

## Recent advances in the treatment of low HDL: In search of novel therapeutics

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The notion that high-density lipoprotein (HDL) is protective of the development of coronary heart disease (CHD) dates back to the early observation of the inverse relationship between plasma HDL-cholesterol (HDL-C) levels and the prevalence of CHD in the 1950s. Subsequent large-scale epidemiologic studies, conducted both in the US and in Europe, consistently substantiated HDL-C levels as being a strong predictor of CHD risk.<sup>1,2</sup> An aggregate analysis of four of the largest US epidemiological studies (Framingham Heart Study, Lipid Research Clinic Prevalence Mortality Follow-up Study, Lipid Research Clinic Primary Prevention Trial, and Multiple Risk Factor Intervention Trial) estimated that each 1 mg/dL (0.02 mM) elevation of HDL-C is associated with a 2%-3% reduction in CHD risk, a magnitude that rivals that for LDL lowering.

Over the past decade, tremendous progress has been made in the recognition of the clinical significance of low HDL-C and the impact of existing lipid-modifying agents in reversing its associated coronary risk. Despite such advances, our current ability to prevent CHD by manipulating plasma HDL continues to lag behind that of lowering LDL. This is in part due to the fact that HDL metabolism and its role in atherogenesis is considerably more complex. Gaps in our knowledge have resulted in unsolved apparent paradoxes, hampering our ability to accurately interpret simple lipoprotein parameters in clinical settings and to effectively institute more targeted therapies. In this issue of *Endocrinology Rounds*, I will report on some of the current advances in our understanding of HDL metabolism and how this new knowledge refines our understanding of current therapies and accelerates the discovery of novel drugs for the clinical management of low HDL and CHD.

### The prevalence of low HDL-C, its impact on CHD, and current drug treatments

In the general population, low HDL-C (< 0.9 mmol/L) is highly prevalent. Suppressed levels of HDL-C are a frequent occurrence in subjects for all hyperlipidemic classes. Low HDL-C is also common in subjects with familial combined hyperlipidemia and familial hyperlipidemia, two familial forms of dyslipidemic states that are associated with a marked increase in CHD risk. Likewise, low HDL-C may occur in isolation – isolated low HDL – with total cholesterol (TC) < 5.2 mmol/L and triglycerides (TG) < 2.8 mmol/L. Nearly 11% of adult men over age 20 in the US, according to the NHANES III population survey, have isolated low HDL-C levels. In the Israeli Ischemic Heart Disease Study of 8000 adult males who were free of heart disease at entry, a total of 31.1% were found to have low HDL-C at entry, and among them, 17% had isolated low HDL.<sup>3</sup> In a large cohort of 85,000 men with previous history of MI



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screened for the Veterans Affairs HDL Intervention Trial (VA-HIT), 58% had LDL-C below 2.5, and among them, 41% had HDL-C <0.9 mmol/L.<sup>4</sup>

The long-term prognosis of patients with low HDL-C differs substantially, depending on whether they have had a previous history of CHD. In the Israeli Ischemic Heart Disease Study cohort, 5-year survival was well above 95% in all lipoprotein subgroups, including those with HDL <0.9 mmol/L and TC >5.2 mmol/L. At the 21-year follow-up, the survival curve separates and individuals with baseline HDL-C below 0.9 mmol/L fair less well compared to their “high-HDL” counterparts. In comparison, the placebo-treated group in the VA-HIT study had a 17% total mortality rate over an average of 5 years.<sup>5</sup>

In recent years, a number of pharmacologic intervention trials have been carried out that target high-risk patients with isolated low HDL-C. In VA-HIT, men with a previous history of CHD and isolated low HDL-C were randomly assigned to gemfibrozil 1200 mg each day or placebo. Compared with placebo, gemfibrozil treatment resulted in a 6% increase in HDL-C, a 31% reduction in TG, and no significant change in LDL-C. At the 5-year follow-up, the treatment group enjoyed a 22% reduction in CHD death and non-fatal MI. Total mortality was also reduced by 10% in the treated group, but was not statistically significant.<sup>5</sup> It is interesting to note that the degree of reduction in clinical events per unit HDL-C increase is astounding. A more detailed analysis of the data suggests that the clinical benefit may not be entirely attributable to the lipid changes.<sup>6</sup>

Other fibrates have also been tested in angiographic trials. In the Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT),<sup>7</sup> 92 post-myocardial infarction male subjects were randomized to bezafibrate vs placebo for 5 years. In the Diabetes Atherosclerosis Intervention Study (DAIS),<sup>8</sup> 731 men and women with type 2 diabetes were randomized to either fenofibrate or placebo for at least 3 years. Active drug treatment in both studies led to more favorable angiographic changes, substantiating the overall beneficial effect of fibrates in the treatment of high-risk patients.

