HDL-therapy: the next big step in the treatment of atherosclerosis?

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Despite significant reductions in mortality with statins, and increasingly lower targets of low-density-lipoprotein cholesterol, two thirds of cardiovascular events cannot be prevented with current treatments. Therefore, a clear need for additional therapeutic interventions to complement the results of low-density-lipoprotein lowering, exists. One prime target for new interventions is high-density lipoprotein (HDL) and/or its apolipoproteins. While lifestyle interventions and well-established drugs such as fibrates and nicotinic acid modestly increase HDL, the most promising current approaches to increase HDL are direct infusion of HDL-like particles (e.g., apolipoprotein Al Milano–phospholipid complexes) and inhibition of one of the key enzymes in HDL metabolism, cholesterol ester transfer protein. These methods have been shown to have dramatic effects on the incidence of atherosclerosis and/or HDL cholesterol. This review will focus on treatments that raise HDL cholesterol or enhance reverse cholesterol transport. Old and new drugs will be discussed as well as combination therapy and novel approaches such as plasma delipidation and recombinant apolipoprotein AI.

Despite the large effort to reduce coronary heart disease (CHD) by diet, drug therapies and even low-density lipoprotein (LDL) apheresis, this disease remains the major cause of death in most industrialized countries. Statins have emerged as the most important therapy to reduce cardiovascular (CV) mortality and morbidity, and have thus been hailed as the penicillin of the 21st century. Unfortunately, however, they do not remove the risk completely and two thirds of all CV events cannot be prevented. The focus on anti–atherosclerotic therapies is, therefore, shifting to other targets. Three such targets for new interventions are:

- HDL
- Its apolipoproteins
- Proteins that are involved in reverse cholesterol transport (RCT)

Clinical evidence for the benefits of raising HDL cholesterol levels

The relationship between low levels of HDL cholesterol (HDL-C) and the development of CHD can be inferred from epidemiologic studies, where even small differences in the level of HDL-C are associated with substantial variations in the risk of major coronary events. Data from the Framingham population indicate that, at any given level of total cholesterol, the relative risk of CHD increases with decreasing levels of HDL-C [1].

In addition, prospective clinical studies have demonstrated a link between low HDL-C and an increased risk of atherosclerosis. The relationship between HDL-C and the incidence of CHD in the Framingham Study, Lipid Research Clinics Prevalence Mortality Follow-up Study, Coronary Primary Prevention Trial control group and the Multiple Risk Factor Intervention Trial control group was examined by Gordon and colleagues [2]. Analysis of these studies demonstrated that for every 1 mg/dl rise in HDL-C, the risk of CHD decreased by 2% in men and 3% in women, and this was independent of LDL cholesterol (LDL-C) [2].

Reverse cholesterol transport

The accumulation of cholesterol within atherosclerotic lesions occurs when the influx of cholesterol (carried by apolipoprotein (apo) B-containing lipoproteins) into the arterial wall exceeds cholesterol efflux [3]. Reverse cholesterol transport (RCT) is a process in which cholesterol from peripheral tissues, such as vessel wall macrophages, is moved to lipid-poor HDLs via the adenosine triphosphate (ATP) binding cassette transporters (ABCA1, ABCG1 and ABCG4) and possibly several other pathways. Lecithin cholesteryl acyl transferase (LCAT) is involved in the esterification of cholesterol, which results in the formation of mature spherical HDL in a reaction catalyzed by LCAT. In the presence of cholesterol ester transfer protein (CETP) cholesterol esters are then either transferred to apoB-containing lipoproteins (in exchange for triglycerides) and ultimately returned to the liver through the LDL receptor pathway or directly...
taken up by the liver via the scavenger receptor, class B, Type 1 (SR-B1). The process of RCT is summarized in Figure 1.

Apart from its role in RCT, HDL has several other important antiatherosclerotic effects such as anti-inflammatory, antithrombotic and antioxidative effects, which are summarized in Figure 2.

