

## Reverse cholesterol transport, high density lipoproteins and HDL cholesterol: recent data

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### SUMMARY

Unlike LDL cholesterol, which is a major cardiovascular risk factor, HDL cholesterol plays an important anti-atherogenic role through reverse cholesterol transport from peripheral cells to the liver. Some recent biochemical and epidemiological data shed light on this key function. In the hereditary Tangier disease with disseminated lipid storage, the main biochemical feature is a dramatically low level of HDL cholesterol. Different mutations in the ATP-binding cassette transporter A1 (ABCA1) gene have been recently described, which interfere with cellular cholesterol efflux. This results in low HDL plasma level, and defective reverse cholesterol transport to the liver. Moreover, selective hepatic uptake of HDL cholesteryl esters by SR-B1, a class B scavenger receptor, also plays a key role. In the follow-up of the PROCAM Study, the relative risk of coronary events is high in a cluster of patients with increased total cholesterol/HDL-cholesterol ratio. In the prospective secondary prevention VA-HIT study, the relative risk of coronary events in patients with low HDL cholesterol levels is decreased of 22% with a treatment by gemfibrozil. If the present available range of drugs targeted at increasing HDL cholesterol levels is rather narrow, future therapies will be encouraging, especially with agonists of PPARs.

**Key-words:** Atherosclerosis · Lipoproteins · HDL-Cholesterol · PPAR · Normolipemic Drugs.

**Fredenrich A, Bayer P. Reverse cholesterol transport, high density lipoproteins and HDL cholesterol: recent data. Diabetes Metab 2003,29,201-5**

### RÉSUMÉ

#### La voie de retour du cholestérol et les lipoprotéines de haute densité (HDL) : données récentes

Contrairement aux lipoprotéines de basse densité (LDL), dont l'élévation est un des facteurs principaux du risque cardio-vasculaire et notamment coronarien, les lipoprotéines de haute densité (HDL), qui assurent le retour du cholestérol à partir des tissus périphériques vers le foie, jouent un rôle anti-athérogène très important. Un certain nombre de données nouvelles, biochimiques, épidémiologiques et thérapeutiques, permettent de mieux comprendre ce rôle. Dans la maladie de Tangier, affection héréditaire rare caractérisée par des dépôts lipidiques ubiquitaires et un taux plasmatique très bas de cholestérol-HDL, il a été décrit récemment différentes mutations du gène du transporteur ABCA1 (ATP binding cassette transporter A1) responsable de l'efflux du cholestérol du milieu intracellulaire vers le milieu extracellulaire. En conséquence, le taux de cholestérol-HDL, et donc le retour du cholestérol vers le foie, sont très diminués. Par ailleurs, la captation du cholestérol-HDL par le foie dépend d'un récepteur hépatique, SR-B1 qui joue également un rôle clé dans ce processus de transport inverse. Sur le plan épidémiologique, le suivi de l'étude allemande PROCAM confirme l'existence d'un sous-groupe de patients à haut risque coronarien caractérisé par un rapport cholestérol total/cholestérol-HDL élevé. Par ailleurs, l'essai clinique prospectif VA-HIT a montré que, chez des patients coronariens avec un taux de cholestérol-HDL bas, un traitement par un fibrate, le gemfibrozil, réduit le risque relatif d'événements coronariens de 22 %. Enfin sur le plan thérapeutique, si la gamme actuelle des hypolipémiants permet une augmentation limitée du taux de HDL, les perspectives pharmacologiques sont encourageantes, et notamment celles tirées des manipulations d'animaux transgéniques pour les récepteurs activés des proliférateurs de peroxisomes (PPARs).

**Mots-clés :** Athérosclérose · Lipoprotéines · Cholestérol-HDL · PPAR · Médicaments hypolipémiants.

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Received: November 29th, 2002; revised: March 10th, 2003

