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COMMENTARY

RAISING HIGH DENSITY LIPOPROTEIN CHOLESTEROL

THE BIOCHEMICAL PHARMACOLOGY OF REVERSE CHOLESTEROL TRANSPORT

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The predictive power of plasma density lipoprotein (HDL*) cholesterol concentration for the development of coronary heart disease (CHD) has now been demonstrated in four large studies in the U.S.A. (Framingham Heart Study, Lipid Research Clinics—Coronary Primary Prevention Trial [LRC-CPPT], LRC Follow-up Study and Multiple Risk Factor Intervention Trial), and similar results have been reported from prospective studies in Norway, Israel, West Germany, Britain and Finland [1]. The collective evidence indicates that the inverse relation of CHD risk to HDL cholesterol is continuous; is independent of the concentrations of very low density (VLDL) and low density (LDL) lipoproteins; is independent of non-lipid risk factors for CHD, such as cigarette smoking, blood pressure, glucose intolerance and obesity; applies to CHD mortality as well as morbidity; is present in both middle-aged and elderly subjects; applies to both sexes; and applies to subjects with, as well as those without, a previous history of myocardial ischemia [1]. In most cohorts the association of CHD with HDL cholesterol has been at least as strong as that with LDL cholesterol. In North American men and women, CHD risk increases on average by 2–3% for each 1 mg/dL decrease in HDL cholesterol (and by 1% for each 1 mg/dL increase in LDL cholesterol) [2]. Angiographic studies and examination of vessels at autopsy have shown that these associations with clinical CHD reflect an underlying association with coronary atherosclerosis [3].

Genetic and pharmacologic studies have implicated a causal effect of HDL metabolism on atherogenesis. In mice variations at gene loci affecting HDL concentration have been found to have major effects on diet-induced atherosclerosis [4]. In cholesterol-fed rabbits atherogenesis was strongly inhibited by BRL26314, a drug which raises HDL cholesterol without significantly affecting levels of other lipoproteins [5]. In humans with familial normotriglyceridemic hypoalphalipoproteinemia (defined as an HDL cholesterol <5th centile), a low synthesis rate of the most abundant HDL protein, apo AI, results in a low plasma HDL cholesterol level and premature CHD [6]. More rarely, complete absence of normal HDL due to major deletions or rearrangements of the apo AI–AIV–CIII gene cluster, leads to severe coronary atherosclerosis at an early age [1, 7, 8]. In the LRC-CPPT, designed to study the effect on CHD incidence of lowering LDL concentration in hypercholesterolemic men, treatment with cholestyramine also raised HDL cholesterol. Although the mean increase was small (3%), proportional hazards analysis showed that this contributed significantly to the reduction of CHD incidence [9]. In the NHLBI Type II Intervention Study, the rise in HDL cholesterol produced by cholestyramine was shown to contribute to a reduced rate of progression of angiographically defined coronary atherosclerosis [10]. In the LRC Follow-up Study, a low cardiovascular disease mortality in post-menopausal women taking estrogens was largely attributable to the associated increase in HDL cholesterol [11]. Most striking were the results of the Helsinki Heart Study, which tested the efficacy in CHD prevention of treating hypercholesterolemic men with gemfibrozil, a drug which can produce a major increase in HDL cholesterol [12]. On the average, gemfibrozil raised HDL cholesterol by 10%, and reduced LDL cholesterol by 9%. Overall the 5 year-incidence of CHD events was reduced by 34% ($P < 0.01$). In a proportional hazards model, the changes in both HDL and LDL contributed to the beneficial outcome, but the rise in HDL cholesterol was the more important. The men who developed the greatest increases in HDL cholesterol, and who accordingly showed the greatest reduction in CHD (47%), were those whose concentrations were initially below 46 mg/dL (a figure which corresponds to the 50th centile in middle-aged American men).

