign. change in HDL2 and HDL3	UC
Mølgaard (1999)	Simva (20/40 mg/d)	hypercholesterolemic (45)	10-year follow-up, HDL2 ↑ by 9% to 25% the first 6 years and then ↓. HDL3 cholesterol showed a persistent elevation during treatment	UC
Guerin et al. (2000)	Atorva (10 mg/d)	CHD (18)	HDL2a↑, HDL3c↓, HDL2b↔, HDL3a↑	density gardient UC
Schaefer et al (2002)	Atorva (20-40 mg/d)	CHD (77)	large HDL ↑	NMR spectroscopy
Asztalos et al. (2002)	Atorva (20/40/80 mg/d)	CHD (86)	HDL2b↑, HDL3↓	2D nondenaturing agarose PAGE
Brousseau et al. (2004)	Atorva (20 mg/d)	low HDL (9)	no significant change	NMR spectroscopy
Kawano et al. (2007)	Pitava (2 mg/d)	hyperlipidemia (29)	HDL2↑, preβ1 ↓, HDL3 ↔	UC and anti-pre-β1 Ab ELISA
Saougos et al. (2007)	Rosuva (10 mg/d)	hyperlipidemia (50)	no significant change	density gradient UC
Harangi et al. (2009)	Atorva (20 mg/d)	Ila and Iib hyperlipid. (33)	HDL3↑, HDL2a and 2b↓	GGE

CHD: coronary heart disease; HDL: high-density lipoprotein; FH: familial hypercholesterolemia, UC: ultracentrifugation, GE: gel electrophoresis, GGE: gradient GE, NMR: nuclear magnetic resonance, ELISA:

Surprisingly, only a few studies investigated the effect of atorvastatin on LDL and HDL subclasses, and the results are not in concordance. Schaefer et al. found that atorvastatin treatment increased the ratio of large HDL2b by 25%, while the medium and small HDL3 did not change significantly, and decreased all LDL subfractions in CHD patients and control subjects (Schaefer et al. 2002). O'Keefe et al reported that atorvastatin did not significantly alter the overall LDL subfraction pattern in dyslipidemic patients, however, in the isolated hypercholesterolemia group, the proportion of LDL - presented as the small dense fraction - increased by 23%. In patients with atherogenic dyslipidemia, the sdLDL (small dense LDL) preponderance was not significantly decreased (O'Keefe et al. 2004). Guerin et al. treated patients with combined hyperlipidemia, and found that atorvastatin treatment reduced the levels of all LDL subspecies, but the reduction in dense LDL levels was the most prominent. Total HDL mass was not modified by atorvastatin; however, the relative proportion of HDL2a was significantly increased, whereas the HDL3c ratio was decreased. The HDL3a ratio was not significantly increased (Guerin et al. 2000). Ai et al. investigated the effect of a maximal dose of atorvastatin on LDL subfractions in hyperlipidemic patients, and reported a significant decrease in small dense LDL levels after treatment (Ai et al 2008). They found that in 9 CHD patients, using five different statins, atorvastatin was the most effective in modifying the HDL subfraction profile, by increasing the concentration of large, cholesterol rich, apoA1 containing HDL2b fraction and decreasing the concentration of the small, ApoA1/A2 containing HDL3 subpopulation (Ai et al 2008). Brousseau et al. found that in 9 patients with low initial HDL levels atorvastatin treatment alone did not significantly affect the distributions of large and small HDL subclasses (Brousseau et al. 2004). We also found that atorvastatin increased the diameter of LDL particles, which may reflect a decrease in the sdLDL fraction. Interestingly, in our study a significant increase was found in HDL3 percentage, while the HDL2a and 2b were decreased. Probably, lower doses of atorvastatin that were applied in our study were not able to restore the physiological proportions of the HDL subclasses, while the higher doses used by Schaefer et al. and Asztalos et al. may have beneficially affected the distributions of the HDL subclasses.

Previous studies showed that there can be a strong variation among different statin agents regarding their effects on other lipoprotein subclasses. Statins potentially lower all LDL subclasses, thus, their net effect on LDL subclasses or size is often only

moderate. However, analyses of published studies suggested a very limited role of pravastatin and simvastatin in modifying LDL size and their subclasses, while fluvastatin and atorvastatin seemed to be much more effective agents. Finally, rosuvastatin seemed to be promising in altering LDL subclasses towards less atherogenic particles (Rizzo and Berneis 2006). Similarly to their various effect on LDL subfractions, different statins may exert different effect on HDL subclasses. Therefore, further studies will be necessary to clarify the role of different statins in modifying HDL subfractions.

Previously van Himbergen et al found that PON1 levels and activities significantly modified the HDL-C increment in familial hypercholesterolaemia (FH) patients undergoing atorvastatin or simvastatin therapy (Himbergen et al. 2005). Recently, Moren et al. analyzed the subfraction distribution of HDL-associated PON1 enzyme activity and stability in hamster cell culture. They found that PON1 was equally distributed between apoA1 and apoA2 containing HDL particles. Particles containing both apoA1 and A2 showed significantly greater resistance to PON1 inactivation arising from oxidation. ApoA1 and A2 were independent determinants of serum PON1 concentration and activity (Moren et al. 2008). It is also known that a higher proportion of PON1 is located in the apoJ containing HDL fraction; although, not all of the plasma paraoxonase is associated with apoJ (Kelso et al. 1994). It has been also reported that PON1 activity is highest in the denser HDL3 and ApoJ containing HDL particles, which are present only in the HDL3 fraction (Himbergen et al, 2005). In agreement with most of the previous studies, we found that PON1 activity significantly increased after atorvastatin treatment (Schaefer et al. 2002, Bergmeier et al. 2004, Paragh et al. 2004). Although, we did not find any correlation between the changes in PON1 activity and HDL subclasses; the significant increase in HDL3 fraction (which consists of the majority of apoJ and apoA1/A2 containing HDL particles) may have contributed to the augmented PON1 activity. Similarly, Vekic et al. could not find any significant correlation between the PON1 activity and HDL particle size or HDL subclass pattern, although the diazoxonase activity of PON1 showed a positive correlation with HDL size. Moreover, they reported an association of PON1 RR phenotype with the formation of smaller, denser HDL3 particles (Vekic et al. 2007).