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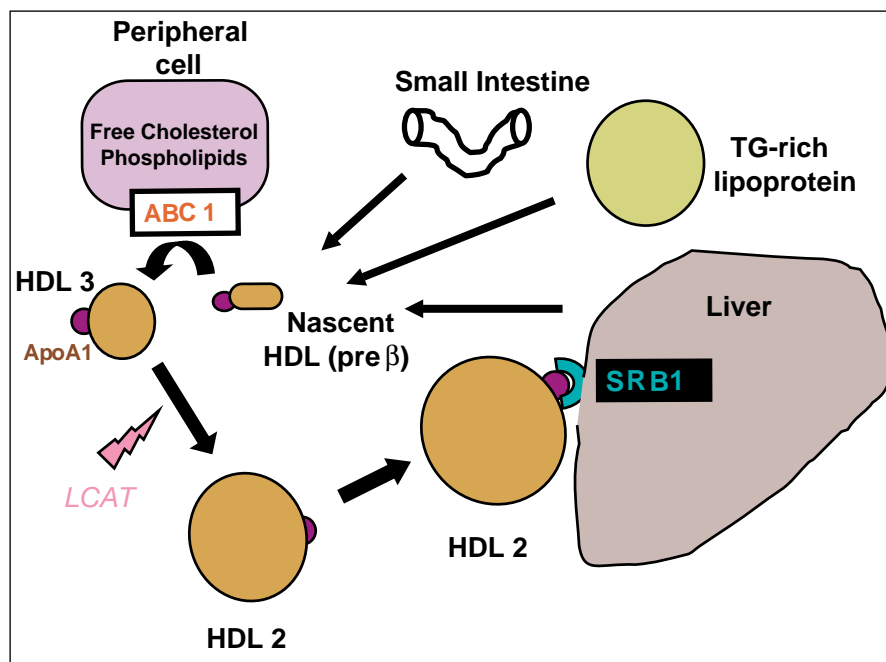
A low plasma level of HDL-cholesterol is a relevant cardio-vascular risk factor [1]. Indeed, the reverse transport from peripheral cells to the liver is the physiological process required to counteract the deposition of cholesterol via very low density and low density lipoproteins (VLDL and LDL). In the reverse transport, high density lipoproteins (HDL) uptake cholesterol from peripheral cells and carry it to the liver. Moreover, HDL particles have many additional antiatherogenic features, such as anti-inflammatory, anti-aggregative, and antioxidative properties [2]. These characteristics account for the recent interest in present and future pharmacological manipulations of plasma HDL-cholesterol levels [3].

### Biochemical data

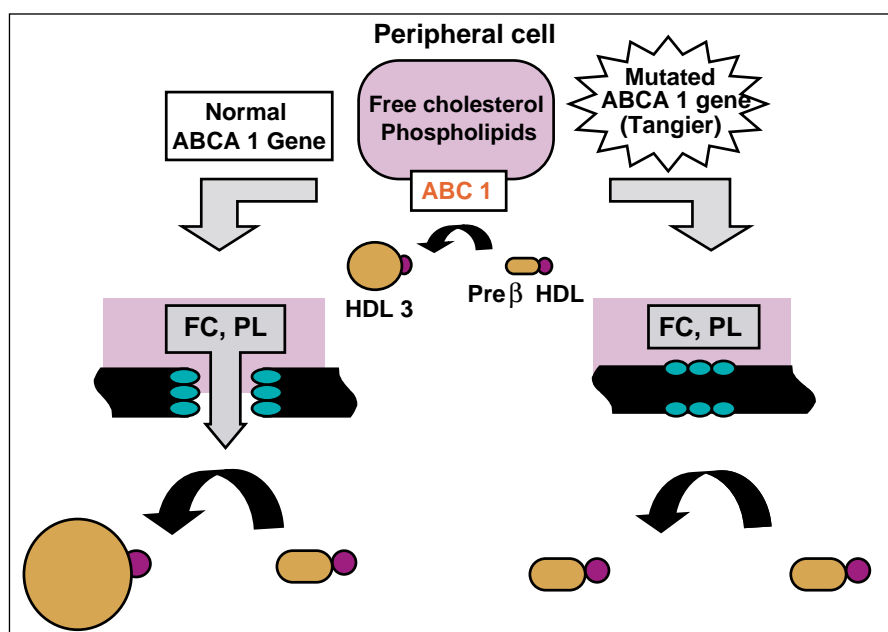
Reverse cholesterol transport from peripheral cells to the liver involves in the first step small particles of discoidal shape, named pre $\beta$ -HDL, synthesized in liver and small intestine, or resulting from hydrolysis of triglyceride-rich particles. These pre $\beta$ -HDL uptake cholesterol from peripheral cells, and their shape change to spherical particles, named HDL3 then HDL2, as they become enriched in esterified cholesterol (via an esterifying enzyme, lecithin cholesterol acyl transferase (LCAT) associated with pre $\beta$ -HDL particles) and phospholipids. The final uptake of HDL2 by the

liver involves a selective receptor, named scavenger receptor B1 (SR-B1) (Fig 1).

