HDL and the progression of atherosclerosis: new insights

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Aims Lowering low-density lipoprotein (LDL) cholesterol or raising high-density lipoprotein (HDL) cholesterol can result in significant cardiovascular benefit, both in terms of reduction of events and also, to a variable extent, of atheromatous lesions. LDL and HDL have opposite roles in body cholesterol regulation and, both reduced deposition (LDL reduction) and increased removal (raised HDL) can improve vascular disease. Very recently, studies using recombinant apolipoprotein AI liposomes (in particular with the mutant apo AIMilano) have shown that direct infusion can effectively remove cellular cholesterol and dramatically reduce established atheromatous plaques in animals and in coronary patients. It has thus become of growing interest, the possibility of raising HDL by pharmacological treatments. This review will attempt to investigate existing and novel methodologies for HDL raising.

Methods and results HDL raising can be achieved by using drugs active on the peroxisome proliferator activated receptor α (PPARα) system, particularly using fibrates or n-3 fatty acids and, possibly more effectively, by using nicotinic acid. The activity of this agent has been traditionally linked to a reduced free fatty acid (FFA) mobilization from adipose tissue. More recently, it has been noted that nicotinic acid can activate the PPAR system, by stimulating all three PPAR isoforms (α, γ, δ). This mode of action seems to more effectively explain the striking HDL-raising properties of the drug.

Conclusions Newly discovered mechanisms of nicotinic acid can explain, on the one hand, reduced atherosclerosis progression in secondary prevention patients treated with statins and, on the other hand, improved body cholesterol mobilization, effectively reducing cholesterol pool sizes.

Introduction: the HDL targets

Raising high-density lipoprotein (HDL) levels or providing exogenous HDL (HDL therapy) are novel areas of therapeutic development in the cardiovascular field. They are based on the strong scientific and clinical evidence linking HDL to cardiovascular protection. Although the 'low HDL-cholesterol' or 'hypoalpha' syndrome is the most frequent lipoprotein abnormality in coronary patients, it has not received as much attention as the hyper LDL-cholesterolaemia. Way back from Framingham Study analyses in 1977, an association was reported between reduced HDL-cholesterol and increased cardiovascular risk vs. an apparent protective effect of elevated HDL-cholesterol in both sexes.¹ A raised cardiovascular risk was detected in subjects with HDL-cholesterol levels below 50 mg/dL for females and below 40 mg/dL for males, but there seemed to be little benefit in HDL-cholesterol levels above 60 mg/dL in either sex.

A direct comparison between the risk associated with increased LDL-cholesterol and reduced HDL-cholesterol had not been clearly provided by any epidemiological study, until the recent ARIC investigation, assessing risk in 12 339 middle-aged participants of both sexes, free of coronary heart disease (CHD), with a 10-year follow-up resulting in 725 CHD events.² In males, comparing event
rates in the lowest quintile of LDL-cholesterol (83 mg/dL) vs. the highest quintile (185 mg/dL), a risk ratio of 2.6 could be found; conversely, from the highest (59.4 mg/dL) to the lowest (28.6 mg/dL) quintile of HDL-cholesterol, there was a 2.9-fold risk rise (Figure 1). Multiplication of risk going from the highest to the lowest HDL-cholesterol quintile is thus at least equivalent (if not higher) vs. the multiplication of risk going from the lowest to the highest LDL-cholesterol quintile. The difference in risk ratios for HDL-cholesterol vs. LDL-cholesterol was even more dramatic for women: risk multiplication from the lowest to the highest LDL-cholesterol levels in fact was 2.9 vs. 5.8 for the highest vs. the lowest HDL-cholesterol quintile. A detailed evaluation of the comparative benefit of LDL lowering vs. HDL raising has been recently provided.3

HDL-cholesterol as a risk-prediction marker has provided, at times, surprising findings: a post hoc evaluation of patients with acute coronary syndromes participating in the MIRACL study has very recently shown that baseline LDL-cholesterol and LDL-cholesterol changes after atorvastatin treatment were not predictive of subsequent events. In contrast, HDL-cholesterol levels at baseline predicted a short-term risk reduction of 1.4% for each 1 mg/dL increment of HDL-cholesterol.4 A similar reduction in risk occurred when evaluating baseline ApoAI instead of HDL-cholesterol. Use of ApoAI vs. HDL-cholesterol for coronary risk evaluation has provided contrasting findings.5 Determination of ApoAI would have the advantage of little analytical influence from concomitant hypertriglyceridaemia or non-fasting conditions. However, a number of reports, including a very recent one from the Women’s Health Study, have shown that HDL-cholesterol levels are at least good if not better predictors than ApoAI.5

