The origin and properties of free cholesterol potential gradients in plasma, and their relation to atherogenesis

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The aqueous solubilities of most lipids in lipoproteins are so low that their transfer between lipoproteins and cell membranes depends on random (collision) or specific (receptor-mediated) physical interaction between donor and acceptor interfaces, or the activity of transfer proteins which serve to increase the solubility of lipids in the aqueous phase (1). However, the solubility of cholesterol, although small (critical micelle concentration (CMC) $\sim 3 \times 10^{-8}$ M) (2), is sufficient for simple Fick diffusion to account for a major part of the observed flux of cholesterol between different lipoprotein particles, or between lipoproteins and cell membranes (**Table 1**). A similar identity has been found for the activation energies of cholesterol transfer between cell membranes and lipoproteins, and between synthetic lipid vesicles. When bidirectional flux rates are equal (for example, between cell membranes and unfractionated native normal plasma, when plasma cholesterol content is not perturbed by esterification), there is no spontaneous net transport of cholesterol into or out of the cell (6). When lecithin:cholesterol acyltransferase (LCAT) in plasma is active, and by esterification reduces the effective concentration (i.e., chemical potential) of cholesterol in the plasma in the face of an unchanged efflux of cholesterol from the adjacent cell membranes (3, 6), its effect overall is to catalyze a compensatory net transport of free cholesterol from the cell to the plasma medium. This is compensated in vivo by cholesterogenesis within the affected cell, and by the entry of new lipoprotein cholesterol from those cells that secrete lipoproteins into plasma, which reduces or nullifies the induced cell-to-plasma chemical potential gradient of cholesterol.

From the same principles, it follows that if the chemical potential of cholesterol in a plasma lipoprotein species is higher than that in the other lipoproteins or in the cell membranes, cholesterol will flow down its gradient to such cells or lipoproteins until their free cholesterol content reaches its new steady state. If the acceptor lipoproteins in plasma (by the activity of LCAT) or cells (by the action of acyl CoA:cholesterol acyltransferase, ACAT) remove the cholesterol transferred from the equilibrium by the synthesis of esterified cholesterol, the flow of cholesterol will continue indefinitely, as long as the supply of cholesterol-rich donor lipoproteins continues. This process will also be governed by the thermodynamic parameters shown in Table 1.

Two arguments in particular can be advanced against a significant role for thermodynamic (i.e., non-receptor) processes in cholesterol transport between plasma lipoproteins and cells, and the regulation of cellular cholesterol metabolism, particularly in relation to the cholesterol accumulation that occurs in atherosclerosis.

i) In the plasma of many of those at risk for atherosclerosis, e.g., hyperbetalipoproteinemics (7), the composition, and, in particular, the cholesterol content, of the lipoproteins is normal.

ii) Only that lipoprotein cholesterol interiorized by receptor mechanisms is effective in mediating changes in cellular cholesterol metabolism (8).

Neither argument, however, is likely to be valid.

Evidence for cholesterol potential gradients in pathological human plasma

In several groups of human subjects at increased risk for atherosclerosis, there is a consistent abnormal pattern of plasma cholesterol metabolism (9–11) (**Table 2**). In particular, there is an inhibition in the transfer of the cholesteryl esters generated by the LCAT reaction to very low and low density lipoproteins (VLDL and LDL), and an inhibition, or even reversal, of net transport of free cholesterol from cell membranes to plasma, in the presence of an undiminished rate of cholesterol esterification by plasma LCAT. In the face of an unchanged

Abbreviations: VLDL, LDL, and HDL, very low density, low density, and high density lipoproteins, respectively; LCAT, lecithin:cholesterol acyltransferase; ACAT, acyl CoA:cholesterol acyltransferase.