Possible reasons for contradictions in the literature

Many of the contradictions in the literature may be attributable to the different assay methods, different study populations, selection bias and ethnic variations. However, there can be other factors.

Today many statins (pravastatin, simvastatin, atorvastatin, fluvastatin, lovastatin, pitavastatin, and rosuvastatin) are clinically available worldwide. Each statin has different pharmacokinetic properties and bioavailability. Their efficacy in modifying lipoprotein levels are also variable. Although there is a body of evidence that statins are equally effective for primary and secondary prevention of cardiovascular disease (Pedersen et al. 1998, Shepherd et al. 1995), in experimental animal models it has been reported that each statin has different pleiotropic effects. For example, solubility in water or alcohol (hydrophilicity or lipophilicity) is one such property related to tissue selectivity of statins (Sirtori 1993). Therefore, their effects on HDL-associated enzymes and HDL subfractions may also differ.

In addition, it is likely that the antatherogenic effect attributed to the process of RCT might be better predicted by assessing the flow of cholesterol through this pathway, than by the concentration of HDL and HDL subfractions or HDL-associated enzyme activities.

VI. SUMMARY

Human serum paraoxonase-1 (PON1) protects lipoproteins against oxidation by hydrolyzing lipid peroxides in oxidized LDL, therefore it may protect against atherosclerosis. Changes in the ratio of HDL subfractions may alter the stability and antioxidant capacity of PON1. PON1 activity variations have been also related to some common polymorphisms in the coding and promoter regions. The PON1-192 polymorphism has the most significant impact on enzyme activity, and its prevalence can be estimated by phenotype distribution analysis.

The aim of the study was to examine the effect of atorvastatin treatment on the distribution of HDL subfractions, LDL size, cholesteryl ester transfer protein (CETP), lecithin:cholesterol acyltransferase (LCAT) and human serum paraoxonase-1 (PON1) activity, and to clarify the role of PON1 phenotypes on the effect of three different

statins on paraoxonase activity and lipid parameters in patients with type IIa and IIb hypercholesterolemia.

Three months of 20 mg/day atorvastatin treatment significantly increased the HDL3 and decreased the HDL2a and HDL2b subfractions. The mean LDL size was significantly increased. The PON1 activity was augmented by the atorvastatin treatment. The CETP activity positively correlated with the HDL2b and negatively correlated with the HDL3 and HDL2a levels. Three months of statin (10 mg/day atorvastatin, 10/20 mg/day simvastatin and 80 mg/day extended-release fluvastatin) treatment significantly increased the paraoxonase activity in every statin-treated group. In patients with AB+BB phenotype the statin treatment was significantly more effective on paraoxonase activity than in the AA group. The statin treatment more effectively decreased the triglyceride levels in the AB+BB group compared to the AA group in the whole study population and in the simvastatin-treated group. The atorvastatin treatment was significantly more effective on apoB levels in patients with AB+BB phenotype than in the AA phenotype group.

These results confirm that atorvastatin normalizes lipid levels and preferentially increases HDL. Our data are consistent with the concept that changes in CETP and LCAT activities during statin treatment may directly alter the ratio of HDL subclasses. Data on PON1 activity and HDL subfraction alterations suggest that the increase in HDL3 ratio may also responsible for the enhanced PON1 activity after atorvastatin treatment. Our results indicate that the PON1 phenotype may be a novel predictive factor for the effectivity of statin treatment on PON1 activity and serum lipid levels; however, different types of statins may exert different effects on these parameters.

VII. NEW RESULTS

We reported the first time that PON1 phenotypes have an impact on the paraoxonase-activating and lipid-lowering effect of three different types of statins:

- All the statins (atorvastatin, simvastatin and fluvastatin) included in this study were able to effectively increase the serum paraoxonase activity and decrease triglyceride levels, however this response seemed to be more significant in

patients with AB+BB PON1 phenotype than in patients bearing AA PON1 phenotype.

- Furthermore, the apoB-lowering effect of atorvastatin was also found to be PON1 phenotype-dependent.

We were the first ones to show correlations between the CETP and LCAT activity and HDL subclasses after atorvastatin treatment:

- We found a significant positive correlation between the CETP activity and the HDL2b ratio when we pooled the results of before and after atorvastatin treatment, while we found a significant negative correlation between CETP activity and HDL3 ratio.
- There was a significant negative correlation between the change in CETP activity and the change in HDL2a ratio.
- We found a significant positive correlation between the change of LCAT activity and the change of HDL2a ratio after atorvastatin treatment.

VIII. REFERENCES

Abbott CA, Mackness MI, Kumar S, Boulton AJM, Durrington PN. Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins.

Arterioscler Thromb Vasc Biol 1995;15:1812-8.

Ai M, Otokozawa S, Asztalos BF, et al. Effects of maximal doses of atorvastatin versus rosuvastatin on small dense low-density lipoprotein cholesterol levels. *Am J Cardiol* 2008;101:315-8.