Such results have established beyond reasonable doubt that the increased susceptibility to CHD experienced by subjects with low HDL cholesterol levels reflects a causal effect of a component of HDL metabolism on atherogenesis, and that this can be

* Abbreviations: HDL, high density lipoprotein; CHD, coronary heart disease; LRC-CPPT, Lipid Research Clinics—Coronary Primary Prevention Trial; VLDL, very low density lipoprotein; LDL, low density lipoprotein; UC, unesterified cholesterol; CE, cholesteryl ester; ACAT, acylCoA:cholesterol acyltransferase; LPL, lipoprotein lipase; LCAT, lecithin:cholesterol acyltransferase; CETP, CE transfer protein; HL, hepatic lipase; and FCR, fractional catabolic rate.

modified pharmacologically to reduce the incidence of disease in hypercholesterolemic men beyond that attainable by reduction of LDL concentration alone. Can we conclude, therefore, that any drug-induced change in HDL cholesterol will have a reciprocal effect on CHD? The answer to this question is almost certainly negative. Several lines of evidence indicate that the metabolic mechanism which underlies a change in HDL cholesterol is probably of critical importance. First, HDL is not a single species of particle, but a complex family of particles which differ in lipid composition, protein composition, size and density, and which have been shown to have different functions in cholesterol transport [13]. Plasma HDL cholesterol as measured in clinical and epidemiologic studies represents the total sum of unesterified and esterified cholesterol in these subclasses, the relative proportions of which vary under different metabolic conditions. From the theoretical standpoint alone, therefore, it would be surprising if the relationship between plasma HDL cholesterol and CHD were simple. Clinical studies have strengthened the notion that the effects on tissue cholesterol pools of changes in HDL are determined by the causative metabolic events [14]. Second, in contrast to all other communities so far examined, in the U.S.S.R. CHD incidence is apparently unrelated to HDL cholesterol [15]. Presumably, therefore, the environmental and/or genetic factors responsible for most of the variance in HDL cholesterol in that society differ from those elsewhere, and do not influence atherogenesis to the same degree. Third, although, as already mentioned, several inherited disorders of apo AI synthesis are known to accelerate atherogenesis, some other forms of familial hypoalphalipoproteinemia do not have such an impact on CHD [16, 17].

Recent research has thrown light on the roles of different HDL subclasses in the three anatomical stages (extravascular, intravascular and intrahepatic) of reverse cholesterol transport [13, 18]. Intracellular cholesterol exists in unesterified (UC) and esterified (CE) forms. There is a continuous cycle of esterification and hydrolysis, catalyzed by cytoplasmic acylCoA:cholesterol acyltransferase (ACAT) and cholesterol esterase. Cholesterol transport from most cells begins with the movement of UC from the plasma membrane to acceptor particles in the interstitial fluid. This probably occurs by desorption–adsorption, but may be facilitated by the translocation of UC from intracellular pools to the membrane in response to the binding of apo AI-containing particles to cell surface receptors [19].

The principal acceptors of UC in the extracellular matrix are probably members of a minor subclass of HDL, characterized by pre-beta mobility on agarose gel electrophoresis (in contrast to the alpha mobility typical of the majority of plasma HDL). Such particles are smaller than other HDL (diameter, <7 nm vs 7.5 to 15 nm), being composed of only one or two molecules of apo AI (vs 3 or 4 in most HDL) in combination with phospholipid and UC, and being essentially devoid of core CEs [20, 21]. Such physicochemical properties, coupled with their prominence in human tissue fluids (including that of artery) [22, 23], make pre-beta HDL the strongest candidates of all lipoproteins for the role of cholesterol acceptors in the extracellular matrix of the arterial wall. This expectation has been supported by studies of the efflux of radiolabeled cholesterol from cultured fibroblasts [20], and by the changes induced in dog peripheral lymph lipoproteins by dietary cholesterol [24]. The mechanisms by which such particles are produced are uncertain, but local production in the extracellular matrix by the action of phospholipases on larger HDL filtered from plasma, by fusion of filtered HDL with discoidal surface remnants released from triglyceride-rich lipoproteins by endothelial-bound lipoprotein lipase (LPL), and/or by fusion of HDL with apo E-containing discoidal lipoproteins secreted by peripheral cells are all possibilities [13, 18, 25]. Similar particles may also be secreted by the liver [26].