In the HDL Atherosclerosis Treatment Study (HATS),<sup>9</sup> high-risk men and women with isolated low HDL-C (HDL-C < 0.9 mmol/L for men and <1.03 for women, LDL <3.75 mmol/L and TG <4.52 mmol/L) were randomized in a 2x2 factorial design to receive simvastatin plus niacin, with and without, antioxidants. Both simvastatin and niacin doses were titrated to pre-specified LDL-C and HDL-C targets. The on-trial changes in LDL-C and HDL-C in the

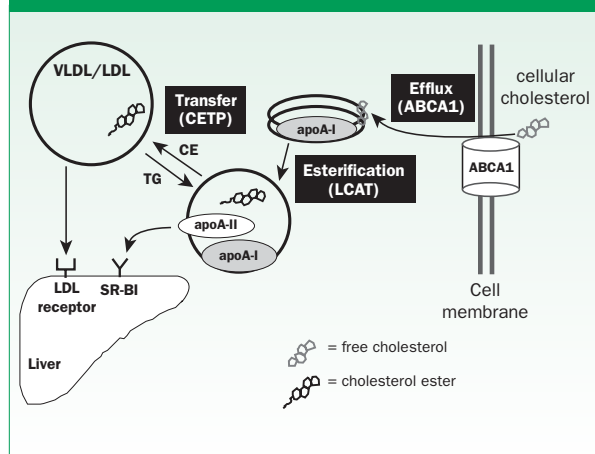
simvastatin-niacin group were -42% and +26%, respectively, compared to the placebo group. At the 3-year follow-up, the total clinical event rate was 24% in the placebo group, but reduced to a remarkable 3% in the simvastatin-niacin group. Use of the prescribed antioxidants alone did not influence either the lipid profile or the clinical event rate, but they did attenuate the rise in HDL-C and the 3-year event rate reduction if given together with simvastatin-niacin. The results of this study are consistent with the hypothesis that the use of niacin, the most efficacious agent in raising HDL-C to-date, acts synergistically with statins and confers additional clinical benefits in this unique high-risk group.

### **HDL metabolism – the Reverse Cholesterol Transport hypothesis and paradoxes**

Circulating HDL particles have the ability to remove cholesterol from peripheral tissues, including lipid-laden macrophages in the vessel walls, and direct them back to the liver for eventual elimination, a process dubbed reverse cholesterol transport (RCT) (Figure 1). It has long been hypothesized that RCT is the main mechanism by which HDL exerts its cardioprotection. The initial step is the efflux of cholesterol. Circulating HDL particles have the ability to remove cholesterol from cell membranes. Multiple pathways have been identified to mediate this process, including simple diffusion and ATP-dependent transport. Recently, a transmembrane transporter – ABCA-1 – has been identified as a key player in facilitating efflux of cholesterol from a broad range of tissues. Kindreds with defective ABCA-1, also known as Tangier Disease, uniformly suffer impaired cholesterol efflux and extremely low plasma HDL-C levels.<sup>10</sup> Intriguingly, despite its perceived importance in reversing atherogenesis by removing cellular cholesterol, Tangier disease subjects are not uniformly predisposed to premature CHD.<sup>11</sup> This clinical observation marks only one of the many examples of how a defect of a single key protein in HDL metabolism may confer a diverse impact on the development of atherosclerosis.

Circulating HDL particles are structurally and compositionally heterogeneous. Most of these particles are spherical, but their size distribution is polymodal with the most dominant fractions being labeled HDL<sub>2</sub> and HDL<sub>3</sub>. However, the HDL species most potent in mediating efflux of cholesterol, known as nascent HDL or pre-β-HDL, constitute only a small fraction of circulating HDL. These particles are made up primarily of phospholipids, and the protein compo-

**Figure 1: Reverse cholesterol transport process**



ment is exclusively apolipoprotein (apo) A-I, the major structural protein of HDL particles. Upon taking up the cell-derived cholesterol, these particles also acquire lecithin cholesterol acyltransferase (LCAT), an enzyme that mediates the esterification of cell-derived, unesterified cholesterol (or free cholesterol (FC)) into cholesterol ester (CE) – the esterification step. This neutral lipid favours entering the core of the HDL particle and continually depleting the HDL of FC, thus sustaining the concentration gradient to continuously mobilize efflux. Continuing accumulation of CE causes the HDL to turn spherical and increase in size. Such cell-derived CE can subsequently be transferred to apoB-containing particles (VLDL, IDL and LDL) in exchange for TG, mediated by the cholesterol ester transfer protein (CETP). While such cell-derived cholesterol may be taken up by the liver through particulate uptake of LDL, recent studies suggest that direct transport of cholesterol from HDL to the liver may take place through a newly described HDL receptor, scavenger receptor class B type I (SR-BI).<sup>12</sup> In addition, TG accumulating in the HDL are continuously hydrolyzed by hepatic lipase (HL). Failure of this hydrolysis step, as seen in hepatic lipase deficient subjects, results in the accumulation of TG-rich HDL particles.