Hypoalpha: the clinical condition

Hypoalpha asymptomatic patients show extensive coronary atheromas upon intravascular ultrasound (IVUS) evaluation vs. the case of patients with an isolated elevation of LDL-cholesterol.7 Carotid intima media thickness (IMT) studies8 indicate that individuals with reduced HDL-cholesterol levels show IMTs similar to those with familial hypercholesterolaemia. Further, hypoalpha patients show reduced arterial vasodilation after various stimuli, thus confirming endothelial protection by HDL.9 Growing evidence indicates that HDL-cholesterol raising may possibly have a more powerful effect on arterial protection than LDL-cholesterol lowering.

A direct correlation between HDL-cholesterol levels and a series of parameters of coronary risk has been attempted by a number of authors. Evidence has been provided for an inverse correlation between HDL-cholesterol levels and C-reactive protein levels in large series of patients.10 In a similar study, also in an Italian population, it could be shown that hypoalpha patients have higher C-reactive protein levels than patients with normal HDL, and the highest levels are found in the hypoalpha patients with coronary disease.11 There appears, however, to be a threshold beyond which higher HDL-cholesterol levels may not make a large difference; this may be the case discussed by Calabresi et al.,12 where plasma cytokine levels (ICAM-I, VCAM-1, E-selectin) appeared to be dramatically elevated in patients with HDL-cholesterol levels below 40 mg/dL, but were not further reduced with HDL-cholesterol exceeding this level.

The importance of the acute raising of HDL levels was recently demonstrated by the direct infusion of AliMilano liposomes, resulting in significant atheroma regression by IVUS evaluation in experimental animals13 as well as in coronary patients.14 Conversely, extreme LDL-cholesterol...
reduction by statins, as reported in the REVERSAL trial, did not lead to any significant reduction in coronary atheromas by IVUS evaluation.\textsuperscript{15}

The clinical observation of rapid atheroma regression following \textit{Al\textsubscript{Milano}} liposome infusion has raised considerable enthusiasm on the concept of ‘HDL-therapy’, and this has led to the development of a number of approaches using the so-called ‘HDL mimetics’ (i.e. large unilamellar vesicles)\textsuperscript{16} to small dextro-rotary peptides (D4F) and HDL with aminoacid or lipid modifications.\textsuperscript{17,18} At present, Apo\textit{Al\textsubscript{Milano}} is the most advanced and probably the most promising product among these and is undergoing a late phase 2 clinical trial, evaluating doses and safety.

**Present-day HDL-cholesterol-raising drugs’ efficacy and differential mechanisms**

HDL can directly remove cellular cholesterol and exert a variety of vascular protective effects (antioxidant, anti-inflammatory, pro-fibrinolytic, and others). Use of drugs raising HDL-cholesterol by different mechanisms (from fibrates to nicotinic acid) has gained support from a variety of clinical studies, some of which are indicative of reduced cardiovascular events/atheroma reduction following treatment. The direct provision of biosynthetic or plasma-extracted HDL, e.g. with \textit{Al\textsubscript{Milano}}, can improve the extent and severity of vascular lesions, with the potential benefit of acting in a short time frame.

The clear evidence of beneficial effect of HDL-therapy has encouraged a re-evaluation of established treatments aimed to elevate HDL-cholesterol levels in order to reduce cardiovascular risk. Two recent studies, Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2, evaluating IMT in coronary patients already at optimal LDL-cholesterol levels,\textsuperscript{19} and the Armed Forces Regression Study (AFREGS), in coronary patients not on statins,\textsuperscript{20} clearly showed that drug-induced HDL-cholesterol elevations \textit{per se} can cause atheroma regression and directly reduce cardiovascular events. Interestingly, in the ARBITER 2 study, all patients were on statins, whereas in the AFREGS, they were mainly on resins, and thus the effects of nicotinic acid were assessed in their absence.