After entry of these particles into blood via peripheral lymph, the cell-derived UC is esterified by lecithin:cholesterol acyltransferase (LCAT), an enzyme that is physically associated with another minor pre-beta migrating HDL subclass, and of which apo AI is the physiologic cofactor [27]. Some of the CE molecules so formed enter the cores of plasma HDL leading to a progressive increase in their size and changes in apoprotein composition. This ultimately includes the acquisition of apo E [28]. Other CE molecules are transferred to apo B-containing lipoproteins via the CE transfer protein (CETP) [29]. This occurs in exchange for triglyceride, which after entering HDL is hydrolyzed by hepatic endothelial lipase (HL). In the final stage CEs are delivered to hepatocytes via several routes: receptor-mediated uptake of apo E-containing HDL; direct transfer from HDL by a mechanism not requiring particle uptake; and receptor-mediated uptake of apo B-containing particles (including LDL) derived from the catabolism of triglyceride-rich lipoproteins [13]. Studies of the kinetics of plasma cholesterol have shown that, in addition, UC is transferred from HDL to hepatocytes, presumably driven by the gradient of chemical potential created by bile acid synthesis, by secretion of UC into bile, and possibly also by the hydrolysis of HDL phospholipid by HL [30].

In view of the importance in atherogenesis of cholesterol metabolism in arterial macrophages and vascular smooth muscle cells [31], it is likely that the effect of HDL metabolism on atherosclerosis reflects its function in reverse cholesterol transport. This is supported by the evidence that the cholesterol content of healthy vascular tissue is a negative function of plasma HDL cholesterol in humans [32–34]. Important questions concern the identity of the rate-limiting step in the transport of cholesterol from arterial tissue, the metabolic processes which control that step, and the extent to which their functional activity is reflected in the plasma total HDL cholesterol concentration.

Several metabolic determinants of HDL cholesterol have been identified in healthy humans. Concentration is related directly to the production rate of apo AI (synthesized in humans only in liver and intestine) [35]. This is particularly so in the absence of hypertriglyceridemia [6], and presumably reflects

the effect of apo AI production on the secretion of nascent HDL particles into the circulation. Of the two major density subclasses of plasma HDL, variation in apo AI synthesis has its greater effect on the more dense, relatively cholesterol-poor, HDL_3 [36].

Plasma HDL cholesterol is also positively correlated with the activities of LPL in skeletal muscle and adipose tissue, and accordingly with the fractional catabolic rates (FCRs) of their substrate lipoproteins: chylomicrons and VLDL [37, 38]. There are probably at least two reasons for this. First, lipolysis of triglyceride-rich lipoproteins results in the transfer of surface material (including UC and phospholipid) to HDL. Second, triglyceride-rich lipoproteins are important acceptors of LCAT-derived CEs, transferred from HDL by CETP. The net effect of LPL and LCAT is to convert the smaller dense HDL_3 subclass into the larger, less dense and more CE-rich HDL_2. HDL cholesterol is additionally, albeit weakly, a positive function of plasma LCAT concentration, reflecting its effect on the rate of esterification of UC in HDL [39].

Other metabolic factors have a negative impact on HDL cholesterol. These include the FCR of apo AI [6, 35], the activity of CETP [40], and the activity of HL [41]. The FCR of apo AI reflects in part the rate of clearance of HDL particles from the circulation, which probably occurs mostly in the liver via receptors for apo E [42]. High FCRs of apo AI occur commonly in subjects with hypertriglyceridemia [6, 37], and this has been shown to reflect an effect of the lipolysis of VLDL on apo AI catabolism [35, 37, 38], the possible mechanism of which will be proposed later. In contrast, the rate of synthesis of VLDL appears to have little or no impact on apo AI metabolism (or HDL cholesterol) [37, 38].