TABLE 1. Thermodynamic constants for free cholesterol unidirectional transfer in cell membranes and model systems

Diffusion	(J, moles·cm^{-2}·sec^{-1})	Activation energy	(ΔG, kcal·mole^{-1})
J_{theor}	2×10^{-15}	ΔG_{theor}	14.0
$J_{cells-plasma}$	1×10^{-14}	$\Delta G_{cells-plasma}$	11.0
$J_{vesicle-vesicle}$	4×10^{-15}	$\Delta G_{vesicle-vesicle}$	8–16

J_{theor} is the diffusion rate calculated from Fick's law ($J = D\Delta c/\chi$) where D is the lateral diffusion constant of free cholesterol (8×10^{-5} cm^2 sec^{-1}), Δc is the critical micelle concentration of free cholesterol (3×10^{-11} moles cm^{-3}), and χ is the thickness of nonexchangeable surface water. $J_{cells-plasma}$ was determined for normal fibroblasts in normolipidemic native human plasma (ref. 3); $J_{vesicle-vesicle}$ is for synthetic lecithin-cholesterol liposomes (ref. 1). ΔG_{theor} was determined as $\Delta G \sim RT\log_e x_w f_w$ where x_w is the aqueous mole fraction and f_w is the activity coefficient (ref. 4) modified as described (ref. 5); $\Delta G_{cells-plasma}$ is for normal fibroblasts in native plasma (ref. 3) and $\Delta G_{vesicle-vesicle}$ is for lecithin-cholesterol liposomes (ref. 1).

unidirectional flux of cholesterol from cell membranes to plasma (9, 10), this indicates a greatly increased (up to 75-fold, ref. 9) influx of free cholesterol from plasma to cells.

Composition of VLDL and LDL in atherogenic hyperlipidemia

Analysis of the mass ratio of free cholesterol and phospholipid (the major determinant of lipoprotein surface properties) (12) by different laboratories has shown a consistently increased free cholesterol content in the VLDL and LDL from the plasma of those groups with abnormal plasma cholesterol transport (**Table 3**) (13–20). The free cholesterol content of these lipoproteins is in each case sufficient to nearly or completely saturate with cholesterol the lipoprotein surface film, as determined by triangular coordinates (11, 12). Furthermore, as shown in Table 2, in all of these groups there is a spontaneous gradient of free cholesterol between VLDL + LDL and HDL. When LCAT activity is inhibited, free cholesterol is spontaneously transported from VLDL + LDL to HDL (11). These data indicate that contrary to the situation in normal plasma (6), the lipoprotein classes in these pathological plasma samples are not in thermodynamic equilibrium. The effective cholesterol concentration of VLDL (secreted by the liver) and its product LDL is significantly increased above that of HDL. Accordingly, plasma from all the affected groups (Table 2) shows both of the prerequisites for an effective thermodynamic transfer of free cholesterol to cell membranes: the presence of a plasma lipoprotein population containing an increased concentration of cholesterol, and the presence of a potential gradient between such lipoproteins and the cell membrane.

Uptake of free cholesterol across cell membranes.

When plasma lipoproteins are enriched synthetically with free cholesterol, and these lipoproteins are incubated with one of several lines of vascular or other cells, cholesterol is taken up into the cells and cholesterol and its esters accumulate (21). Unmodified normal lipoproteins are without effect on cell cholesterol under the same conditions. Balance studies indicate that almost all of the cholesterol interiorized from cholesterol-enriched lipoproteins is in the unesterified form (22). However, once within the cell, this cholesterol enters fully into the intracellular metabolic reactions of cholesterol in the same manner as did cholesterol interiorized via receptor pathways (7). These data indicate that free cholesterol interiorized by transfer from cholesterol-rich lipoproteins across cell membranes can be esterified and stored.

Uptake of cholesterol from native plasma across cell membranes.

It has been repeatedly shown that native plasma from cholesterol-fed primates and other animal models of

TABLE 2. Abnormalities of plasma cholesterol metabolism in atherogenic hyperlipidemias

	Net Transport	Esterification	Transfer	Cholesterol Potential Gradient
Noninsulin-dependent diabetes mellitus	Reduced/reversed	Normal	Reduced	VLDL, LDL → HDL
Hyperbetalipoproteinemia	Reduced/reversed	Normal	Reduced	VLDL, LDL → HDL
Dysbetalipoproteinemia	Reduced	Increased	Reduced	VLDL, LDL → HDL
Hyper TG with PVD/CVD	Reduced/reversed	Normal	Reduced	VLDL, LDL → HDL

Rates are expressed relative to those in normolipidemic plasma. Diabetes, noninsulin-dependent diabetes mellitus (ref. 10); Hyperbeta, familial hyperbetalipoproteinemia; dysbeta, dysbetalipoproteinemia, hyper TG with PVD/CVD, hypertriglyceridemia with peripheral or coronary vascular disease. Methods for determination of individual metabolic parameters, refs. 10 and 11.