Asztalos BF, Batista M, Horvath KV, Cox CE, Dallal GE, Morse JS, Brown GB, Schaefer EJ. Change in alpha1 HDL concentration predicts progression in coronary artery stenosis. *Arterioscler Thromb Vasc Biol* 2003;23:847-52.

Asztalos BF, Collins D, Cupples LA, Demissie S, Horvath KV, Bloomfield HE, Robins SJ, Schaefer EJ. Value of high-density lipoprotein (HDL) subpopulations in predicting recurrent

cardiovascular events in the Veterans Affairs HDL Intervention Trial. *Arterioscler Thromb Vasc Biol* 2005;25:2185-91.

Asztalos BF, Cupples LA, Demissie S, Horvath KV, Cox CE, Batista MC, Schaefer EJ. High-density lipoprotein subpopulation profile and coronary heart disease prevalence in male participants of the Framingham Offspring Study. *Arterioscler Thromb Vasc Biol* 2004;24:2181-7.

Asztalos BF, Horvath KV, McNamara JR, Roheim PS, Rubinstein JJ, Schaefer EJ. Effects of atorvastatin on the HDL subpopulation profile of coronary heart disease patients. *J Lipid Res* 2002;43:1701-7

Aviram M, Billecke S, Sorenson R, Bisgaier C, Newton R, Rosenblat M, Eroglu J, Hsu C, Dunlop C, La Du BN. Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: selective active of human paraoxonase alloenzymes Q and R. *Arterioscler Thromb Vasc Biol*. 1998;10:1617–1624.

Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *J Clin Invest*. 1998 Apr 15;101(8):1581-90.

Aviram M. Macrophage foam cell formation during early atherogenesis is determined by the balance between pro-oxidants and anti-oxidants in arterial cells and blood lipoproteins. *Antioxid Redox Signal*. 1999 Winter;1(4):585-94.

Aviram M, Rosenblat M. Paraoxonases and cardiovascular diseases: pharmacological and nutritional influences. *Curr Opin Lipidol*. 2005 Aug;16(4):393-9.

Ayub A, Mackness MI, Arrol S, Mackness B, Patel J, Durrington PN. Serum paraoxonase after myocardial infarction. *Arterioscl Thromb Vasc Biol* 1999;19:330-5.

Ballantyne C, Blazing M, Hunningake D, et al. Effect on high-density lipoprotein cholesterol of maximum doses of simvastatin and atorvastatin in patients with hypercholesterolemia: results of the Comparative HDL Efficacy and Safety Study (CHESS). *Am Heart J* 2003;146:862–9.

Balogh Z, Seres I, Harangi M, Kovács P, Kakuk G, Paragh G. *Diabetes Metab*. Gemfibrozil increases paraoxonase activity in type 2 diabetic patients. A new hypothesis of the beneficial action of fibrates? 2001 Nov;27(5 Pt 1):604-10.

Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, Lopez-Sendon J, Mosca L, Tardif JC, Waters DD, Shear CL, Revkin JH, Buhr KA, Fisher MR, Tall AR, Brewer B; ILLUMINATE Investigators. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med*. 2007 Nov 22;357(21):2109-22.

Bellosta S, Paoletti R, Corsini A. Safety of statins. Focus on clinical pharmacokinetics and drug interactions. *Circulation* 2004; Suppl III: 50-7.

Bergmeier C, Siekmeier R, Gross W. Distribution spectrum of paraoxonase activity in HDL fractions. *Clin Chem* 2004;50:2309-15.

Biggadike K, Angell RM, Burgess CM, Farrell RM, Hancock AP, Harker AJ, Irving WR, Ioannou C, Procopiou PA, Shaw RE, et al. Selective plasma hydrolysis of glucocorticoid g-lactones and cyclic carbonates by the enzyme paraoxonase. *J Med Chem*. 2000;43:19–21.

Billecke S, Draganov D, Counsell R, Stetson P, Watson C, Hsu C, La Du BN. Human serum paraoxonase (PON1) isoenzymes Q and R hydrolyse lactones and cyclic carbonate esters. *Drug Metab Dispos*. 2000;28:1335–1342.

Blanche PJ, Gong EL, Forte TM, Nichols AV. Characterization of human high-density lipoproteins by gradient gel electrophoresis. *Biochim Biophys Acta*. 1981; 665(3):408-19.

Blatter M-C, James RW, Messmer S, Barja F, Pometta D. Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-85: identity of K-85 with paraoxonase. *Eur J Biochem*. 1993;211:871– 879.

Brites F, Zago V, Verona J, Muzzio ML, Wikinski R, Schreier L. HDL capacity to inhibit LDL oxidation in well-trained triathletes. *Life Sci*. 2006 May 22;78(26):3074-81.

Brophy VH, Jampsa RL, Clendenning JB, McKinstry LA, Jarvik GP, Furlong CE. Effects of 5' regulatory-region polymorphisms on paraoxonase-gene (PON1) expression. *Am J Hum Genet*. 2001 Jun;68(6):1428-36.

Brousseau ME, Schaefer EJ, Wolfe ML, et al. Effects of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. *N Engl J Med* 2004;350:1505-15.

Broyles FE, Walden CE, Hunninghake DB, Hill-Williams D, Knopp RH. Effect of fluvastatin on intermediate density lipoprotein (remnants) and other lipoprotein levels in hypercholesterolemia. *Am J Cardiol* 1995;76:129A-35A.

Campos H, Genest JJ Jr, Blijlevens E, et al. Low density lipoprotein particle size and coronary artery disease. *Arterioscler Thromb* 1992;12:187-95

Chen Q, Reis SE, Kammerer CM, McNamara DM, Holubkov R, Sharaf BL, Sopko G, Pauly DF, Merz CN, Kamboh MI; WISE Study Group. Association between the severity of angiographic coronary artery disease and paraoxonase gene polymorphisms in the National Heart, Lung, and Blood Institute-sponsored Women's Ischemia Syndrome Evaluation (WISE) study. *Am J Hum Genet*. 2003 Jan;72(1):13-22.