The negative correlation between HDL cholesterol and CETP activity can be explained by the function of the latter in the transport of CE from HDL to other lipoproteins [29]. The negative correlation with HL activity may also be related to this process. As already indicated, this enzyme hydrolyzes triglyceride that has been transferred to HDL from chylomicrons and VLDL in exchange for CEs [43]. Thus, the concerted actions of CETP and HL serve to slow the accumulation of core lipids in HDL particles [44]. The negative association of HDL cholesterol with HL may also reflect the putative function of HL in promoting the release of UC from HDL to hepatocytes by hydrolyzing HDL surface phospholipid. However, as familial absence of HL is characterized by HDL particles with a high triglyceride/CE ratio, but relatively normal UC/phospholipid ratio [45], the effect on core lipid composition appears to be predominant.

In attempting to identify which of these determinants of HDL cholesterol concentration also have significant impacts on reverse cholesterol transport, and are therefore appropriate targets for pharmacologic intervention, we need to consider them in relation to the mechanism by which cholesterol is removed from arterial cells, and the insights that we have into the nature of the association between HDL metabolism and risk of CHD. The latter may be gleaned from several sources: the disturbance of HDL composition that is characteristic of CHD patients; differences in lipoprotein metabolism between CHD cases and controls; HDL metabolism and atherogenesis in familial disorders affecting HDL cholesterol concentration; the effects on HDL metabolism of agents that have been shown to reduce CHD in humans via an increase in HDL cholesterol; and studies of experimental atherosclerosis in animals.

The rate of efflux of UC from cultured cells has been shown to be influenced, under different conditions, by the composition of the HDL particles in the culture medium (the lipid-poor apo AI-rich subclasses, especially pre-beta HDL, being the most effective); the concentration of HDL particles; the extent of their binding to the plasma membranes of the cells; and the rate of esterification of UC in HDL by LCAT [13]. The latter, in turn, is determined *in vitro* partly by the mass of LCAT present, and partly by the rate of transfer of CEs from HDL to triglyceride-rich lipoproteins by the CETP, thereby releasing LCAT from end-product inhibition [27, 46]. Such observations *in vitro* may be misleading, however, and it cannot be assumed that any one of these factors is rate-limiting *in vivo* in the extracellular matrix of the artery wall.

For several reasons, it seems unlikely that cholesterol transport in arterial tissue is greatly influenced by physiologic variations in plasma LCAT activity. First, in contrast to plasma, tissue fluids contain little LCAT enzyme [47]. Accordingly, little esterification of cell-derived UC occurs in HDL particles until after they have entered the blood circulation via peripheral lymph [48]. Second, familial LCAT absence leads to only a modest increase in atherosclerosis [49]. In this condition, cholesterol accumulation occurs principally in erythrocytes, spleen, bone marrow and renal glomeruli, suggesting that LCAT is more important for the removal of UC from cells exposed to plasma than from those exposed to tissue fluid [18]. Third, no significant correlation was found between plasma LCAT activity and the severity of angiographically defined coronary artery disease [50].

It is also unlikely that the efflux of UC from cells is enhanced by retention of CEs in HDL, secondary to low CETP activity. On the contrary, by increasing the mean diameter of HDL particles, and thereby reducing their volume of distribution, it may even have the opposite effect. However, by reducing the transfer of CEs to triglyceride-rich lipoproteins, and hence eventually to LDL, low CETP activity would be expected to increase the direct transfer of CEs from HDL to hepatocytes, and to reduce the recycling of cholesterol back to other cells, the overall effect being to increase net reverse cholesterol transport from such tissues. This may explain why certain species in which high HDL cholesterol levels result from deficiency of CETP activity (e.g. dogs and rats) are also resistant to atherogenesis [51]. Familial deficiency of CETP activity in humans leads to very high plasma HDL cholesterol concentrations, due to the accumulation of CE-rich HDL [52]. No history or clinical evidence of CHD was found in a large kindred with this condition [52]. Owing to its rarity, however, it is not yet certain that it confers protection against atherogenesis.