Current strategies to increase HDL

Lifestyle changes

The key lifestyle changes to increase HDL-C are weight loss, reduction in saturated fat intake, increase of physical activity, smoking cessation and alcohol in moderate amounts. These approaches can increase HDL-C by up to 20%. Saturated fatty acids are, in general, associated with increased HDL levels, whereas substituting polyunsaturated fats for saturated fats decreases HDL levels. The former is due to decreased catabolism of HDL apo-AI, while the latter is associated with increased catabolism of HDL and increased expression of SR-B1. For every 3 kg (7 lb) of weight loss, HDL-C levels increase 1 mg/dl [4]. In a study by Moffatt and colleagues, smokers had HDL-C levels 5–20% lower than nonsmokers and HDL-C levels returned to normal within 0–60 days after smoking cessation [5]. Kokkinos and colleagues reported a clear dose–response relationship between aerobic exercise (running) and HDL-C levels in healthy men [6]. Alcohol also increases HDL-C levels in a dose-dependent manner [7] and this has been shown for both wine and beer. In many CHD patients, however, lifestyle changes alone are not successful.

Drugs

Statins

In addition to the dose-dependent reduction of LDL-C levels, statins show beneficial effects across the lipid profile and can raise HDL-C by 5–10%. A direct comparison of the lipid modifying effects of atorvastatin, pravastatin, lovastatin, fluvastatin and simvastatin was performed in the CURVES study (Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, fluvastatin and simvastatin was performed in the CURVES study (Comparative dose efficacy study of atorvastatin versus simvastatin, pravastatin, lovastatin, fluvastatin and simvastatin in patients with...
HDL-therapy for the treatment of atherosclerosis – REVIEW

hypercholesterolemia) [8]. Rosuvastatin and Simvastatin seem to have the most beneficial effects on HDL-C [9]. However, if the primary objective is to raise HDL-C, statins are usually not the drugs of first choice. A comparison of the effects of different lipid-modifying drugs on HDL-C levels is outlined in Table 1.

Fibrates
Fibrates are PPAR ( Peroxisome Proliferator Activated Receptor) α agonists, that can increase HDL by up to 20%. They have also been shown to increase RCT by altering gene expression, resulting in increases in ABCA1, SR-B1, apo-AI and lipoprotein lipase. The Helsinki Heart Study showed a significant association of increasing HDL-C with gemfibrocil and reduction of CHD events [10]. The Veterans Affairs cooperative studies program HDL Intervention Trial (VA-HIT) assessed the effect of raising HDL-C levels with gemfibrocil on CHD risk in patients with low levels of both LDL-C (<140 mg/dl) and HDL-C (<40 mg/dl). This study demonstrated a significant increase of 1.9 mg/dl in HDL-C, which was associated with a reduction of 22% in nonfatal myocardial infarction (MI) or death due to CHD. However, since only a 5 mg/dl increase in HDL-C was significantly associated with nonfatal MI or death, it is not clear whether the increase in HDL-C or the pleiotropic effects of gemfibrocil were responsible for the reduction in events [11]. The largest fibrate trial to date, the FIELD (Fenofibrate [Tri-Cor®] Intervention and Event Lowering in Diabetes) study, is due to be reported at the end of this year.

Niacin
Of all available lipid-lowering drugs, niacin has the best HDL-increasing properties. Niacin can increase HDL by up to 35% [12] In addition, niacin leads to significant reductions in LDL, lipoprotein-a and triglycerides (TG) and has been shown to have long-term mortality benefits in CHD patients [12]. Unfortunately, although niacin is poorly tolerated owing to a high incidence of side effects such as flushing and gastrointestinal symptoms. Extended-release niacin, which is available in some countries has a much better tolerability and may also be combined with statins.

Intestinal-acting drugs
Ezetimibe, a selective cholesterol absorption inhibitor that blocks the uptake of dietary and biliary cholesterol by preventing its transport through the intestinal wall, increases HDL-C by 3–5%. It is well tolerated when administered with a statin or fibrate with additive effects [13].

Estrogens
Estrogens increase HDL-C by approximately 10% by increasing apo-AI production and decreasing HDL catabolism through inhibition
of hepatic lipase (HL) and SR-B1. While meta analyses of observational studies suggested that HRT lowers risk for CHD in postmenopausal women, two recent controlled intervention studies showed that a combination of unconjugated estrogen and progesterone acetate did not reduce, or even increase coronary event rates in postmenopausal women, despite their beneficial effects on plasma lipoproteins [14,15].

**Glitazones**

Agonists of PPAR-α (fibrates) and PPAR-γ (glitazones) are already used as drugs for prevention of atherosclerosis and treatment of diabetes. More potent PPAR agonists (superfibrates) are currently being developed. Muriglitazar, for example, a novel PPAR agonist has recently been shown to decrease TG by 30% and increase HDL by 20% when used in combination with metformin [16]. However, the effects of these drugs on cardiovascular outcomes have not yet been shown.