The continual removal of FC from cells, its esterification, transfer of the neutral lipids, and the hydrolysis of HDL-TG, constitute a dynamic steady state, and would prescribe a continuous spectrum of sizes and compositions of HDL. However, most mature HDL fall into 2 distinct densities, the more dense HDL<sub>3</sub> and the buoyant HDL<sub>2</sub>. This polymodal distribution of HDL may be a result of distinct conformational properties of its major structural protein apoA-

I. Interestingly, a detailed evaluation of the epidemiologic data suggests that HDL<sub>2</sub> has a stronger inverse relationship with CHD risk. However, this method of separating HDL subspecies by density has yet to be of sufficient discriminating power to be accepted for general clinical use. From the apoprotein composition point of view, circulating HDL particles have also been divided into 2 major fractions based on its apoprotein composition, Lp-AI (HDL containing only apoA-I) and LpAI-AII (HDL containing both apoA-I and apoA-II, the latter being another important structural apoprotein in HDL). Many lines of evidence suggest that LpAI, which includes pre- $\beta$ -HDL, is the main fraction mediating most of the cardioprotective actions.

### Validating the RCT model

Despite the attractiveness of the model, experimental validation of the cardioprotective role of HDL via RCT in humans has proven to be difficult, in part because of its complex metabolism. The most direct evidence that HDL-C is cardioprotective comes from transgenic mouse model studies. Rubin et al<sup>13</sup> generated transgenic mice that over-express human apoA-I, leading to a markedly increased plasma level of HDL-C and a substantial reduction in atherosclerotic lesions. A similar cardioprotective effect was also demonstrated in transgenic rabbits that over-express human apoA-I.<sup>14</sup> In addition, the increased level of plasma HDL was able to increase cholesterol efflux from cells by standard in vitro efflux assays.<sup>14</sup> In humans, while therapeutic measures for selectively raising apoA-I levels remains elusive, data from numerous epidemiologic studies and intervention trials all show a consistent inverse relationship between plasma apoA-I levels and the risk of CHD.<sup>15</sup>

Paradoxically, in humans, severe HDL deficiency syndromes, caused by a variety of genetic defects in the apoA-I gene, fail to uniformly show susceptibility to premature CHD.<sup>16</sup> In fact, one particular apoA-I mutation, apoA-I Milano, seems to confer cardioprotection despite causing severe HDL deficiency. The mechanisms underlying these “exceptions to the rule” remain poorly understood.<sup>17</sup> It has been suggested that efficient cholesterol efflux may still take place despite such marked reductions in the steady-state level of HDL-C. To date, this notion remains speculative.

These surprising observations naturally raise the question whether raising HDL-C should be implemented uniformly in all subjects with low HDL-C. The answer to the question is likely to be complex.

Many lines of evidence suggest that HDL may confer non-RCT-related anti-atherogenic effects, including a direct impact on endothelial dysfunction, expression of adhesion molecules, and antioxidative effects,<sup>18</sup> suggesting that a sustained high level of HDL is likely of benefit. More in depth understanding of RCT and the anti-atherogenic activities of HDL may provide additional insights.

### **Cholesterol esterification**

The role of LCAT in atherosclerosis is also controversial. LCAT is central to the RCT process. By mediating the esterification of FC, LCAT promotes the “maturation” of the nascent HDL on the one hand, and maintains a concentration gradient of FC on the other. In both humans and in animal models, LCAT activity is inversely associated with plasma HDL-C levels. Deficiency of LCAT is therefore expected to result in severe reductions in HDL-C with the residual HDL being the nascent HDL. This is, in fact, the case in subjects with complete LCAT deficiency caused by mutations in the LCAT gene. Surprisingly, these affected subjects are not particularly prone to premature CHD. Rather, a high prevalence of chronic progressive nephropathy is noted among these subjects. Animal models available to-date have not clarified the controversy.

The impact of an over-expression of the human LCAT gene on the development of atherosclerosis is species-dependent, despite a uniform increase in HDL-C levels. Over-expression of human LCAT gene in rabbits results in an attenuation of atherosclerosis, but a comparable level of over-expression in a transgenic mouse model leads to a paradoxical increase in atherosclerosis. The latter case is attributed to an accumulation of atypically large HDL particles in mice because these animals lack the enzyme CETP. A concomitant over-expression of CETP in these human LCAT transgenic mice partially corrects the accumulation of the large HDL, and reverses the increase in atherosclerosis.<sup>19</sup> These findings suggest that excessive build-up of HDL through LCAT, in the absence of CETP, may be pro-atherogenic.