In the present state of knowledge, the effects on reverse cholesterol transport and atherogenesis in humans of increasing HDL by inhibiting HL activity cannot be predicted. Both high and low activities of HL have been reported in post-heparin plasma from patients with CHD [53, 54]. Familial deficiency of the enzyme in humans has been reported to be associated with a high prevalence of CHD [45]. On the other hand, stimulation of HL activity in non-human primates by administration of a progestin did not increase atherosclerosis in spite of a reduction of HDL cholesterol [55].

In contrast to the foregoing, the effect on atherogenesis of alteration in apo AI synthesis seems more clear. It has already been mentioned that inherited disorders in which apo AI synthesis is diminished or absent appear to be invariably associated with premature CHD [1, 6–8]. Newly synthesized apo AI is secreted by liver cells as a component of both discoidal and small spherical particles [56]. The latter may include pre-beta HDL, to which reference has already been made as the probable physiologic acceptor of UC in arterial tissue. In non-human primates, species differences in susceptibility to diet-induced atherosclerosis have been found to be associated with reciprocal differences in apo AI mRNA abundance in liver and intestine, and in nascent HDL secretion by perfused livers [57]. Repeated intravenous injections of a plasma fraction rich in apo AI have been shown to protect cholesterol-fed rabbits from atherosclerosis [58]. Although more epidemiologic data would be of value, apo AI concentration probably predicts risk of CHD in humans [59]. Two drugs that have been shown in prospective clinical trials to reduce CHD incidence and/or coronary atherogenesis in part by raising HDL cholesterol also increase apo AI synthesis [9, 10, 12]. In the case of cholestyramine, this is the only known effect on HDL metabolism [60]. In the case of gemfibrozil, an increase in LPL activity also contributes to the rise in HDL cholesterol [61, 62]. There is evidence that CHD is also reduced by long-term medication with two other classes of agent that probably stimulate apo AI synthesis: enzyme-inducing anticonvulsants (e.g. phenytoin) [63] and, in post-menopausal women, estrogens (which also suppress HL activity) [11, 64]. This collective evidence points strongly to a major impact of apo AI synthesis rate on atherogenesis.

The significance of LPL activity and triglyceride-rich lipoprotein catabolism in the present context is particularly intriguing. Epidemiologically, HDL cholesterol is negatively correlated with plasma triglycerides. In CHD victims, low HDL cholesterol and raised triglyceride levels frequently coexist [59]. On the other hand, hypertriglyceridemia itself is, at most, only a weak risk factor for CHD in the absence of a low HDL cholesterol [65]. As it is the FCR, and not the synthetic rate, of VLDL that influences HDL cholesterol concentration, these data raise the possibility that reverse cholesterol transport through HDL may be stimulated by the lipolysis of chylomicrons and VLDL. This would be compatible with the evidence that the severity of angiographically defined coronary artery disease is negatively correlated with post-heparin LPL activity [50]; that CHD victims have lower rates of heparin-induced lipolysis *in vivo* than healthy subjects [66, 67]; and that the composition of HDL in CHD victims shows similarities to that in healthy subjects with low LPL activities [59]. Consideration of the cholesterol contents of chylomicrons and their core remnants also lends support to this possibility [68]. One possible mechanism for such an effect is provided by the evidence that heparin-induced lipolysis of chylomicrons in humans increases the plasma concentration of pre-beta HDL [69], probably reflecting the formation of small apo AI–phospholipid complexes consequent upon the transfer of surface phospholipid from the chylomicrons to HDL [70]. As vascular endothelium is rich in LPL, this process may increase the concentration of cholesterol acceptors in adjacent tissue fluids. Where the number of such particles is rate-limiting in the efflux of UC from cells, as is likely in arterial tissue [18], this would result in local enhancement of reverse cholesterol transport.

