Cholesterol transfer from mitochondrial membranes and cells to human and rat serum lipoprotein fractions

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Abstract We have investigated the transfer of [^{14}C]cholesterol from labeled bovine heart mitochondria and Friend erythroleukemic cells to high density lipoprotein (HDL), low density lipoprotein (LDL), and very low density lipoprotein (VLDL) fractions from human and rat plasma. The lipoprotein fractions were obtained by molecular sieve chromatography of plasma on agarose A-5m columns. For either membrane system, the highest rate of [^{14}C]cholesterol transfer was observed with the human and the rat HDL fraction. Since the mitochondria lack the receptors for HDL, one may conclude that the observed preferential transfer is not governed by a receptor-controlled interaction of HDL with the membrane. Under conditions where the pool of free cholesterol in the lipoprotein fractions was the same, HDL was a much more efficient acceptor of [^{14}C]cholesterol from mitochondria than LDL or VLDL. Similarly, transfer of [^{14}C]cholesterol proceeded at a higher rate to HDL than to sonicated egg phosphatidylcholine (PC) vesicles, even under conditions where there was a tenfold excess of the vesicle-PC pool over the HDL phospholipid pool. This preferred transfer of [^{14}C]cholesterol to HDL cannot be explained by a random diffusion of monomer cholesterol molecules. Rather, it shows that HDL has a specific effect on this process in the sense that it most likely enhances the efflux of cholesterol from the membrane. Treatment of HDL with trypsin reduced the rate of [^{14}C]-cholesterol transfer by 40–50%, indicating that protein component(s) are involved. One of these components appears to be apoA-I, as this protein was shown to enhance the transfer of [^{14}C]cholesterol from mitochondria to lipid vesicles. — **van Heusden, G. P. H., J. W. P. M. van Schijndel, and K. W. A. Wirtz.** Cholesterol transfer from mitochondrial membranes and cells to human and rat serum lipoprotein fractions. *J. Lipid Res.* 1989. **30:** 1357–1364.

Supplementary key words apolipoprotein A-I • high density lipoprotein • reverse cholesterol transport

Plasma high density lipoprotein (HDL) plays an important role in the transport of cholesterol from peripheral tissues to the liver (reverse cholesterol transport) (1–3). The first step in this process is the uptake of free cholesterol from the cellular plasma membrane by circulating HDL (3–11). Subsequently, cholesterol is esterified by lecithin: cholesterol acyltransferase, resulting in a net flux of cholesterol from the plasma membrane to HDL (1–3).

The mechanism by which HDL extracts cholesterol from the cell membrane is poorly understood. Several extrahepatic cell types have receptors for HDL (12,13). Binding of HDL to these receptors may facilitate movement of cholesterol from the cell surface to HDL. On the other hand, evidence has been provided that cholesterol can be taken up by HDL without binding to its receptor (14). According to a proposed mechanism, cholesterol monomers may diffuse from the cell membrane through the aqueous phase to circulating HDL (11, 14–18). Since HDL has a relatively high affinity for lipids (19,20), it is equally possible that HDL takes up cholesterol by direct contact with the cell membrane.

In the present study we have compared HDL from human and rat serum with other serum lipoprotein fractions for its capacity to take up [^{14}C]cholesterol from a subcellular membrane fraction (i.e., bovine heart mitochondria) and from intact cells (i.e., Friend erythroleukemic cells). To this end, the various lipoprotein fractions were isolated by gel filtration in order to prevent the dissociation of apolipoproteins from the lipoprotein structure as was previously observed for the ultracentrifugal method (21–25). It was shown that the uptake of [^{14}C]cholesterol from both mitochondria and Friend cells proceeds much faster by HDL than by other lipoprotein fractions. Since mitochondria lack receptors for lipoproteins, we conclude that the preferred uptake of cholesterol by HDL is a property inherent to this lipoprotein. It was also shown that apoA-I makes HDL an effective receptor for cholesterol, possibly by enhancing the rate at which cholesterol leaves the donor membrane.