**Rimonabant**

A novel endocannabinoid receptor blocker that induces weight loss has also been shown to increase HDL by up to 10% [17].

**Drug combinations**

A combination of lovastatin and extended-release niacin, has been demonstrated to produce greater effects on LDL-C, HDL-C and TG levels than either of the two drugs alone: HDL-C levels were increased by 30%, while LDL-C decreased by 47% [18]. A prospective RCT: AIM-HIGH, partially funded by the National Institutes of Health will tell us whether the combination is more effective than simvastatin alone. Several other combination therapies, such as metformin–fenofibrate and simvastatin–ezetimibe among others, are currently being tested in Phase III and IV trials and are expected to have additive effects on HDL-C.

**Novel pharmacological treatments increasing reverse cholesterol transport**

**Cholesterol ester transfer protein inhibition**

Inhibition of one of the key enzymes of RCT, CETP with JTT-705 has been shown to lead to regression of atherosclerosis in animal models of atherosclerosis [19]. In humans one such CETP inhibitor, torcetrapib, has been shown to increase plasma HDL by up to 100% and apo-AI by 35% [20]. This increase in apo-AI is due to decreased fractional catabolic rate of apo-AI [20]. In subjects with low HDL-C levels, CETP inhibition with torcetrapib markedly increased HDL-C levels and also decreased LDL cholesterol levels, both when administered as monotherapy and in combination with a statin [21]. It is not clear to date whether the large, cholesterol and apoE-rich particles formed by CETP inhibition are truly functional in RCT and have antiatherogenic properties. End-point trials currently being conducted with torcetrapib will determine whether this drug provides additional benefits to statin therapy. Statins also reduce CETP, other approaches to inhibit CETP include small molecules and vaccines.

**Plasma delipidation**

Extracorporeal delipidation technology has been used to remove lipids from lipoproteins such as HDL in the bloodstream and then return the delipidated HDL particles back to the body. These delipidated HDL particles then pick up excess lipids from the artery walls and transport them to the liver (Figure 3) [22]. The lipids are then processed and excreted naturally from the body. This has been shown to lead to regression of atherosclerosis and mobilization of adipose tissue in animal studies [23,24].

**Direct high-density-lipoprotein-therapy:** apo-AI /synthetic peptides/apo-AI Milano, phospholipid complexes

Currently, the most direct approach to increase HDL is the direct infusion of HDL, recombinant apo-AI or apo-AI phospholipid complexes. Several apo-AI analogs: AI, pro apo-AI, recombinant apo-AI Milano, as well as mimetic peptides and small molecules such as: ETC-462, ETC-216, 5F, D-4F, are currently under investigation. In one such study, apo-AI/phosphatidyl

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**Table 1. Effects of lipid-modifying drugs on HDL-C levels.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>% Increase in HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacin</td>
<td>15–35%</td>
</tr>
<tr>
<td>Fibrates</td>
<td>10–15%</td>
</tr>
<tr>
<td>Estrogens</td>
<td>10–15%</td>
</tr>
<tr>
<td>Statins</td>
<td>5–10%</td>
</tr>
<tr>
<td>Ezetrol</td>
<td>3–5%</td>
</tr>
<tr>
<td>Rimonabant</td>
<td>5–10%</td>
</tr>
<tr>
<td>Torcetrapib</td>
<td>50–100%</td>
</tr>
<tr>
<td>Glitazones</td>
<td>5–20%</td>
</tr>
</tbody>
</table>

*Adapted from [30].
HDL-C: High-density lipoprotein cholesterol.*
Choline discs were infused over 4 h into seven healthy men [25]. The authors concluded that the infusion of apo-AI/phosphatidyl complexes resulted in an increased intravascular production of small pre-β-HDL \textit{in vivo} and that this was associated with an increase in the efflux and esterification of unesterified cholesterol from fixed tissues.

In another study, Ericksson and colleagues explored the effect of pro apo-AI/phospholipid complexes on the fecal sterol excretion as the final step in the reverse cholesterol transport pathway [26]. There was a 30% increase in fecal bile salt excretion and a 39% increase in neutral sterol excretion, corresponding to the removal of approximately 500 mg/dl excess of cholesterol after infusion. Lathosterol, a marker for the rate of cholesterol synthesis \textit{in vivo}, was unchanged, suggesting that the net increase in cholesterol excretion reflected an enhanced RCT.