Cherki M, Berrougui H, Drissi A, Adlouni A, Khalil A. Argan oil: which benefits on cardiovascular diseases? *Pharmacol Res*. 2006 Jul;54(1):1-5.

Christidis DS, Liberopoulos EN, Kakafika AI, Miltiadows GA, Liamis GL, Kakaidi B, Tselepis AD, Cariolou MA, Elisaf MS. Effect of paraoxonase 1 polymorphisms on the response of lipids and lipoprotein-associated enzymes to treatment with fluvastatin.

Arch Med Res 2007; 38: 403-410.

Collet X, Tall AR, Serajuddin H, et al. Remodeling of HDL by CETP in vivo and by CETP and hepatic lipase in vitro results in enhanced uptake of HDL CE by cells expressing scavenger receptor B-I. *J Lipid Res* 1999;40:1185-93

Davies HG, Richter RJ, Keifer M, Broomfield CA, Sowalla J, Furlong CE. The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. *Nat Genet.* 1996 Nov;14(3):334-6.

Deakin S, Leviev I, Guernier S, James RW. Simvastatin modulates expression of the PON1 gene and increases serum paraoxonase: a role for sterol regulatory element-binding protein-2. *Arterioscler Thromb Vasc Biol* 2003; 23: 2083-9.

Deakin SP, James RW. Genetic and environmental factors modulating serum concentrations and activities of the antioxidant enzyme paraoxonase-1. *Clin Sci (Lond).* 2004 Nov;107(5):435-47.

Deakin S, Moren X, James RW. Very low density lipoproteins provide a vector for secretion of paraoxonase-1 from cells. *Atherosclerosis.* 2005 Mar;179(1):17-25.

Deakin S, Guernier S, James RW. Pharmacogenetic interaction between paraoxonase 1 gene promoter polymorphism C-107T and statin. *Pharmacogen Genom* 2007; 17: 451-457.

Draganov DI, Teiber JF, Speelman A, Osawa Y, Sunahara R, La Du BN. Human paraoxonases (PON1, PON2, and PON3) are lactonases with overlapping and distinct substrate specificities. *J Lipid Res.* 2005 Jun;46(6):1239-47.

Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2001 Apr;21(4):473-80.

Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase polymorphism. *Am J Hum Genet.* 1983 Nov;35(6):1126-38.

Fazio S, Linton MF. Sorting out the complexities of reverse cholesterol transport: CETP polymorphisms, HDL, and coronary disease. *J Clin Endocrinol Metab.* 2006 Sep;91(9):3382-8.

Forti N, Diament J. High-Density Lipoproteins: Metabolic, clinical, epidemiological and therapeutic intervention aspects. An update for clinicians *Arq Bras Cardiol* 2006; 87 : 614-622

Fuhrman B, Koren L, Volkova N, Keidar S, Hayek T, Aviram M. Atorvastatin therapy in hypercholesterolemic patients suppresses cellular uptake of oxidized-LDL by differentiating monocytes. *Atherosclerosis.* 2002 Sep;164(1):179-85.

Fuhrman B, Volkova N, Aviram M. Postprandial serum triacylglycerols and oxidative stress in mice after consumption of fish oil, soy oil or olive oil: Possible role for paraoxonase-1 triacylglycerol lipase-like activity. *Nutrition.* 2006 Sep;22(9):922-30.

Gaidukov L, Rosenblat M, Aviram M, Tawfik DS. The 192R/Q polymorphs of serum paraoxonase PON1 differ in HDL binding, lipolactonase stimulation, and cholesterol efflux. *J Lipid Res.* 2006 Nov;47(11):2492-502.

Giroux LM, Davignon J, Naruszewicz M. Simvastatin inhibits the oxidation of low-density lipoproteins by activated human monocyte-derived macrophages. *Biochim Biophys Acta.* 1993 Jan 10;1165(3):335-8.

Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation.* 1989;79:8-15.

Guerin M, Lassel TS, Le Goff W, Farnier M, Chapman MJ. Action of atorvastatin in combined hyperlipidemia: preferential reduction of cholesteryl ester transfer from HDL to VLDL1 particles. *Arterioscler Thromb Vasc Biol* 2000;20:189-97.

Hachem SB, Mooradian AD. Familial dyslipidaemias: an overview of genetics, pathophysiology and management. *Drugs.* 2006;66(15):1949-69.

Hasselwander O, McMaster D, Fogarty DG, Maxwell AP, Nichols DP, Young IS. Serum paraoxonase and platelet-activating factor acetylhydrolase in chronic renal failure. *Clin Chem* 1998;44:179-81.

Harangi M, Seres I, Varga Z, Emri G, Szilvássy Z, Paragh G, Remenyik E. Atorvastatin effect on high-density lipoprotein-associated paraoxonase activity and oxidative DNA damage. *Eur J Clin Pharmacol.* 2004 Dec;60(10):685-91.

Harangi M, Mirdamadi HZ, Seres I, Sztanek F, Molnár M, Kassai A, Derdák Z, Illyés L, Paragh G. Atorvastatin effect on the distribution of high-density lipoprotein subfractions and human paraoxonase activity. *Transl Res.* 2009;153(4):190-8.