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; PC, phosphatidylcholine; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Materials

[^{14}C]Cholesterol (56 mCi/mmol) was obtained from the Radiochemical Centre (Amersham, UK) Egg phosphatidylcholine (PC), phosphatidic acid (derived from egg PC), sphingomyelin (from bovine brain), and cholesterol were purchased from Sigma (St. Louis, MO). Trypsin and trypsin inhibitor were products of Boehringer (Mannheim, GFR).

Plasma samples

Human plasma obtained from the blood of healthy volunteers was mixed immediately with 1/5 volume of 18 mM glucose/100 mM sodium citrate, pH 7.2. Rat plasma was prepared similarly from blood collected from male Wistar rats.

Gel filtration chromatography of plasma

Ten ml of rat or human plasma mixed with 10% (w/v) sucrose was applied to a 6% agarose A-5m column (2 × 105 cm: Bio-Rad, Richmond, CA), equilibrated with 0.15 M NaCl containing 2 mM sodium phosphate, pH 7.4, 0.01% NaN_3, and 1 mM EDTA (buffer A) (25,26). The elution was performed at 4°C at a flow rate of 10 ml/h and fractions of 5 ml were collected.

Labeling of bovine heart mitochondria with [^{14}C]cholesterol

Bovine heart mitochondria were isolated as previously described (27). An aliquot of the mitochondrial suspension (20 μmol of phospholipid) was incubated with sonicated vesicles consisting of phosphatidylcholine–[^{14}C]cholesterol (450,000 dpm)–sphingomyelin–phosphatidic acid (78:10:10:2, mol/mol; equivalent to 3.2 μmol total lipid) and 80 μg of pure nonspecific lipid transfer protein (28) in a volume of 4 ml of 0.25 M sucrose–10 mM Tris-HCl, pH 7.4–1 mM EDTA (SET) for 60 min at 37°C. After the incubation a cushion of 2 ml of 14.3% (w/v) sucrose was layered under the incubation mixture and the mitochondria were pelleted by centrifugation (10 min at 10,000 *g*). The mitochondria were washed once with 8 ml of SET and resuspended in 0.8 ml of SET. This suspension contained between 50,000 and 70,000 dpm [^{14}C]cholesterol. The vesicles were prepared by mixing the lipids in chloroform–methanol 1:1 (vol/vol), followed by evaporation of the solvent under a stream of nitrogen, addition of SET-buffer up to a total lipid concentration of 1 mM, and sonication for 10 min at 0°C using a Branson sonifier at 60 W.

Labeling of Friend erythroleukemic cells with [^{14}C]cholesterol

Friend erythroleukemic cells were maintained in Eagle's essential medium supplemented with 10% fetal calf serum (29). [^{14}C]Cholesterol (4 μCi in 0.04 ml of toluene) was added to 50 ml of culture and after 20 h the cells were harvested by centrifugation (5 min at 1,000 *g*). The cells were washed three times in 0.9% (w/v) NaCl and the final cell pellet was resuspended in 2 ml of 0.9% NaCl. This suspension contained approximately 14,000 dpm [^{14}C]cholesterol.

Uptake of [^{14}C]cholesterol from mitochondria and cells

Lipoprotein fractions or lipid vesicles were pipetted into an Eppendorf tube to a total volume of 0.36 ml of buffer A. [^{14}C]Cholesterol-labeled mitochondria or cells (0.04 ml) were added and the mixture was incubated for 60 min at 37°C, with occasional vortexing. After the incubation, cells or mitochondria were pelleted by centrifugation (5 min in a microfuge) and the supernatant was transferred to a scintillation vial. The radioactivity was quantitated after addition of 5 ml of emulsifier scintillation fluid (Packard, Brussels, Belgium). Each incubation was performed in duplicate; the values were corrected for blank incubations lacking either lipoprotein or vesicles.