In a study by Nissen and colleagues, 5 weekly infusions of a complex consisting of apo-AI Milano and phospholipids resulted in significant regression of atherosclerosis in patients with acute coronary syndromes. The total atheroma volume decreased by 4.2% on intravascular ultrasound [27]. However, the study group was rather small and there is currently no evidence that infusion of apo-AI Milano is more beneficial than wild-type apo-AI or phospholipid complexes.

Apo-AI mimetic peptides have also been given orally, which has been shown to inhibit the formation of atherosclerotic plaques in a hyperlipidemic mouse model [28].

**Nuclear receptors & transcription factors**

Other targets for antiatherosclerotic therapy are the transcription factors PPAR-\(\alpha\), -\(\gamma\), -\(\sigma\), liver X receptor (LXR)-\(\alpha\), retinoid X receptor (RXR)-\(\alpha\), and other orphan members of the nuclear receptor gene family. Natural agonists of LXR-\(\alpha\) (oxysterols) and RXR\(\alpha\) (retinoids) improve cholesterol efflux from macrophages, but also induce hypertriglyceridemia, which is why synthetic agonists, which only exert nonhepatic effects are currently being investigated [29].

**Future targets for high-density lipoprotein therapies**

Apart from the above mentioned strategies, three main targets for potential antiatherogenic treatments involving HDL metabolism have emerged. Stimulation of apo-AI synthesis and

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Image: Figure 3. Plasma delipidation.

Extracorporeal delipidation technology to remove lipids from lipoproteins such as HDL in the bloodstream and then return the delipidated HDL particles back to the body. These delipidated HDL particles pick up excess lipids from the artery walls and transport them to the liver.

HDL: High-density lipoprotein; VLR:
Executive summary

Clinical evidence for the benefits of raising high-density lipoprotein (HDL)-cholesterol (C) levels
- Epidemiological and prospective clinical studies have demonstrated a link between low HDL-C and an increased risk of atherosclerosis.

Current strategies to increase HDL
- Statins, estrogen and ezetimibe have mild HDL-increasing properties (up to 10%).
- Lifestyle interventions, fibrates and glitazones produce a moderate increase in HDL-C (up to 20%).
- Nicotinic acid and some combination therapies have good HDL raising effects (up to 30%).

Novel pharmacologic treatments increasing reverse cholesterol transport
- Currently the most promising approaches to increase HDL include direct infusion of HDL-like particles and inhibition of one of the key enzymes in HDL metabolism, cholesterol ester transfer protein (CETP).
- Both strategies have shown regression of atherosclerosis in animals. The former has also shown regression of atherosclerosis in humans.

Future targets for HDL therapies
- Other exciting approaches include oral HDL mimetic small molecules, autologous delipidation of HDL, drugs that affect nuclear hormone receptors (RXR and LXR) and combined peroxisome proliferator activated receptor-alpha and -gamma agonists (superfibrates).

Future perspective
Large end-point trials, underway with some of the approaches discussed, like CETP inhibitors, will determine whether these drugs have additional mortality benefits over statin therapy alone. If they should achieve this, the combination of a statin and a CETP inhibitor will become an important tool towards the treatment of atherosclerosis. It is also likely that PPAR-α and -γ agonists (superfibrates) will show their benefits in RCT trials and these will be broadly available in patients with diabetes and metabolic syndromes. Other approaches being developed such as orally bioavailable small molecules that resemble HDL and delipidation of autologous HDL appear very promising based on in vitro and animal results. Since some of these therapies are very expensive and will not be available to the broad population for some time, we must remind ourselves that lifestyle modification and well-established drugs such as fibrates and nicotinic acid are both efficient and cost effective methods of raising HDL-C and enhancing RCT.

Bibliography
Papers of special note have been highlighted as either of interest (•) or of considerable interest (★★) to readers.
• First study to show mortality benefit with fibrates.
• Landmark fibrate study
• First study to show mortality benefit with nicotinic acid.
• Landmark study revealing the risk of coronary heart disease with estrogen and progestin.
• Very impressive study showing regression of human coronary atherosclerosis with direct high-density-lipoprotein therapy.

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