### **Transfer of neutral lipids**

Cholesterol ester transfer protein (CETP) was initially described for its action in mediating the exchange of neutral lipids among lipoprotein classes, and as such, occupies a crucial role in the RCT process. Esterified cholesterol (CE) in HDL synthesized by LCAT is transferred to apoB-containing VLDL, IDL and LDL particles in exchange for TG. While the CE being transferred to LDL is delivered to the liver for catabo-

lism, the TG in HDL is subject to hydrolysis by hepatic lipase in HDL. Excess CETP activity results in accelerated catabolism of HDL and is the main mechanism that mediates secondary HDL deficiency in hypertriglyceridemia. Likewise, it has been suggested that excess transfer of neutral lipids between LDL and VLDL causes the formation of small, dense LDL. Both phenotypes are particularly prevalent in insulin resistant/diabetic states. In mouse models, over-expression of CETP leads to significant hyperlipidemia, presumably due to excessive CE transfer to the apoB-containing particles, favouring deposition in the vessel wall. Elevated CETP activity has been described in humans based on genetic polymorphism, but its association with increased propensity for CHD has not been reported. However, several lines of evidence suggest that CETP activity is elevated in many subjects at high risk of CHD, including syndrome X.<sup>20,21</sup>

Deficiency of CETP is associated with elevated HDL-C levels. The initial studies on a number of Japanese CETP-deficient kindreds suggest that the associated elevation in HDL-C might contribute to longevity. Subsequent population studies indicate that low CETP and premature CHD is prevalent in Japan.<sup>22</sup> To-date, the role of CETP in atherogenesis remains controversial. The exact relationship may be influenced by a number of metabolic factors. The idea of designing a CETP inhibitor as a therapeutic agent is attractive but, based on our current knowledge, its potential impact on atherosclerosis is somewhat difficult to predict.

### **Scavenger receptor class B type I (SR-BI) – an HDL receptor**

The identification of this multi-ligand transmembrane receptor as the first *bona fide* receptor for HDL was reported in 1996.<sup>12</sup> Unlike its counterpart, the LDL receptor which binds and mediates cellular uptake of the entire LDL particle, SR-BI binds HDL and mediates only selective uptake of the CE content of HDL particles. Selective uptake involves transfer to the cell of the CE from the lipoprotein hydrophobic core, but not the apoproteins. After lipid transfer, the lipid-depleted lipoprotein particle is released from the cell surface and re-enters the circulation.

Several lines of evidence suggest that SR-BI is a physiologically relevant HDL receptor. In mice, the species for which SR-BI was initially described, SR-BI is distributed in a wide variety of tissues, but most abundantly in the steroidogenic tissues and the liver. In the former, SR-BI-mediated uptake of CE provides the bulk of the cholesterol needed as substrate for

steroidogenesis. Hepatic over-expression of SR-BI in mice results in a marked reduction in plasma HDL-C levels. Likewise, targeted disruption of the SR-BI gene in mice leads to a marked increase in plasma HDL-C. An immediate question is, of course, how does altered expression of SR-BI in the mouse liver influence the development of atherosclerosis? Is expression of SR-BI pro-atherogenic because it reduces plasma HDL-C, or is it anti-atherogenic because it accelerates RCT by promoting removal of circulating HDL in mice? After cross-breeding these transgenic and knockout mice into appropriate hyperlipidemic, atherosclerosis-prone backgrounds, namely apoE- and LDL receptor-knockout mice, it has been found that hepatic over-expression of SR-BI attenuates atherosclerosis, whereas a complete absence of SR-BI greatly accelerates it. However, the interpretation of these findings requires caution. It has been shown that, in addition to mediating selective uptake of CE, SR-BI has also been shown to mediate bi-directional flux of FC and phospholipids between HDL and cells. With these animal models, it would be of great interest to further explore and localize the main site where SR-BI exerts its anti-atherogenic actions.

The anti-atherogenic effects of increased expression of SR-BI in the liver of mice clearly raises the possibility that SR-BI would be a novel therapeutic target, either through pharmacologic modulation, or gene therapy. However, much less is known about the human version of SR-BI (hSR-BI or CLA-1). To address this issue, Ueda's laboratory in Kyoto created a transgenic mouse model that over-expresses hSR-BI (CLA-1) using its endogenous promoter for gene expressions.<sup>23</sup> This group reported that the hSR-BI gene preferentially expresses both in the liver and in the brain, but much less abundantly in the steroidogenic tissues. Furthermore, the hepatic gene expression produces a variant of the SR-BI receptor whose action is that of mediating RCT. On the other hand, brain expression resulted in an alternate variant (SR-BII) whose function has yet to be identified. This finding is exciting since hSR-BI also appears to preferentially express in the liver to promote RCT, making the exploration of the increasing expression of hSR-BI as a therapeutic goal a promising pursuit.