Uptake of [^{14}C]cholesterol by HDL treated with trypsin

Rat or human HDL (0.2 ml of fraction 42 or 46, respectively, see Fig. 1) was incubated with trypsin (0.05 mg) in 0.3 ml of buffer A for 30 min at 37°C. Upon addition of soybean trypsin inhibitor (0.18 mg) in 0.05 ml buffer A, [^{14}C]cholesterol-labeled mitochondria (0.04 ml) were added and the uptake of cholesterol was measured as described above. Control incubations were carried out in the presence of trypsin and trypsin inhibitor.

Isolation of apolipoproteins by chromatofocusing

Human HDL (fractions 44–47; see Fig. 1B) was delipidated (30) and the apolipoproteins were dissolved in 10 ml of 25 mM imidazole, pH 7.4–1 mM dithioerythritol–7.2 M urea. The mixture was applied to a PBE-94 (Pharmacia, Uppsala, Sweden) column (1 × 30 cm), equilibrated with 25 mM imidazole, pH 7.4–7.2 M urea. The proteins were eluted with Polybuffer 74 (1:8, pH 4.0) containing 7.2 M urea as described by McLeod et al. (31). Fractions were neutralized with 1 M Tris and dialyzed overnight against 10 mM sodium phosphate, pH 7.2–150 mM sodium chloride. Fractions were analyzed for the protein composition using sodium dodecylsulfate polyacrylamide gel electrophoresis (32).

Analytical procedures

Protein was determined according to the procedure of Lowry et al. (33). Free cholesterol was determined with an enzymatic method (CHOD-iodide method, Merckotest, Merck, Darmstadt, GFR). Phospholipid was quantitated

by determination of lipid phosphorus (34) after extraction (35).

RESULTS

Rat and human plasma were fractionated by gel filtration on agarose A-5m columns. The fractions were analyzed for protein composition (SDS-PAGE) as well as for their content of free cholesterol. For rat plasma (**Fig. 1A**, upper panel), a prominent cholesterol-containing peak was present in the void volume (fraction number 22), representative of VLDL and chylomicrons (26). Two additional peaks were observed with maxima at fraction numbers 36 and 44. These peaks represent the LDL and HDL fractions, respectively, as was confirmed by the presence of apoB and apoA-I in these fractions. Analysis of the fractions from human plasma (Fig. 1B, upper panel) showed three cholesterol-containing peaks as well, representative of VLDL, LDL, and HDL (maxima at fraction numbers 24, 32, 46, respectively). In this instance, the bulk of free cholesterol was present in LDL. For both rat and human plasma, the HDL fraction had the lowest free cholesterol content.

In order to analyze these column fractions for their ability to take up cholesterol from membranes, the fractions (aliquots of 0.2 ml) were incubated for 60 min with bovine heart mitochondria, labeled with [^{14}C]cholesterol. It is shown in Figs. 1A and B (lower panels) that both rat and human plasma have one major peak active in the uptake of [^{14}C]cholesterol. This peak coincides with the fractions containing HDL. It is to be noted that the peak in rat plasma elutes from the column earlier than the peak in human plasma. This is in agreement with the fact that rat HDL particles are larger than human HDL particles (36). Furthermore, the incorporation of [^{14}C]cholesterol into human HDL (fraction 46) increased with time up to 60 min, indicating that rates of incorporation were measured. These rates were low for those fractions from rat and human plasma that contained VLDL and LDL (fractions 24 and 32, respectively). It is evident that the rate at which [^{14}C]cholesterol is incorporated into the lipoprotein particles is not related to the pool of free cholesterol, as this pool is larger in rat and human VLDL and human LDL than in HDL (see Figs. 1A and B). On the basis of cholesteryl ester, it was found that upon sedimentation of the mitochondria the recovery of human HDL and VLDL was on the order of 70–80% as compared to 50–60% for LDL. The uptake of [^{14}C]cholesterol as reported in Fig. 1 has not been corrected for this incomplete recovery. In another experiment the lipoprotein-containing fractions from human plasma (fractions 24, 32, and 46, see Fig. 1B) were incubated with intact Friend erythroleukemic cells labeled with [^{14}C]cholesterol. In **Fig. 2** it can be seen that HDL takes up [^{14}C]cholesterol much faster than LDL and VLDL. This shows that whether subcellular membranes or intact cells were used, HDL is the most efficient acceptor for [^{14}C]cholesterol.