TABLE 3. Free cholesterol/phospholipid mass ratio of plasma VLDL and LDL in atherogenic hyperlipidemias

Normolipidemic Controls	Dysbeta-lipopro-teinemia	Hyper-lipopro-teinemia	Noninsulin-Dependent Diabetes	Refer-ence
VLDL				
0.37	0.45			12
0.28–0.35	0.44–0.70			13
0.29	0.43			14
0.43	0.64			15
0.38			0.50	11
LDL				
ND	0.52			14
0.39		0.54		16
0.40		0.48		17
0.43		0.48		18
0.36		0.52		19
0.41			0.50	11

Values were calculated where necessary from the individual values of free cholesterol and total phospholipid mass in the original compositional data; ND, not determined.

experimental atherosclerosis is enriched in free cholesterol and promotes the accumulation of cholesterol and its esters in vascular and other cells (23–25). Normolipemic plasma did not result in the accumulation of cellular cholesterol. This difference is not due simply to the increased concentration of cholesterol in the hyperlipidemic plasma, since the same result is obtained when plasma from normal and cholesterol-fed animals is diluted to equivalent medium cholesterol concentration. Even when LDL receptors are maximally up-regulated by lipoprotein-deficient serum (26), the accumulation of cholesterol from hypercholesterolemic LDL within the cells is greater than can be accounted for by endocytosis, since in these experiments:

i) the uptake of cholesterol exceeded the proportionate uptake of LDL protein; this is unlikely to result from preferential uptake of a lipid-rich LDL subfraction, since a particle with the twofold increased cholesterol/protein ratio that would be required would not have the density characteristics of LDL.

ii) the uptake of protein from hyperlipidemic LDL by the receptors was actually decreased relative to the uptake of normal LDL, although only the former catalyzed the accumulation of cellular cholesterol; and

iii) cholesterol was accumulated from hyperlipidemic LDL even in receptor-deficient cells.

Similar studies have also been carried out with hyperlipemic human plasma, under conditions where the transfer of free and esterified cholesterol across cell membranes could be separately determined (9, 10). The following data indicate that such uptake is mediated by a nonreceptor mechanism.

i) It is unchanged in receptor-deficient cells.

ii) Cholesterol is often transferred mostly or exclusively in the unesterified form (e.g., in diabetics, and those with hyperbetalipoproteinemia).

iii) The uptake of free cholesterol is inversely proportional to the net transport of cholesterol from cell membranes to plasma (**Fig. 1**), i.e., directly proportional to the influx of cholesterol from plasma to cells in the face of unchanged unidirectional flux from cells to plasma (9, 10).

Finally, it was shown directly by balance studies that free cholesterol taken across the cell membrane was esterified and stored intracellularly (9).

These data indicate clearly that it is the free cholesterol potential gradient in the plasma of those at increased risk for atherosclerosis (Table 2) that drives the transport of free cholesterol into cells. It has been shown that, in diabetes, it is the increased free cholesterol concentration of VLDL and LDL that also inhibits the transfer of