Harel M, Aharoni A, Gaidukov L, Brumshtein B, Khersonsky O, Meged R, Dvir H, Ravelli RB, McCarthy A, Toker L, Silman I, Sussman JL, Tawfik DS. Structure and evolution of the serum paraoxonase family of detoxifying and anti-atherosclerotic enzymes. *Nat Struct Mol Biol.* 2004 May;11(5):412-9

Heinecke JW. Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis. *Atherosclerosis.* 1998 Nov;141(1):1-15.

Herrick JB. Certain clinical features of sudden obstruction of the coronary arteries. *JAMA.* 1912;59:2015–2020.

Himbergen TM, van Tits LJ, Voorbij HA, de Graaf J, Stalenhoef AF, Roest M. The effect of statin therapy on plasma high-density lipoprotein cholesterol levels is modified by paraoxonase-1 in patients with familial hypercholesterolaemia. *J Intern Med* 2005;258:442-9.

Huesca-Gómez C, Carreón-Torres E, Nepomuceno-Mejía T, et al. Contribution of cholesteryl ester transfer protein and lecithin:cholesterol acyltransferase to HDL size distribution. *Endocr Res* 2004;30:403-15.

Humbert R, Adler DA, Distèche CM, Hassett C, Omiecinski CJ, Furlong CE. The molecular basis of the human serum paraoxonase activity polymorphism.

Nat Genet. 1993 Jan;3(1):73-6.

Ikeda Y, Suehiro T, Inoue M, Nakauchi Y, Morita T, Arai K, Hiroyuki I, Kumon Y, Hushimoto K.

Serum paraoxonase activity and its relationship to diabetic complications in patients with noninsulin- dependent diabetes mellitus.

Metabolism 1998;47:598-602

James RW, Leviev I, Ruiz J, Passa P, Froguel P, Garin MC. Promoter polymorphism T(-107)C of the paraoxonase PON1 gene is a risk factor for coronary heart disease in type 2 diabetic patients.

Diabetes. 2000 Aug;49(8):1390-3.

Jakubowski H. Calcium-dependent human serum homocysteine thiolactone hydrolase.

J Biol Chem. 2000;275:3957–3962.

Jarvik GP, Rozek LS, Brophy VH, Hatsukami TS, Richter RJ, Schellenberg GD, Furlong CE. Paraoxonase (PON1) Phenotype Is a Better Predictor of Vascular Disease Than Is PON1192 or PON155 Genotype Arterioscler Thromb Vasc Biol 2000; 20: 2441-.

Jarvik GP, Jampsa R, Richter RJ, Carlson CS, Rieder MJ, Nickerson DA, Furlong CE. Novel paraoxonase (PON1) nonsense and missense mutations predicted by functional genomic assay of PON1 status.

Pharmacogenetics. 2003 May;13(5):291-5.

Jordan-Starck TC, Witte DP, Aronow BJ, Harmony JAK. Apolipoprotein J: a membrane policeman?

Curr Opin Lipidol. 1992;3:75– 85.

Kalmar T, Seres I, Balogh Z, Kaplar M, Winkler G, Paragh G. Correlation between the activities of lipoprotein lipase and paraoxonase in type 2 diabetes mellitus. Diabetes Metab 2005; 31: 574-80.

Kassai A, Illyés L, Mirdamadi HZ, Seres I, Kalmár T, Audikovszky M, Paragh G. The effect of atorvastatin therapy on lecithin:cholesterol acyltransferase, cholesteryl ester transfer protein and the antioxidant paraoxonase.

Clin Biochem. 2007 Jan;40(1-2):1-5.

Kelso GJ, Stuart WD, Richter RJ, Furlong CE, Jordan-Starck TC, Harmony JAK. Apolipoprotein J is associated with paraoxonase in human plasma.

Biochemistry. 1994;33:832– 839.

Kempen HJ, van Gent CM, Buytenhek R, Buis B. Association of cholesterol concentrations in low-density lipoprotein, high-density lipoprotein, and high-density lipoprotein subfractions, and of apolipoproteins AI and AII, with coronary stenosis and left ventricular function. J Lab Clin Med 1987;109:19-26.

Khersonsky O, Tawfik DS. Structure-reactivity studies of serum paraoxonase PON1 suggest that its native activity is lactonase.

Biochemistry. 2005 Apr 26;44(16):6371-82.

Kleemann R, Kooistra T. HMG-CoA reductase inhibitors: effects on chronic subacute inflammation and onset of atherosclerosis induced by dietary cholesterol.

Curr Drug Targets Cardiovasc Haematol Disord. 2005 Dec;5(6):441-53.

Klerkx AH, El Harchaoui K, van der Steeg WA, Boekholdt SM, Stroes ES, Kastelein JJ, Kuivenhoven JA. Cholesteryl ester transfer protein (CETP) inhibition beyond raising high-density lipoprotein cholesterol levels: pathways by which modulation of CETP activity may alter atherogenesis.

Arterioscler Thromb Vasc Biol. 2006 Apr;26(4):706-15.

Kural BV, Orem C, Uydu HA, Alver A, Orem A. The effects of lipid-lowering therapy on paraoxonase activities and their relationships with the oxidant-antioxidant system in patients with dyslipidemia.

Coron Artery Dis. 2004 Aug;15(5):277-83.

La Du BN, Eckerson HW. The polymorphic paraoxonase/arylesterase isozymes of human serum. *Fed Proc* 1984; 43: 2338-41.

La Du BN, Adkins S, Kuo CL, Lipsig D. Studies on human serum paraoxonase/arylesterase. *Chem Biol Interact* 1993; 87: 25-34

La Du BN. Structural and functional diversity of paraoxonases.

Nat Med. 1996;2:1186 –1187

Lagrost L, Athias A, Herbeth B, et al. Opposite effects of cholesteryl ester transfer protein and phospholipid transfer protein on the size distribution of plasma high density lipoproteins. Physiological relevance in alcoholic patients. *J Biol Chem* 1996;271:19058-65.