As shown in Figs. 1A and B, there is no apparent relationship between the rate of [^{14}C]cholesterol uptake and the amount of free cholesterol in the lipoprotein fractions. This observation was confirmed by determining the [^{14}C]-cholesterol uptake from labeled mitochondria as a function of free cholesterol in the lipoprotein fractions (**Fig. 3**). At each cholesterol concentration, both rat and human HDL were much more efficient in this uptake process than LDL and VLDL. Esterification of cholesterol may enhance the flux of labeled cholesterol to HDL. However, under our conditions of incubation, less than 2% of the [^{14}C]-cholesterol in rat and human HDL was recovered as cholesteryl ester.

It is well established that phospholipid vesicles will take up cholesterol from membranes (11). We compared human HDL with phospholipid vesicles for their ability to take up [^{14}C]cholesterol from mitochondria (**Fig. 4A**) and Friend cells (Fig. 4B). The vesicles consisted of either egg PC alone or egg PC–sphingomyelin–phosphatidic acid 88:10:2 (mol %) or egg PC–cholesterol–sphingomyelin–phosphatidic acid 58:30:10:2 (mol %). It is evident that, as a function of phospholipid added, human HDL is a much better acceptor of [^{14}C]cholesterol than any of the phospholipid vesicles tested. This was also found to be true for vesicles prepared from total lipids extracted from HDL (data not shown). Even at a tenfold excess of vesicle PC over HDL phospholipid, the uptake of [^{14}C]cholesterol by HDL was still approximately twofold higher.

The data in Figs. 3 and 4 suggest that, in addition to lipids, protein components of the HDL particle may be instrumental in the observed cholesterol uptake. This point was addressed by incubating human and rat HDL with trypsin. As shown in **Table 1**, trypsin treatment diminished the ability of HDL to take up cholesterol from mitochondria by 40–50%. In order to determine what protein components in HDL were involved in this process, human HDL was delipidated and the protein components were separated by chromatofocusing in the presence of 7.2 M urea. After dialysis the protein fractions (0.2-ml aliquots) were tested for their ability to enhance the uptake of [^{14}C]cholesterol from mitochondria by egg PC vesicles. As shown in **Fig. 5** a distinct stimulatory activity was observed in fractions 33–35 (pH 5.4–5.3). The major component in these fractions was apoA-I (see Fig. 5, insert C). In addition, activity was detected in fractions 26 to 28 (pH 5.9–5.8). For unknown reasons, activity in the latter fractions differed greatly among preparations. In order to show that apoA-I has stimulatory activity, this protein was purified to homogeneity by fractionation of fractions 33–35 on a Sephadex G-75 column. As shown in **Fig. 6**, the uptake of [^{14}C]cholesterol from mitochondria by PC vesicles increased linearly with the amount of apoA-I added.

It is of note that preincubation of the vesicles with apoA-I overnight at 37°C (37) had no effect on the extent of [^{14}C]-cholesterol uptake. At present it is not known whether the observed effect is caused by free apoA-I or by a complex formed between this protein and vesicle lipids. On the other hand, we have observed that apoA-I itself has some ability to bind cholesterol. In the absence of vesicles, binding of [^{14}C]cholesterol from mitochondria to apoA-I was about one-third of that observed in the presence of vesicles.