### **Cholesterol efflux**

Unregulated uptake of oxidatively-modified LDL by macrophages and their transformation into foam cells plays a central role in the development of athero-

sclerotic lesions. The reversal of lipid accumulation in these cells by inducing the efflux of cholesterol is, therefore, a logical approach in the retardation of lesion formation.

Tangier disease is a rare autosomal recessive disorder characterized by the accumulation of CE in reticuloendothelial tissues, including macrophages, and an extremely low plasma HDL-C (<0.2 mM). Efflux of cholesterol from cells is defective in affected subjects. Frequently, these patients also have very low levels of LDL and a mild degree of fasting TG. Premature CHD has been reported in a number of kindreds, but is not uniform among all those affected. The molecular defect of Tangier disease has been identified as a mutation of the ATP binding cassette type I (ABCA-1) gene.<sup>10</sup> Subsequent studies reveal that ABCA-1 facilitates efflux of both cellular free cholesterol and phospholipids to lipid acceptors in the interstitium, including lipid-poor apoA-I and apoE, resulting in the formation of pre- $\beta$ -HDL. The ABCA-1 transporter appears to mediate cellular trafficking of cholesterol, shuttling cholesterol from the interior of the cell to the surface for efflux. More importantly, gene expression of ABCA-1 is highly regulated. Both cAMP and cellular lipid load upregulates ABCA-1. More recently, ABCA-1 was found to be a target gene for the nuclear receptor, liver X receptor (LXR).<sup>24</sup> LXR was initially considered one of the orphan receptors because it is a receptor cloned without a known ligand. Recent studies reveal that a number of intracellular oxysterols, a class of enzymatically-mediated oxidized cholesterols, are naturally-occurring potent ligands for LXR. This finding not only explains how cellular lipid loading may upregulate this transporter, but more importantly, it also identified a pathway amenable to pharmacologic modulation. LXR upregulates its target genes, including ABCA-1, by binding to a specific region of the gene promoter in partnership with another generic receptor molecule called RXR. It is apparent that ligands to either LXR or RXR may be used to modulate ABCA-1 expression.

### **Proof of principle studies**

In order to show that increased expression of ABCA-1, especially in macrophages, directly influences the severity of atherosclerosis, a transgenic mouse over-expressing human ABCA-1 in both the liver and macrophages has been generated. Atherosclerotic lesions were induced by feeding these mice a high-fat diet, as well as by breeding them into the

severely hyperlipidemic apoE knockout-mouse background. In the former case, an over-expression of hABCA-1 resulted in a reduction in atherosclerotic lesions that is compatible with the hypothetical role of ABCA-1 in promoting accelerated efflux of cholesterol from macrophages. Intriguingly, in the absence of apoE, over-expression of macrophage hABCA-1 resulted in a paradoxical increase in atherosclerosis.<sup>25</sup> This finding is somewhat surprising and a definitive explanation is not available. However, it is conceivable that the local presence of apoE, in and around the macrophages, is crucial, perhaps acting as an acceptor for the effluxed cholesterol to form pre- $\beta$ -HDL. Without a doubt, this surprising finding raises the alarm that the effect of increased expression of ABCA-1 on the development of atherosclerosis may be sensitive to the lipid milieu of the organism.

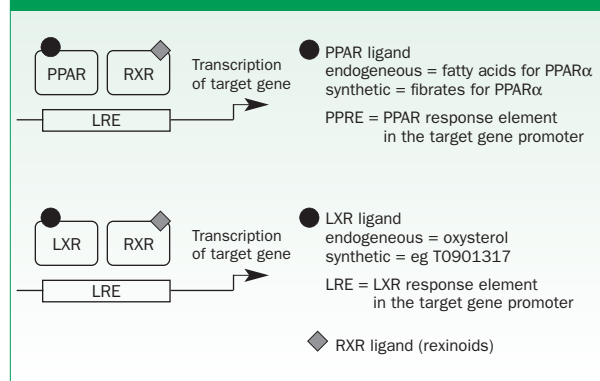
## The role of nuclear receptors in HDL metabolism and atherogenesis

### Novel LXR/RXR ligands.

To-date, a great deal of investigative effort has been devoted to either screening for or synthesizing small molecules to act as ligands for either LXR or RXR. The effect of a number of “first generation” ligands has been reported. Treatment of mice with LXR and RXR ligands both lead to a significant reduction in aortic atherosclerotic lesions. In one study, the RXR agonist (called rexinoids) appears to be more potent. In either case, plasma HDL-C levels are not significantly altered in the treated animals compared with their respective untreated controls. One explanation for the “added” effectiveness of rexinoids may be due to the fact that RXR forms transcriptionally active dimers, not only with LXR, but also with peroxisomal proliferator activator receptors (PPARs). Previous studies have shown that PPAR $\alpha$  in vessel-wall macrophages have a direct effect in the upregulation of ABCA-1.