Lamarche B, Moorjani S, Cantin B, Dagenais GR, Lupien PJ, Després JP. Associations of HDL2 and HDL3 subfractions with ischemic heart disease in men. Prospective results from the Québec Cardiovascular Study. *Arterioscler Thromb Vasc Biol* 1997;17:1098-105.

Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *BMJ* 2003; 326: 1423-7.

Leviev I, Deakin S, James RW. Decreased stability of the M54 isoform of paraoxonase as a contributory factor to variations in human serum paraoxonase concentrations.

J Lipid Res. 2001 Apr;42(4):528-35.

Levy RI, Brensike JF, Epstein SE, et al. The influence of changes in lipid values induced by cholestyramine and diet on progression of coronary artery disease: results of NHLBI Type II Coronary Intervention Study. *Circulation* 1984;69:325-37.

Lusis AJ. Atherosclerosis.

Nature. 2000 Sep 14;407(6801):233-41.

Mackness MI, Walker CH. "A"-esterase activity in the lipoprotein fraction of sheep serum.

Biochem Pharmacol. 1981;30:903–906.

Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, Durrington PN. Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. *Atherosclerosis* 1991;86:193-9.

Mackness MI, Abbott CA, Arrol S, Durrington PN. The role of high density lipoprotein and lipid-soluble antioxidant vitamins in inhibiting low-density lipoprotein oxidation.

Biochem J. 1993;294:829–835

Mackness MI, Durrington PN. High density lipoprotein, its enzymes and its potential to influence lipid peroxidation. *Atherosclerosis*. 1995;115:243–253.

Mackness B, Durrington PN, Mackness MI. Human serum paraoxonase. *Gen Pharmacol*. 1998 Sep;31(3):329-36.

Mackness B, Mackness MI, Arrol S, Turkie W, Julier K, Abuashia B, Miller JE, Boulton AJM, Durrington PN. Serum paraoxonase (PON1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. *Atherosclerosis* 1998;139:341-9.

Mackness B, Davies GK, Turkie W, Lee E, Roberts DH, Hill E, Roberts C, Durrington PN, Mackness MI. Paraoxonase status in coronary heart disease: are activity and concentration more important than genotype? *Arterioscler Thromb Vasc Biol*. 2001 Sep;21(9):1451-7.

Mackness MI, Mackness B, Durrington PN. Paraoxonase and coronary heart disease. *Atheroscler Suppl*. 2002 Dec;3(4):49-55.

Mackness B, Durrington P, McElduff P, Yarnell J, Azam N, Watt M, Mackness M. Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study. *Circulation*. 2003 Jun 10;107(22):2775-9.

Malin R, Laaksonen R, Knuuti J, Janatuinen T, Vesalainen R, Nuutila P, Lehtmaki T. Paraoxonase genotype modifies the effect of pravastatin on high-density lipoprotein cholesterol. *Pharmacogenetics* 2001; 11: 625-633.

McElveen J, Mackness MI, Colley CM, Peard T, Warner S, Walker CH. Distribution of paraoxon hydrolysing activity in the serum of patients after myocardial infarction. *Clin Chem* 1986;32:671-3.

Mertens A, Holvoet P. Oxidized LDL and HDL: antagonists in atherothrombosis. *FASEB J*. 2001 Oct;15(12):2073-84.

Mineo C, Deguchi H, Griffin JH, Shaul PW. Endothelial and antithrombotic actions of HDL. *Circ Res*. 2006 Jun 9;98(11):1352-64.

Mirdamadi HZ, Sztanek F, Derdak Z, Seres I, Harangi M, Paragh G. 2008, The human paraoxonase-1 phenotype modifies the effect of statins on paraoxonase activity and lipid parameters. *Br J Clin Pharmacol* 66:366-74.

Moren X, Deakin SP, Liu ML, Taskinen MR, James RW. HDL subfraction distribution of paraoxonase-1 and its relevance to enzyme activity and resistance to oxidative stress. *J Lipid Res* 2008;49:1246-53.

Movva R, Rader DJ. Laboratory assessment of HDL heterogeneity and function. *Clin Chem* 2008;54:788-800

Mölgaard J, Wärjerstam-Elf S, Olsson AG. Efficacy and safety of simvastatin for high-risk hypercholesterolemia. *Am J Cardiol* 1999;83:1043-8.

Musliner TA, Krauss RM. Lipoprotein subspecies and risk of coronary disease. *Clin Chem* 1988;34:B78-83.

Navab M, Hama-Levy S, Van Lenten BJ, Fonarow GC, Cardinez CJ, Castellani LW, Brennan ML, Lusis AJ, Fogelman AM, La Du BN. Mildly oxidized LDL induces an increased apolipoprotein J/paraoxonase ratio. *J Clin Invest.* 1997 Apr 15;99(8):2005-19.

O'Keefe JH Jr, Captain BK, Jones PG, Harris WS. Atorvastatin reduces remnant lipoproteins and small, dense low-density lipoproteins regardless of the baseline lipid pattern. *Prev Cardiol* 2004;7:154-60.

Oda MN, Bielicki JK, Ho TT, Berger T, Rubin EM, Forte TM. Paraoxonase 1 overexpression in mice and its effect on high-density lipoproteins. *Biochem Biophys Res Commun.* 2002 Jan 25;290(3):921-7.

Ohashi R, Mu H, Wang X, Yao Q, Chen C. Reverse cholesterol transport and cholesterol efflux in atherosclerosis. *Q J Med.* 2005 Dec;98(12):845-56.