The first step has been recently unravelled by new data coming from patients affected by Tangier disease. This hereditary affection, known since the 60s, is clinically characterized by a spread infiltration of cholesteryl esters in hematopoietic organs, as tonsils, lymph nodes and spleen. The main biochemical trait of this disease is a very low plasma level of both HDL-cholesterol and apolipoprotein A1 (apoA1). Genetic analysis of a cluster of members of a same family has recently shown mutations of the gene of the ATP binding cassette transporter 1 (ABC1 or ABCA1). ABCA1 allows the transmembrane transport of free cholesterol and phospholipids from peripheral cells into pre $\beta$ -HDL [4-6]. In patients with Tangier disease, the presence of one or several mutations leads to conformational changes of the transporter, which impede the lipid transfer to nascent HDL particles. Therefore, reverse cholesterol transport to liver is greatly decreased (Fig 2). As these patients are characterized by a low anti-atherogenic HDL-cholesterol plasma level, they would be expected to suffer from premature and diffuse atherosclerosis, however this is not the case in the majority of Tangier individuals. This paradox can be partially explained by the absence of correlation between the amount of cholesterol headed to the liver and the plasma level of HDL-cholesterol. Two explanations are possible for this discrepancy: first, the rate of selective uptake of cholesterol by the



**Figure 1**  
Global view of reverse cholesterol transport. See text for details.



**Figure 2**  
Molecular mechanism of Tangier disease. When ABCA1 gene is mutated, the transport of free cholesterol (FC) and phospholipids (PL) from peripheral cells onto pre $\beta$ -HDL through cellular membrane is hindered.

hepatic receptor SRB1 [7], which is a critical parameter in the regulation of HDL-cholesterol plasma level, second, the diversity of genes involved in the reverse transport, as ApoA1, cholesterol ester transfer protein, LCAT, lipoprotein lipase, and hepatic lipase genes [8].

## Epidemiological data

Several epidemiological studies have shown a strong negative correlation between plasma level of HDL-cholesterol and risk of coronary heart disease. More than 40% of patients with myocardial infarction have a low HDL-cholesterol plasma level [9]. In the German prospective study led in Munster (PROCAM), the relative risk of coronary heart disease is 6 times higher in patients with HDL-cholesterol level below 35 mg/dl [10]. In two prospective studies of cardio-vascular protection led with a fibrate, gemfibrozil, the Helsinki Heart Study [11], in primary prevention, and the VA-HIT study [12], in secondary prevention, the increase of plasma HDL-cholesterol level in the treated group was correlated with the reduction of the incidence of cardio-vascular events, and particularly of coronary disease.

However, the strength of the correlation between HDL-cholesterol level and cardio-vascular risk depends on other risk factors. Obviously, in most patients low HDL-cholesterol is part of the metabolic syndrome, which associates dyslipidemia (low HDL-cholesterol level and hypertriglyceridemia), hypertension, abnormal glucose tolerance or type 2 diabetes, and abdominal obesity [13]. A state of insulin resistance is considered as the common substrate of these features, so the low plasma level of HDL-cholesterol is

not the unique factor accounting for the increased cardio-vascular risk of these patients.

In prospective studies led with statins, as 4S [14], CARE [15], and the recently published HPS [16], cardio-vascular protection in the treated group was not correlated with increase of HDL-cholesterol level, but with decrease of LDL-cholesterol plasma level. An additional explanation accounting for this weak correlation between HDL-cholesterol level and cardio-vascular risk can be provided by the numerous anti-atherogenic properties of HDL particles, which are able to modulate the apparently over-simple effect of the sole plasma level of HDL-cholesterol (inhibition of monocyte chemotaxis and endothelial adhesion of leucocytes, inhibition of endothelial dysfunction, apoptosis, LDL oxidation, complement activation, stimulation of prostacyclin production and NO synthesis) [17].

For all these reasons, the causal link between HDL-cholesterol level and cardio-vascular mortality and morbidity is not fully established to date, and appears surely weaker than the well-established correlation existing with LDL-cholesterol level.