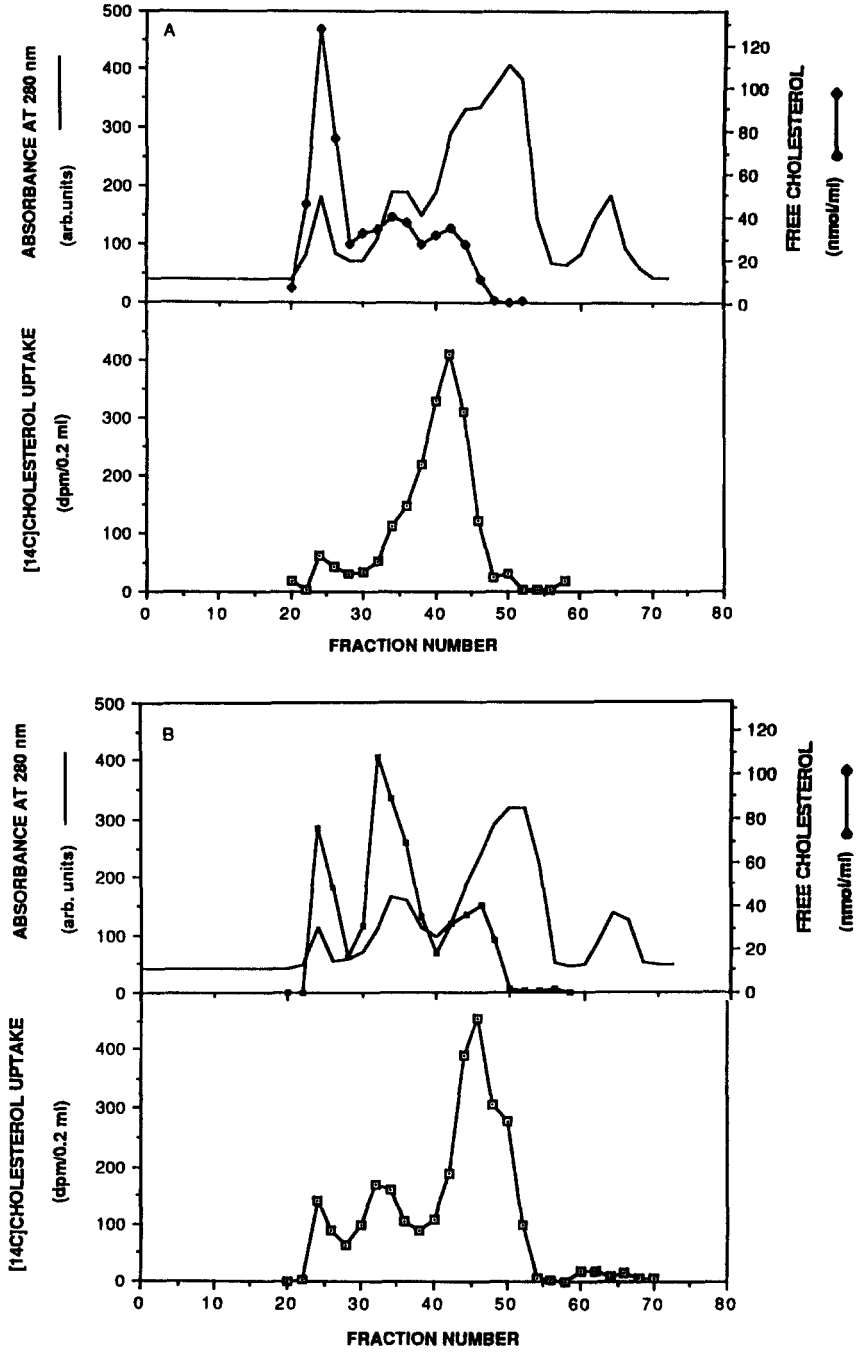

Fig. 1. Free cholesterol content and uptake of [^{14}C]cholesterol from mitochondria by lipoprotein fractions obtained by molecular sieve chromatography of rat and human plasma. Ten ml of rat (A) or human (B) plasma was fractionated on a column of agarose A-5m (2 × 105 cm; fractions of 5 ml). The free cholesterol content of the fractions (0.1-ml aliquots) was determined using an enzymatic method (♦, upper panels). [^{14}C]Cholesterol uptake from labeled mitochondria was measured by incubating the plasma fractions (0.2-ml aliquots) with labeled mitochondria (1 μmol of phospholipid; 2500 dpm of [^{14}C]cholesterol) for 60 min at 37°C, followed by sedimentation of the mitochondria by centrifugation (⊡, lower panels).