In addition to ABCA-1, LXR simultaneously and transcriptionally upregulates another important target gene for cellular lipid metabolism, namely, the sterol response element binding protein-1 (SREBP-1). Like its cousin gene SREBP-2, that coordinately upregulates both LDL receptor and HMG-CoA reductase genes in response to cellular depletion of cholesterol, SREBP-1 coordinately upregulates the program of genes involved in hepatic synthesis of fatty acids, leading to an increase in VLDL production and hypertriglyceridemia. Much effort has been directed at searching for similar compounds that might either bypass or minimize the hypertriglyceridemic effects.

**Figure 2: Mode of action of nuclear receptors PPARs and LXR**



### Peroxisomal proliferator activator receptors (PPARs)

To date, three functionally distinct PPAR proteins, PPAR $\alpha$ , PPAR $\delta/\beta$ , and PPAR $\gamma$  have been identified as members of this subfamily of nuclear receptors. These receptors each have their own selective pattern of tissue distribution. These subtypes of receptors share a number of low-affinity, unsaturated fatty acids as endogenous ligands. A number of high-affinity, synthetic ligands, fibrate drugs for PPAR $\alpha$ , and the thiazolidenedione class for PPAR $\gamma$ , have been in widespread clinical use, the former primarily for the management of dyslipidemia, and the latter for treatment of insulin resistance. Similar to the previously described nuclear receptors, PPARs, after forming heterologous dimers with RXR, exert their biological actions by transcriptionally regulating their individual sets of target genes.

Clinically, fibrate drugs raise plasma HDL-C, lower TG, and in most cases, lower LDL-C. These changes can be largely explained by the coordinate regulation of a series of PPAR $\alpha$  responsive genes. Hepatic upregulation of both apoA-I and apoA-II contribute to the raising of HDL-C. Meanwhile, a simultaneous upregulation of the lipoprotein lipase gene and the down-regulation of the apoC-III gene synergistically lowers plasma TG, which may in turn further raise HDL-C.<sup>26</sup> Furthermore, PPAR $\alpha$  has been shown to act locally, especially on macrophages, to promote cholesterol efflux by upregulating the ABCA-1 pathway and the SR-BI/CLA1 pathway,<sup>27</sup> the former through activation of LXR.<sup>28</sup> Therefore, in addition to modulating plasma lipoproteins, fibrate drugs may also exert direct anti-atherogenic effects by promoting cholesterol efflux.<sup>29</sup>

PPARs are also expressed in atherosclerotic lesions. PPAR $\alpha$  inhibits the expression of a number of proinflammatory genes. PPAR $\alpha$  also inhibits expression of adhesion molecules like VCAM-1. Likewise, PPAR $\gamma$  activation also inhibits expression of adhesion

molecules. PPAR $\gamma$  activation in macrophages and foam cells has been shown to inhibit the expression of activated genes such as iNOS, MMP-9, and scavenger receptor A. In a recent study, treatment of apoE knockout mice with troglitazone for 2 months resulted in a significant reduction of atherosclerotic lesions with the only lipid alteration being an increase in HDL-C in the treated group.<sup>30</sup>

Recently, a novel high-affinity, selective synthetic PPAR $\delta$  agonist has been reported. The design of this synthetic compound was based on combinatorial chemistry.<sup>31</sup> In cultured macrophages, treatment with this compound led to increased expression of ABCA-1 and apoA-I-specific cholesterol efflux. Given to obese rhesus monkeys, a primate model for syndrome X, this PPAR $\delta$  agonist resulted in a simultaneous correlation of several phenotypes, including elevation of HDL-C, reduction of plasma TG, increase in LDL particle size, and lowering of fasting insulin. The ability of a single, small molecule to correct a myriad of metabolic and cardiovascular markers through a single receptor is clearly astounding. The remarkable similarity in clinical phenotypes between obese rhesus monkeys and humans with a metabolic syndrome holds great promise for this experimental agent to be of benefit to humans. In this same report, the authors suggest that due to the lack of specificity with fibrate drugs in binding to the various PPAR receptors and the relative potency, the observed beneficial outcomes in many of the clinical trials using fibrates might have derived their effect predominantly through PPAR $\delta$  action. As always, clinicians should welcome such novel findings with great enthusiasm on the one hand, but in the meantime, critically evaluate the validity of the data and their translatability to human use.