Oka T, Kujiraoka T, Ito M, Nagano M, Ishihara M, Iwasaki T, Egashira T, Miller NE, Hattori H. Measurement of human plasma phospholipid transfer protein by sandwich ELISA. *Clin Chem.* 2000 Sep;46(9):1357-64.

Ota K, Suehiro T, Arai K, Ikeda Y, Kumon Y, Osaki F, Hasimoto K. Effect of pitavastatin on transactivation of human serum paraoxonase 1 gene. *Metabolism* 2005; 54: 142-50.

Patel BN, Mackness MI, Harty DW, Arrol S, Boot-Handford RP, Durrington PN. Serum esterase activities and hyperlipidaemia in the streptozotocin-diabetic rat. *Biochim Biophys Acta* 1990;1035:113-6.

Gy. Paragh, I. Seres, Z. Balogh, Zs.Varga, I. Kárpáti, J. Mátyus, L.Újhelyi, Gy. Kakuk: The serum paraoxonase activity in patients with chronic renal failure and hyperlipidemia [1] *Nephron*, 80: 166-170, 1998

Paragh G, Törocsik D, Seres I, Harangi M, Illyés L, Balogh Z, Kovács P. Effect of short term treatment with simvastatin and atorvastatin on lipids and paraoxonase activity in patients with hyperlipoproteinaemia. *Curr Med Res Opin.* 2004 Aug;20(8):1321-7.

Pedersen TR, Olsson AG, Faergeman O, Kjekshus J, Wedel H, Berg K, Wilhelmsen L, Haghfelt T, Thorgeirsson G, Pyörälä K, Miettinen T, Christophersen B, Tobert JA, Musliner TA, Cook TJ. Lipoprotein changes and reduction in the incidence of major coronary heart disease events in the Scandinavian Simvastatin Survival Study (4S) *Circulation* 1998; 97: 1453-60

Pepine CJ. Systemic hypertension and coronary artery disease. *Am J Cardiol.* 1998 Aug 6;82:21-24.

Rader DJ. Inhibition of cholesteryl ester transfer protein activity: a new therapeutic approach to raising high-density lipoprotein. *Curr Atheroscler Rep.* 2004 Sep;6(5):398-405

Rader DJ., Alexander ET., Weibel GL, Billheimer J., Rothblat GH
The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis

Journal of Lipid Research, Vol. 50, S189-S194, April 2009

Richter RJ, Furlong CE. Determination of paraoxonase (PON1) status requires more than genotyping.
Pharmacogenetics. 1999 Dec;9(6):745-53.

Rizzo M, Berneis K. The clinical relevance of low-density-lipoproteins size modulation by statins.
Cardiovasc Drugs Ther. 2006 Jun;20(3):205-17.

Rosenson RS, Otvos JD, Freedman DS. Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial. Am J Cardiol 2002;90:89-94.

Saini HK, Xu YJ, Arneja AS, Tappia PS, Dhalla NS. Pharmacological basis of different targets for the treatment of atherosclerosis.
J Cell Mol Med. 2005 Oct-Dec;9(4):818-39.

Salonen JT, Salonen R, Seppänen K, Rauramaa R, Tuomilehto J. HDL, HDL2, and HDL3 subfractions, and the risk of acute myocardial infarction. A prospective population study in eastern Finnish men. Circulation 1991;84:129-39.

Schaefer EJ, McNamara JR, Tayler T, et al. Effects of atorvastatin on fasting and postprandial lipoprotein subclasses in coronary heart disease patients versus control subjects. Am J Cardiol 2002;90:689-96.

Segrest JP, Harvey SC, Zannis V. Detailed molecular model of apolipoprotein A-I on the surface of high-density lipoproteins and its functional implications. Trends Cardiovasc Med 2000;10:246-52.

Sentí M, Aubó C, Elosua R, Sala J, Tomás M, Marrugat J. Effect of physical activity on lipid levels in a population-based sample of men with and without the Arg192 variant of the human paraoxonase gene.
Genet Epidemiol. 2000 Mar;18(3):276-86.

Senti; M, Tomás M, Anglada R, Elosua R, Marrugat J, Covas MI, Fitó M. Interrelationship of smoking, paraoxonase activity, and leisure time physical activity: a population-based study.
Eur J Intern Med. 2003 May;14(3):178-184.

Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, Castellani LW, Furlong CE, Costa LG, Fogelman AM, Lusis AJ. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis.
Nature. 1998 Jul 16;394(6690):284-7.

Shimoji E, Zhang B, Fan P, Saku K. Inhibition of cholesteryl ester transfer protein increases serum apolipoprotein (apo) A-I levels by increasing the synthesis of apo A-I in rabbits. Atherosclerosis 2004;172:247-57

Simons LD
Interrelations of lipids-lipoproteins with coronary artery disease mortality in 19 countries.
Am J Cardiol. 1986;27:50-106.

Singh AT, Rainwater DL, Haffner SM, et al. Effect of diabetes on lipoprotein size. Arterioscler Thromb Vasc Biol 1995;15:1805-11.

Sirtori CR. Tissue selectivity of hydroxymethylglutaryl coenzyme A (HMG CoA) reductase inhibitors.

Pharmacol Ther. 1993 Dec;60(3):431-59.

Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. *N Engl J Med* 1991;325:373-81.

Sweetnam PM, Bolton CH, Yarnell JW, Bainton D, Baker IA, Elwood PC, Miller NE. Associations of the HDL2 and HDL3 cholesterol subfractions with the development of ischemic heart disease in British men. The Caerphilly and Speedwell Collaborative Heart Disease Studies. *Circulation* 1994;90:769-74.