It may be pertinent that two drugs which stimulate LPL activity, clofibrate [71] and gemfibrozil [61, 62], have been shown to reduce CHD incidence in hypercholesterolemic men. In the WHO Cooperative Trial, treatment with clofibrate diminished the 5-year incidence of CHD by 20% [72]. Unfortunately, however, the extent to which this outcome was related to changes in HDL cholesterol was not examined. As already discussed, in the Helsinki Heart Study, gemfibrozil reduced CHD by a mechanism that was clearly related to the rise in HDL cholesterol, treatment being particularly effective in subjects with initially high triglyceride and low HDL cholesterol levels [12].

High LPL activity and a high VLDL FCR are associated not only with a high HDL cholesterol, but also with a lesser increase in plasma total apo AI concentration, secondary to a decrease in the FCR of the apoprotein [37]. The opposite picture is seen in hypertriglyceridemic hypoalphalipoproteinemia [6, 37]. These observations could be explained by the foregoing scheme, according to which lipolysis would increase the recycling of apo AI between the majority of plasma HDL and the pre-beta HDL of tissue fluids, leading to reduced catabolism of apo AI by hepatocytes as a component of the apo-E containing plasma HDL particles. The FCR of mature plasma HDL particles may have little impact on reverse cholesterol transport, as two inherited disorders characterized by low HDL cholesterol levels and high apo AI FCRs (Tangier disease and apo AI Milano) appear to be associated with little or no increase in CHD [16, 17, 73]. There have been no measurements of the concentrations of apo AI-containing particles in the tissue fluids of patients with these conditions. As nicotinic acid appears to increase HDL cholesterol by slowing HDL catabolism [74, 75], the effect of this drug on reverse cholesterol transport is uncertain.*

* *Note added in proof:* Since this article was completed, evidence has been presented that the HDL-raising activity of nicotinic acid (given in combination with colestipol) slows the progression of angiographically quantified coronary atherosclerosis in men with elevated levels of apolipoprotein B (Brown BG, Lin JT, Schaefer SM, Kaplan CA, Dodge HT and Albers JJ, Niacin or lovastatin, combined with colestipol, regress coronary atherosclerosis and prevent clinical events in men with elevated apolipoprotein B. *Circulation* **80**: II-266, 1989).

Thus, of the known metabolic determinants of plasma HDL cholesterol concentration in humans, it seems likely that stimulation of apo Al synthesis will be an effective mode of action for future drugs aimed at stimulating reverse cholesterol transport. Accordingly, the possibility that the increase in HDL produced by hepatic microsomal enzyme-inducers may inhibit atherogenesis is worthy of further investigation. The increases in apo Al synthesis produced by gemfibrozil and, to a lesser extent, by cholestyramine are presumed to occur in hepatocytes and/or intestinal epithelial cells. Although expression of the apo Al gene in other cell types has been demonstrated in the human fetus [76], postnatal expression in extra-splanchnic tissues is known to occur only in birds, in whose skeletal muscle it plays an important role in the excretion of excess cholesterol [77]. The possibility of increasing cholesterol mobilization from human macrophages and vascular smooth muscles by re-activation of apo Al genes in those cells has not been explored.

At present there is insufficient information to enable the consequences of increasing HDL cholesterol by mechanisms other than stimulation of apo Al synthesis to be predicted with confidence. This is particularly true of modifications of LCAT concentration, HL activity and HDL catabolism. The possibility that the lipolysis of triglyceride-rich lipoproteins in capillaries may increase the production of small apo Al-containing cholesterol-acceptor particles from HDL in peripheral tissues is one avenue that is worth pursuing. Drugs which increase LPL activity are already available which could provide the basis for developing more potent agents. The possibility that inhibition of CETP activity in plasma may also have a favourable effect on net reverse cholesterol transport also deserves priority.