## Summary

Over the past decade, tremendous progress has been made in the clinical management of low HDL for preventing CHD using existing lipid-modifying agents, significant breakthroughs have deepened our understanding of the complex metabolic pathways involved in HDL metabolism. Unraveling the crosstalk between various classes of nuclear receptors has provided exciting opportunities for designing novel therapeutics to treat this high-risk metabolic syndrome.

From the clinical management point of view, HDL-C is officially incorporated into the global risk assessment for both the revised Canadian<sup>32</sup> and US<sup>33</sup> guidelines for the management of dyslipidemias.

However, partly due to the reasons stated earlier, establishing treatment targets for HDL-C remains unavailable. It is hoped that with a better understanding of how individual players in the HDL metabolic pathways impact on the development of atherosclerosis, and the availability of more effective therapeutics, practical treatment targets for HDL can be developed.

## Suggested references

1. Castelli WP. Cholesterol and lipids in the risk of coronary artery disease – the Framingham Heart Study. *Can J Cardiol* 1988;4, Suppl A:5A-10A.
2. Assmann G, Schulte H, von Eckardstein A, Huang Y. High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. *Atherosclerosis* 1996;124 Suppl:S11-20.
3. Goldbourt U, Yaari S, Medalie JH. Isolated low HDL cholesterol as a risk factor for coronary heart disease mortality. A 21-year follow-up of 8000 men. *Arterioscler Thromb Vasc Biol* 1997;17:107-113.
4. Rubins HB, Robins SJ, Collins D, et al. Distribution of lipids in 8,500 men with coronary artery disease. Department of Veterans Affairs HDL Intervention Trial Study Group. *Am J Cardiol* 1995; 75:1196-1201.
5. Rubins HB, Robins SJ, Collins D, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. *N Engl J Med* 1999;341:410-418.
6. Rubins SJ, Collins D, Wittes JT, et al. Relation of gemfibrozil treatment and lipid levels with major coronary events: VA-HIT: a randomized controlled trial. *JAMA* 2001;285:1585-1591.
7. Ericsson CG, Nilsson J, Grip L, Svane B, Hamsten A. Effect of bezafibrate treatment over five years on coronary plaques causing 20% to 50% diameter narrowing (The Bezafibrate Coronary Atherosclerosis Intervention Trial [BECAIT]). *Am J Cardiol* 1997;80:1125-1129.
8. DAIS investigators. Effect of fenofibrate on progression of coronary-artery disease in type 2 diabetes: the Diabetes Atherosclerosis Intervention Study, a randomised study. *Lancet* 2001; 357:905-910.
9. Brown BG, Zhao XQ, Chait A, et al. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med* 2001;345:1583-1592.
10. Hayden MR, Clee SM, Brooks-Wilson A, Genest J Jr, Attie A, Kastelein JJ. Cholesterol efflux regulatory protein, Tangier disease and familial high-density lipoprotein deficiency. *Curr Opin Lipidol* 2000;11:117-122.
11. Clee SM, Kastelein JJ, van Dam M, et al. Age and residual cholesterol efflux affect HDL cholesterol levels and coronary artery disease in ABCA1 heterozygotes. *J Clin Invest* 2000;106:1263-1270.
12. Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* 1996;271:518-520.
13. Rubin EM, Ishida BY, Clift SM, Krauss RM. Expression of human apolipoprotein A-I in transgenic mice results in reduced plasma levels of murine apolipoprotein A-I and the appearance of two new high-density lipoprotein size subclasses. *Proc Natl Acad Sci USA* 1991;88:434-438.
14. Brousseau ME, Hoeg JM. Transgenic rabbits as models for atherosclerosis research. *J Lipid Res* 1999;40:365-375.
15. Gotto AM Jr, Whitney E, Stein EA, et al. Relation between baseline and on-treatment lipid parameters and first acute major coronary events in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS). *Circulation* 2000;101: 477-484.