Moreover, the French Health Agency (AFSSAPS), in its advices for good clinical practice [www.afssaps.sante.fr], states that the main parameter for prevention efficiency in dyslipidemic individuals is LDL-cholesterol plasma level, and founds its recommendations for the management of these patients on this level. But the Agency considers that HDL-cholesterol levels constitute a modulator of physician's clinical judgement: a plasma level below 35 mg/dl is an additional risk factor, and, conversely, a level above 60 mg/dl is a protective feature. In the recently published NCEP ATP III guidelines [18], greater emphasis is placed on low HDL

cholesterol levels than in the previous version. The level below which HDL-cholesterol is considered to be a coronary heart disease risk factor is revised from 35 to 40 mg/dl.

## Therapeutic data and emerging treatments

Several healthy and dietetic actions are useful to increase plasma levels of HDL-cholesterol. Among these, physical exercise (although intensive practice is mandatory to significantly increase HDL-cholesterol level), smoking cessation (smokers have a HDL-cholesterol level of 5 to 9 mg/dl lower than non-smokers) [19], and weight loss [HDL-cholesterol increases of 0.8 mg/dl as body mass index decreases of 1 kg/m<sup>2</sup>] [20]. Current normolipidemic drugs have a differential effect according to their respective class [21]. Colestyramine increases HDL-cholesterol level from 3 to 5%, statins from 5 to 15%, and fibrates from 10 to 15%. Magnitude of these effects is mild, especially if compared to the effect on LDL-cholesterol level, which is reduced from 15 to 30% with colestyramine, from 30 to 50% with statins, and from 10 to 15% with fibrates. Moreover, colestyramine can increase triglycerides level, which may counteract the anti-atherogenic effect of increasing HDL level.

Therefore an emerging need appears now to develop drugs able to increase more drastically plasma levels of HDL-cholesterol and improve reverse cholesterol transport. Several tracks are currently studied: activators of ApoA1 synthesis, CETP inhibitors, and agonists of the nuclear receptors known as PPARs. Three isoforms of PPARs are actually known, PPAR $\alpha$ , PPAR $\gamma$  and PPAR $\delta$ . Activation of PPAR $\alpha$  by fibrates, which are a potent ligand, explains their hypolipidemic effects, particularly the decrease of triglycerides level and increase of HDL-cholesterol level. Activation of PPAR $\alpha$  targets some genes which play a major role in the regulation of triglycerides and HDL levels, as ApoA1 synthesis, stimulation of lipoprotein lipase, and inhibition of apolipoprotein CIII [22]. PPAR $\gamma$  is involved in the terminal adipocyte differentiation and in insulin sensitivity, and is the target of new antidiabetic drugs known as thiazolidinediones.

A new research pathway concerns the less known isoform PPAR $\delta$ ; it is involved in the initial adipocyte differentiation, but its precise role has not been yet elucidated. Some recent animal studies brought evidence that its pharmacological activation could lead to an increase of HDL-cholesterol levels of a great extent in animal models. In db/db insulin resistant mice, PPAR $\delta$  activation leads to an increase of HDL-cholesterol level and HDL/non-HDL-cholesterol ratio, without any relevant change in triglycerides level [23]. In insulin resistant rhesus monkeys, pharmacological activation of PPAR $\delta$  drastically increases HDL-cholesterol levels of about 50%, with concomitant reduction

of triglycerides, shift in LDL subclasses to large and buoyant particles, and decrease of insulin plasma level [24].

## Conclusion

Knowledge of the atherosclerosis formation process has greatly progressed in the past 30 years, due to major advances in the LDL receptor pathway, which also have led to the discovery and marketing of statins. But it is time now for (re)discovering the major anti-atherogenic role of reverse cholesterol transport from peripheral cells to the liver, involving HDL particles. However, full epidemiological evidence is lacking to state that HDL-cholesterol is an independent cardiovascular risk factor, especially due to its inverse correlation with triglycerides levels and its association with other risk factors part of the metabolic syndrome. New biochemical data are helpful to a better understanding of the initial step of reverse transport, by unravelling the role of recently discovered mutations of the ATP binding cassette transporter A1. Lastly, recent studies have focused on the modulation of HDL plasma levels by pathways involving nuclear receptor PPAR $\delta$ ; pharmacological activation of this nuclear receptor leads in animal studies to more favourable changes in HDL/LDL ratio than those provided by the normolipidemic drugs available to date.

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