Tailleux A, Duriez P, Fruchart JC, Clavey V. Apolipoprotein A-II, HDL metabolism and atherosclerosis.

Atherosclerosis. 2002 Sep;164(1):1-13.

Tall AR, Sammett D, Vita GM, Deckelbaum R, Olivecrona T. Lipoprotein lipase enhances the cholesteryl ester transfer protein-mediated transfer of cholesteryl esters from high density lipoproteins to very low density lipoproteins. *J Biol Chem* 1984;259:9587-94.

Tomás M, Sentí M, García-Faria F, Vila J, Torrents A, Covas M, Marrugat J. Effect of simvastatin therapy on paraoxonase activity and related lipoproteins in familial hypercholesterolemic patients.

Arterioscler Thromb Vasc Biol. 2000 Sep;20(9):2113-9.

Tsimihodimos V, Karabina SA, Tambaki AP, Bairaktari E, Goudevenos JA, Chapman MJ, Elisaf M, Tselepis AD. Atorvastatin preferentially reduces LDL-associated platelet-activating factor acetylhydrolase activity in dyslipidemias of type IIA and type IIB.

Arterioscler Thromb Vasc Biol. 2002 Feb 1;22(2):306-11.

Turban S, Fuentes F, Ferlic L, Brugada R, Gotto AM, Ballantyne CM, Marian AJ. A prospective study of paraoxonase gene Q/R192 polymorphism and severity, progression and regression of coronary atherosclerosis, plasma lipid levels, clinical events and response to fluvastatin. *Atherosclerosis* 2001; 154: 633-40.

Tward A, Xia YR, Wang XP, Shi YS, Park C, Castellani LW, Lusi AJ, Shih DM. Decreased atherosclerotic lesion formation in human serum paraoxonase transgenic mice.

Circulation. 2002 Jul 23;106(4):484-90.

Van Lenten BJ, Wagner AC, Navab M, Fogelman AM. Oxidized phospholipids induce changes in hepatic paraoxonase and ApoJ but not monocyte chemoattractant protein-1 via interleukin-6.

J Biol Chem. 2001 Jan 19;276(3):1923-9.

van der Steeg WA, Holme I, Boekholdt SM, Larsen ML, Lindahl C, Stroes ES, Tikkanen MJ, Wareham NJ, Faergeman O, Olsson AG, Pedersen TR, Khaw KT, Kastelein JJ. High-density lipoprotein cholesterol, high-density lipoprotein particle size, and apolipoprotein A-I: significance for cardiovascular risk: the IDEAL and EPIC-Norfolk studies. *J Am Coll Cardiol* 2008;51:634-42

van Tits LJ, van Himbergen TM, Lemmers HL, de Graaf J, Stalenhoef AF. Proportion of oxidized LDL relative to plasma apolipoprotein B does not change during statin therapy in patients with heterozygous familial hypercholesterolemia.

Atherosclerosis. 2006 Apr;185(2):307-12.

Vaughan C.J. and Gotto AM.: Update on Statins: 2003 Circulation. 2004;110:886-892.

Vekic J, Topic A, Zeljkovic A, Jelic-Ivanovic Z, Spasojevic-Kalimanovska V. LDL and HDL subclasses and their relationship with Framingham risk score in middle-aged Serbian population. Clin Biochem 2007;40:310-6.

Vincent-Viry M, Sass C, Bastien S, Aguillon D, Siest G, Visvikis S. PON1-192 phenotype and genotype assessments in 918 subjects of the Stanislas Cohort Study. Clin Chem Lab Med 2003; 41: 535-40

Winkler K, Abletshauser C, Hoffmann MM, Friedrich I, Baumstark MW, Wieland H, März W. Effect of fluvastatin slow-release on low density lipoprotein (LDL) subfractions in patients with type 2 diabetes mellitus: baseline LDL profile determines specific mode of action. J Clin Endocrinol Metab 2002; 87: 5485-90.

Yu S, Yarnell JW, Sweetnam P, Bolton CH. High density lipoprotein subfractions and the risk of coronary heart disease: 9-years follow-up in the Caerphilly Study. Atherosclerosis 2003;166:331-8.

IX. PUBLICATIONS

Publications serving as the basis of the dissertation:

1. **Mirdamadi HZ**, Sztanek F, Derdak Z, Seres I, Harangi M, Paragh G. The human paraoxonase-1 phenotype modifies the effect of statins on paraoxonase activity and lipid parameters. Br J Clin Pharmacol. 2008;66(3):366-74. (IF: 2.681)
2. Harangi M, **Mirdamadi HZ**, Seres I, Sztanek F, Molnár M, Kassai A, Derdák Z, Illyés L, Paragh G. Atorvastatin effect on the distribution of high-density lipoprotein subfractions and human paraoxonase activity. Transl Res. 2009;153(4):190-8. (IF:1.325)

Other publications:

1. Kassai A, Illyés L, **Mirdamadi HZ**, Seres I, Kalmár T, Audikovszky M, Paragh G. The effect of atorvastatin therapy on lecithin:cholesterol acyltransferase, cholesteryl ester transfer protein and the antioxidant paraoxonase. Clin Biochem. 2007;40(1-2):1-5. (IF:2.072)
2. Kassai A, **HZ Mirdamadi**, Seres I, Kalmár T, Paragh Gy: Az atorvastatin kezelés hatása a lecitin-koleszterin-acil-transzferázra és koleszterin-észter-transzfer-proteinre. Metabolizmus 2006; IV. 1:7-11

X. KEYWORDS

dyslipidemia, atherosclerosis, statin, paraoxonase1, high-density lipoprotein, low-density lipoprotein, genotype, phenotype

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APPENDIX