16. Ng DS, Leiter LA, Vezina C, Connelly PW, Hegele RA. Apolipoprotein A-I Q[-2]X causing isolated apolipoprotein A-I deficiency in a family with analphalipoproteinemia. *J Clin Invest* 1994;93:223-229.
17. Wang WQ, Moses AS, Francis GA. Cholesterol mobilization by free and lipid-bound apoAI(Milano) and apoAI(Milano)-apoAII heterodimers. *Biochemistry* 2001;40:3666-3673.
18. Van Lenten BJ, Navab M, Shih D, Fogelman AM, Lusis AJ. The role of high-density lipoproteins in oxidation and inflammation. *Trends Cardiovasc Med* 2001;11:155-161.
19. Foger B, Chase M, Amar MJ, et al. Cholesteryl ester transfer protein corrects dysfunctional high density lipoproteins and reduces aortic atherosclerosis in lecithin cholesterol acyltransferase transgenic mice. *J Biol Chem* 1999;274:36912-36920.
20. Arai T, Yamashita S, Hirano K, et al. Increased plasma cholesteryl ester transfer protein in obese subjects. A possible mechanism for the reduction of serum HDL cholesterol levels in obesity. *Arterioscler Thromb* 1994;14:1129-1136.
21. Hibino T, Sakuma N, Sato T. Higher level of plasma cholesteryl ester transfer activity from high-density lipoprotein to apo B-containing lipoproteins in subjects with angiographically detectable coronary artery disease. *Clin Cardiol* 1996;19:483-486.
22. Hirano KI, Yamashita S, Nakajima N, et al. Genetic cholesteryl ester transfer protein deficiency is extremely frequent in the Omagari area of Japan: marked hyperalphalipoproteinemia caused by CETP gene mutation is not associated with longevity. *Arterioscler Thromb Vasc Biol* 1997;17:1053-1059.
23. Kashima T, Ueda Y. Different expression pattern of human and mouse gene encoding SR-BI and II in human CLA-I transgenic mouse. Abstract. DALM, New York, 2001.
24. Venkateswaran A, Laffitte BA, Joseph SB, et al. Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR alpha. *Proc Natl Acad Sci USA* 2000;97:12097-12102.
25. Joyce CW, Amar MJ, Lambert G, et al. The ATP binding cassette transporter A1 (ABCA1) modulates the development of aortic atherosclerosis in C57BL/6 and apoE-knockout mice. *Proc Natl Acad Sci USA* 2001; Dec 18 [epub ahead of print].
26. Fruchart JC, Staels B, Duriez P. The role of fibric acids in atherosclerosis. *Curr Atheroscler Rep* 2001;3:83-92.
27. Chinetti G, Gbaguidi FG, Griglio S, et al. CLA-1/SR-BI is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors. *Circulation* 2000;101:2411-2417.
28. Chinetti G, Lestavel S, Bocher V, et al. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat Med* 2001;7:53-58.
29. Rader DJ. Raising low HDL-C: Treatment strategies. Abstract. DALM, New York, 2001.
30. Neve BP, Fruchart JC, Staels B. Role of the peroxisome proliferator-activated receptors (PPAR) in atherosclerosis. *Biochem Pharmacol* 2000;60:1245-1250.
31. Oliver WR Jr, Shenk JL, Snaith MR, et al. A selective peroxisome proliferator-activated receptor agonist promotes reverse cholesterol transport. *Proc Natl Acad Sci USA* 2001;98:5306-5311.
32. Fodor JG, Frohlich JJ, Genest, Jr JGG, McPherson R, for the Working Group on Hypercholesterolemia and Other Dyslipidemias. Recommendations for the management and treatment of dyslipidemia. *CMAJ* 2000;162:1441-1447.
33. NCEP. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA* 2001;285:2486-2497.

## Upcoming Meetings

1-3 February, 2002

**American Diabetes Association  
49<sup>th</sup> Annual Advanced Postgraduate Course**  
San Francisco, California  
CONTACT: ADA Meeting Services Department  
Tel: 703 549-1500 Ext. 2022  
E-mail: meetings@diabetes.org

6-9 March, 2002

**National Osteoporosis Foundation 5<sup>th</sup> International  
Symposium  
Clinical Advances in Osteoporosis**  
Honolulu, Hawaii  
CONTACT: Website: www.nof.org  
Tel: 202-223-2226  
E-mail: tso@nof.org

10-14 May, 2002

**10F World Congress on Osteoporosis**  
Lisbon, Portugal  
CONTACT: Centro de Congressos de Lisboa  
Tel: +351 21 360 14 00  
Fax: +351 21 363 94 50  
E-mail: evelised@aip.pt  
Website: www.iofcongress.org/congress\_info.php

14-18 June, 2002

**American Diabetes Association 62<sup>nd</sup> Annual Meeting and  
Scientific Sessions**  
San Francisco, California  
CONTACT: ADA Meeting Services Department  
Tel: 703 549-1500 Ext. 2134  
E-mail: meetings@diabetes.org

19-22 June, 2002

**Endocrine Society's 84<sup>th</sup> Annual Meeting**  
San Francisco, California  
CONTACT: Beverley Glover  
E-mail: Bglover@endo-society.org  
Website: www.endo-society.org

2-5 October 2002

**Canadian Diabetes Association and the Canadian Society  
of Endocrinology Metabolism  
Professional Conference and Annual Meetings**  
Vancouver, British Columbia  
CONTACT: Helena Miekus  
Tel: 416 363-0177 Ext. 571  
Fax: 416 363-7465  
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