There are at present two major drug classes for raising HDL-cholesterol levels, plus one in development. Fibrates have a well-defined stimulating activity on the peroxisome proliferator receptor \(\alpha\) (PPAR\(\alpha\)) system.\textsuperscript{21} By PPAR\(\alpha\)-associated mechanisms, fibrates stimulate triacylglyceride breakdown, i.e. they activate lipoprotein lipase (LPL) and also reduce ApoCIII expression (an inhibitor of LPL); as a consequence, they raise HDL-cholesterol levels. These mechanisms, together with anti-inflammatory properties\textsuperscript{22} shared by other PPAR\(\alpha\) agonists, characterize their clinical action and potential therapeutic benefit. Nicotinic acid still has an unclear mechanism, which will be discussed in detail elsewhere in this supplement. Finally, cholesterol ester transfer protein (CETP) antagonists (JTI-7051 and torcetrapib), by blocking the reverse cholesterol transport system, raise HDL-cholesterol to very high levels,\textsuperscript{23} but their therapeutic benefit is still unclear.

A general mechanism of HDL-cholesterol elevators is that of stimulating fecal steroid excretion. This mechanism, of course, contrasts with that of statins, which generally reduce fecal steroid elimination, most likely because of their inhibition of biosynthesis, \textit{per se} somewhat reducing sterol pools. Three studies that have specifically investigated body cholesterol movements after statins have produced negative findings. Grundy and Bilheimer\textsuperscript{24} investigated fecal excretion of neutral and acidic steroids in five heterozygote FH patients before and during treatment with lovastatin. In three out of five, neutral and acidic steroid excretions were reduced, whereas there were no alterations in the other two (Figure 2A). These findings were replicated by carrying out a whole-body cholesterol turnover study in nine hypercholesterolemic patients before and after

![Figure 2](https://academic.oup.com/eurheartjsupp/article-abstract/8/suppl_F/F4/446946/446946)

**Figure 2** Comparison between fecal steroid excretions as neutral steroids or bile acids following treatment with (A) HMGCoA reductase inhibitor (lovastatin), drawn from data presented by Grundy et al.\textsuperscript{24}; (B) torcetrapib with and without atorvastatin (fecal steroid excretion corrected by the plant sterol intake elimination), drawn from data presented by Brousseau et al.\textsuperscript{25}; (C) infusion of recombinant proapoA-I (4 g of protein) in familial hypercholesterolemic subjects, reproduced from Eriksson et al.\textsuperscript{28} with permission from Lippincott, Williams & Wilkins (www.lww.com).
15 months of lovastatin therapy (40 mg/day),\(^2^5\) again showing no significant changes in the sizes of rapidly, slowly, and total body exchangeable sterol pools. Finally, in a study in normal lipidemic volunteers\(^2^6\) on lovastatin (40 mg bid) in the presence of either a low- or high-cholesterol diet, as expected, biliary cholesterol secretion, fecal output of endogenous neutral sterols, cholesterol balance, and systemic cholesterol input (sum of cholesterol synthesis + absorbed dietary cholesterol) were reduced. Vascular benefits of statins should, therefore, be attributed to a stabilization of body cholesterol pools, and biliary cholesterol lowering may also potentially be of benefit in gallstone patients.\(^2^7\)

Two studies have evaluated the effects of a direct infusion of recombinant human apoA-I formulated into liposomes on fecal steroid excretion. In patients with severe FH, single dose (4 g of protein) of recombinant apoA-I induced a clear increase of cholesterol mobilization, resulting in increased fecal neutral and bile acids\(^2^8\) (Figure 2B). In a second study,\(^2^9\) the effects of an i.v. infusion of human apoA-I phosphatidylcholine (PC) discs (molar ratio 1:150) were compared with those of apoA-I/PC discs, in normal lipidemic recipients. In both conditions, there was an increase of small pre-β-HDL in plasma and lymph, an increase of both plasma lathosterol and fecal neutral steroids and, more so, of bile acids, in particular, chenodeoxycholate and derivatives (+302% at 24 h and still 192% at the seventh day after the end of the infusion).

Contrasting findings have, instead, been provided with torcetrapib, the most advanced CETP antagonist. In a study where torcetrapib was given together with atorvastatin, the association, as expected, led to a reduction of cholesterol mobilization and increased cholesterol pools after cholestyramine treatment.\(^3^0\) The drug also reversed the apparent increase in acidic steroids, witnessing enhanced cholesterol mobilization.\(^3^1\) The mechanism of the HDL-raising by nicotinic acid: new insights

Nicotinic acid, had shown its lipid-lowering properties well over 50 years ago. These occur at relatively high dosages (above 1 g/day) and are best achieved with retard formulations, which also provide the optimal tolerability.\(^3^2\) Traditionally, the mechanism of the lipid-lowering effect, in particular, triglyceride reduction and the remarkable HDL-raising property, have been ascribed to an antilipolytic activity.\(^3^3\) This has been recently attributed to the interaction with a well-defined G/protein-coupled receptor in adipose tissue.\(^3^4\) Very recently, it has been also indicated that the GPR109A receptor (HM74A in humans and PUMA-G in mice) mediates the skin-flushing response to nicotinic acid.\(^3^5\)