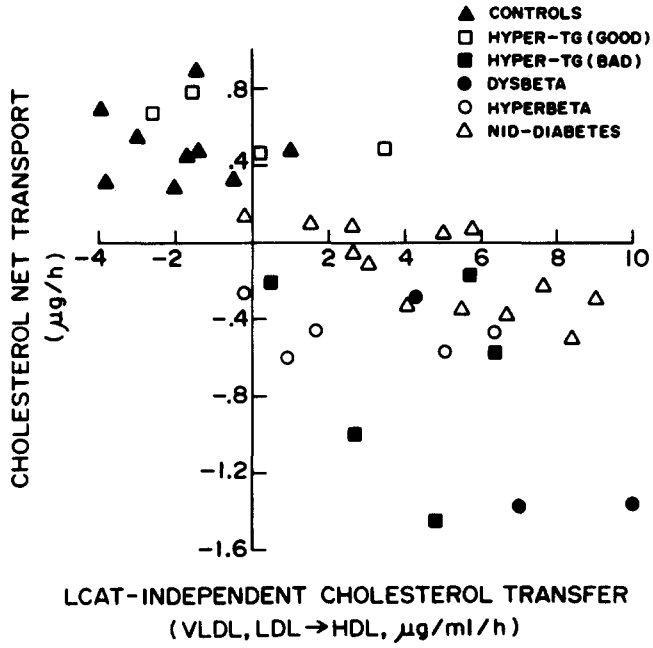

Fig. 1. The relationship between VLDL, LDL → HDL chemical potential gradient and cholesterol net transport. The chemical potential gradient of free cholesterol was determined in native plasma in which LCAT had been inhibited (11). VLDL and LDL were removed from plasma before and after incubation (60 min, 37°C) by precipitation with dextran sulfate–$MgCl_2$, and the increase in supernatant (HDL) free cholesterol was determined fluorimetrically (6). Cholesterol net transport was determined from the difference in the rate of LCAT-mediated decrease of plasma free cholesterol during incubation (60 min, 37°C) in the presence and absence of normal human fibroblasts (6, 9). Human subjects are those from references 9–11. Hyper-TG (good), hypertriglyceridemia without symptoms of vascular disease; hyper-TG (bad), hypertriglyceridemia with vascular disease; dysbeta, dysbetalipoproteinemia; hyperbeta, hyperbetalipoproteinemia; NID diabetes, noninsulin-dependent diabetes.

LCAT-derived cholesteryl esters from HDL to these acceptor lipoproteins. The consistent inverse relationship between cholesteryl ester transfer rate and the magnitude of the VLDL, LDL → HDL free potential gradient (**Fig. 2**) suggest that the same factor mediates both major abnormalities of plasma cholesterol metabolism in the study populations.

Significance of plasma-to-cell cholesterol gradients in vivo

Thermodynamic gradients of the kind shown experimentally in human and animal hyperlipidemias have properties that make them attractive candidates to explain the accumulation of cholesterol and its esters in atherosclerosis.

i) as purely physical forces, they are not subject to the down-regulation that limits the uptake of cholesterol via the major receptor pathways (26).

ii) They involve a nonsaturable pathway, so that risk would increase without limit with increasing plasma cholesterol content; and the greatest rates of influx would be adjacent to the highest concentrations of lipoprotein cholesterol, i.e., in the vascular bed.

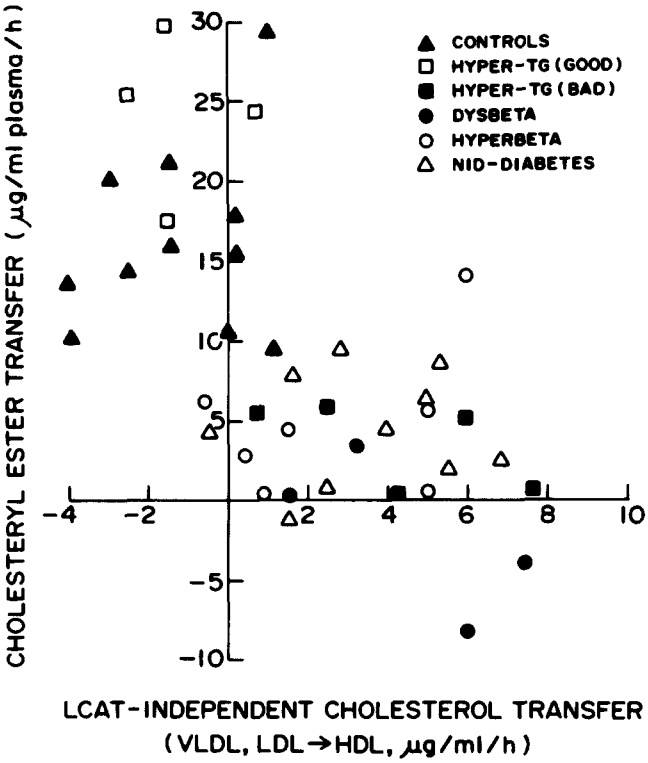

Fig. 2. The relationship between VLDL, LDL → HDL chemical potential gradient and cholesteryl ester transfer. Cholesteryl ester transfer from HDL to VLDL + LDL was determined as in reference 9, and the free cholesterol potential gradient was determined as described in the legend to Fig. 1. Human subjects are those from references 9–11. Abbreviations are as given in the legend to Fig. 1.

iii) They provide a unified mechanism to explain cellular cholesterol accumulation in the tissues of those with different acquired or inherited risks of vascular disease.

Thermodynamic free cholesterol gradients should be taken into account as one of the mechanisms catalyzing the unregulated entry of cholesterol into cells.

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