With the exception of LPL activity, the metabolic factors considered so far have been either intra-splanchnic (apo Al synthesis, HDL catabolism, HL activity) or intravascular (LCAT, CETP), and as such may have widespread effects on cholesterol transport from many tissues. Much neglected in metabolic studies have been the potential roles of intracellular and extracellular–extravascular factors in regulating cholesterol transport within peripheral tissues. As the cholesterol requirements of different cell types are likely to vary, it is possible that local factors have an important homeostatic function. As cholesterol can leave cells only as UC, the activities of neutral cholesterol esterase and ACAT may have very significant effects. Peripheral synthesis of apo E may also be important. Like several other cell types [78], macrophages synthesize and secrete discoidal particles containing phospholipid and apo E [79]. A homeostatic function of this process in regulating colesterol balance is supported by the fact that the rate of synthesis of apo E in tissues increases with increasing cholesterol content [79, 80]. If such discs are able to fuse in interstitial fluid with filtered plasma HDL to release small apo Al-containing particles, in a manner analogous to processes observed in artificial systems *in vitro* [26] and already discussed in relation to the fusion of the surface remnants of triglyceride-rich lipoproteins with HDL, the rate of production of apo E in peripheral cells may have a significant local effect on reverse cholesterol transport. Alternatively, or additionally, UC may be excreted from cells as a component of the apo E discs themselves. Stimulation of peripheral apo E synthesis may represent one mode of action of probucol, which has been reported to increase apo E mRNA levels in rabbit spleen, to reduce the mean particle size of plasma HDL in humans, and to increase the mobilization of cholesterol from cultured fibroblasts in the presence of HDL [81]. As increased fusion of apo E discs with plasma HDL may also enhance the clearance of CE-rich HDL from plasma via hepatic apo E receptors, such a mode of action is compatible with the reduction of plasma HDL colesterol which occurs when hypercholesterolemic patients are given probucol, and the correlation observed in such patients between the decrease in HDL cholesterol and that in tendon xanthoma thickness [81]. This illustrates the important point that it is theoretically possible for a drug to increase reverse cholesterol transport without this being accompanied by a rise in plasma total HDL cholesterol; and likewise that a change in HDL cholesterol need not invariably reflect a parallel change in cholesterol flux from tissues through HDL.

The increase in the cholesterol content of cultured macrophages that occurs when these cells are incubated with acetylated LDL has been shown also to increase the number of binding sites for apo E-free HDL particles in the plasma membrane [19, 82]. Similar results were obtained when cultured vascular smooth muscle cells were incubated with LDL [83]. Other data suggest that the binding of HDL to cell surfaces facilitates the transfer of UC to HDL, either by stimulating the movement of UC from intracellular pools to the plasma membrane [19], or by mediating the endocytosis of HDL [82]. Thus, the possibility that reverse cholesterol transport from developing atherosclerotic lesions may be augmented by pharmacologic stimulation of the synthesis of HDL receptors in vascular cells warrants consideration.

As a general strategy, selective stimulation of reverse cholesterol transport in the arterial wall, without effects in other tissues, might represent the ideal approach, except when there is evidence of cholesterol overload elsewhere. Drugs which have such a mechanism of action may have little or no effect on plasma HDL cholesterol, because of the small overall effect on total cholesterol transport through plasma. For the prevention or reversal of early atherosclerotic lesions, when most cholesterol is intracellular, a specific effect within macrophages and/or smooth muscle cells has attractions. In more advanced lesions, when much cholesterol is extracellular, a local action that increases the number of cholesterol acceptors in the extracellular matrix of vascular tissue might be more appropriate. A dual action in both splanchnic and arterial tissues may represent the optimum.

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