Previous studies by the present author’s group,\(^3^6\) using a very powerful long-acting inhibitor of lipolysis (acipimox), showed that this drug has an antilipolytic activity at least 10-fold higher vs. nicotinic acid and, in addition, it maintains the activity for a prolonged period. Acipimox blocks lipolysis for up to 9–12 h vs. the very transient inhibitory activity of standard formulations of nicotinic acid (less than 3 h). The antilipolytic activity of standard nicotinic acid is also immediately followed by a rebound of free fatty acid (FFA) levels at least 2-fold higher vs. baseline.\(^3^7\) Acipimox, in spite of this extraordinary antilipolytic activity, proved to be a poorly active lipid-lowering medication, with a modest triglyceride-reducing effect and a HDL-raising activity not better than \(+10\%\).\(^3^8\)\(^,\)\(^3^9\) It seems, therefore, rather unlikely that these striking differences in antilipolytic and lipid-lowering activities could address to the antilipolytic effect as a major mechanism of nicotinic acid. In addition, very recently, Lena Vega \textit{et al}.\(^4^0\) showed that Niaspan\(^\text{®}\), a very well tolerated, prolonged-release formulation of nicotinic acid, also inhibits lypolysis not longer than 3–4 h, and 9 h after administration, FFA levels are clearly above baseline. In spite of this, the triglyceride-lowering effect and, in particular, the HDL-cholesterol-raising activity (+21% in non-diabetic subjects and +28% in diabetic patients) are certainly far better than those of acipimox.

In view of the scepticism on the antilipolytic mechanism of nicotinic acid, other investigators have explored alternative pathways. Rubic \textit{et al}.\(^4^1\) have recently shown that by exposing human monoyctoid cells to nicotinic acid, this resulted in markedly increased expression of ABCA1 and PPARγ. ABCA1 activation is opposite to the effect reported by Sone \textit{et al}.\(^4^2\) in a macrophage cell line exposed to fluvastatin, both in basal conditions and after stimulation with 22-hydroxycholesterol.

However, potentially the most exciting findings have been recently reported by Watt \textit{et al}.\(^4^3\) in muscles of healthy individuals before exercise. Volunteers were administered nicotinic acid with the objective of reducing lipolysis during the ensuing exercise. The administration of nicotinic acid, surprisingly, led to a marked increase of PPARα and PPARδ mRNA levels in muscle (Figure 3). This rise was similar to that noted after exercise and was accompanied by an increased expression of the PPAR co-activator 1-α (PGC1α) mRNA. The authors concluded that ‘nicotinic acid ingestion decreased FFA availability but it promoted induction of PPARα/δ and PGC1α gene expression to a similar degree as prolonged exercise’.\(^4^4\) The induction of PPARγ has been very recently ascribed to an indirect mechanism of nicotinic acid, i.e. an induction of the prostaglandin synthesis pathway via the HM74A receptor.\(^4^5\)

These very exciting data point out that possibly nicotinic acid may act as a ‘fraudulent fatty acid’.\(^4^6\) Fraudulent fatty acids are fatty acid-like molecules not metabolized by mitochondria but activating the peroxisomal system, thus leading to metabolic consequences similar to those
exerted by nicotinic acid. In particular, among fraudulent fatty acids, nicotinic acid (or most likely nicotinoyl CoA, the major metabolite) is the only one activating all three peroxisomal isotypes as well as ABCA1.

Conclusions

The very novel findings with nicotinic acid, indicative of a more extensive mode of action vs. presently available HDL-raising drugs, are an additional area of interest for studies on the potential of HDL raising in the clinic.

Conflict of interest: none declared.

References


Figure 3: Real-time PCR analysis of PPARα and PPARδ from muscle of healthy individuals before and after 1 h of moderate exercise with (NA) or without (CON) prior administration of nicotinic acid (10 mg/kg) [©Society for Endocrinology (2004), reproduced with permission]. Suppression of plasma FFAs upregulates PPARα and PPARβ and PPAR coactivator 1alpha in human skeletal muscle, but not lipid regulatory genes. *P < 0.05, different from the corresponding value for CON. †P < 0.05, different from the pre-exercise value.