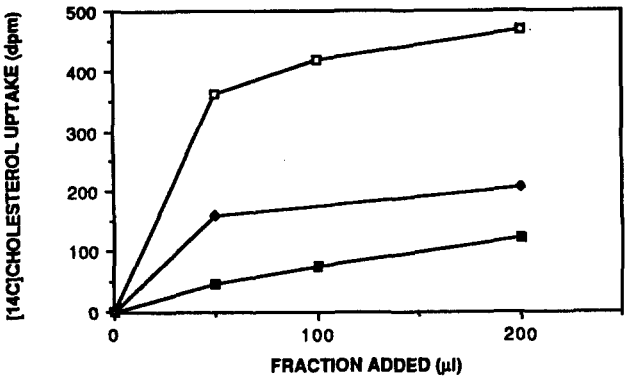

Fig. 2. Uptake of [^{14}C]cholesterol from Friend erythroleukemic cells by human lipoprotein fractions. Human lipoprotein fractions were isolated by molecular sieve chromatography and the indicated amounts of HDL (□, fraction 45; see Fig. 1), LDL (♦, fraction 33), and VLDL (■, fraction 24) were incubated with 0.04 ml of [^{14}C]cholesterol-labeled Friend erythroleukemic cells (14,000 dpm of [^{14}C]cholesterol) for 60 min at 37°C. The uptake of [^{14}C]cholesterol was measured after sedimentation of the cells by centrifugation.

ing apoA-I lipoprotein fraction (38). This labeling is difficult to reconcile with a simple diffusion mechanism. In a recent study on the cholesterol efflux from red blood cells to different acceptor particles including serum lipoproteins, it was concluded that cholesterol transfer occurred by an activation-collision mechanism (39).

Under conditions where the free cholesterol pool was identical in the serum lipoproteins, transfer of [^{14}C]cholesterol was much faster to HDL than to LDL and VLDL (Fig. 3). This preferential labeling cannot simply be explained by the aqueous diffusion model, as the rate-limiting step of this model is the dissociation of the lipid monomer from the membrane interface (11). That this model cannot explain the results was even more clearly shown in experiments in which the transfer of [^{14}C]cholesterol to HDL was compared with the transfer to phospholipid vesicles (Fig. 4). At a tenfold excess of vesicle over HDL phospholipid, transfer of [^{14}C]cholesterol to HDL was still twofold higher. This clearly shows that factors

DISCUSSION

In this study we fractionated rat and human serum lipoproteins by gel filtration over agarose A-5m to identify the components of plasma most effective in cholesterol removal from membranes. We chose the agarose column method since it is known that ultracentrifugation of serum lipoproteins can result in dissociation of some apolipoproteins (21–25). An additional advantage of fractionation by gel filtration is that the lipoprotein classes VLDL, LDL, and HDL are well separated in a single run, facilitating their direct comparison. In order to measure the ability of these lipoprotein fractions to take up free cholesterol, assay systems were developed in which bovine heart mitochondria or Friend erythroleukemic cells prelabeled with [^{14}C]cholesterol were used as donor systems. With either donor system we could demonstrate that, in both human and rat plasma, HDL was by far the most effective acceptor for cholesterol. Since mitochondria do not have receptors for HDL, the cholesterol uptake from this donor system cannot be dependent on a specific binding. Rather, this preferential binding reflects an inherent property of the HDL particle. This observation is in agreement with the conclusions of Karlin et al. (14) that binding of HDL to its receptor is not required for an effective uptake of cholesterol.

It has been well established with model membrane systems that cholesterol can move between interfaces by an aqueous diffusion mechanism (11). Along this line it has been strongly argued that the efflux of cholesterol from cells to HDL occurs by monomer diffusion (11,14,16,18). On the other hand, incubation of human skin fibroblasts labeled with [^{3}H]cholesterol with normal human plasma yielded initially a preferential labeling of a pre-β-migrat-

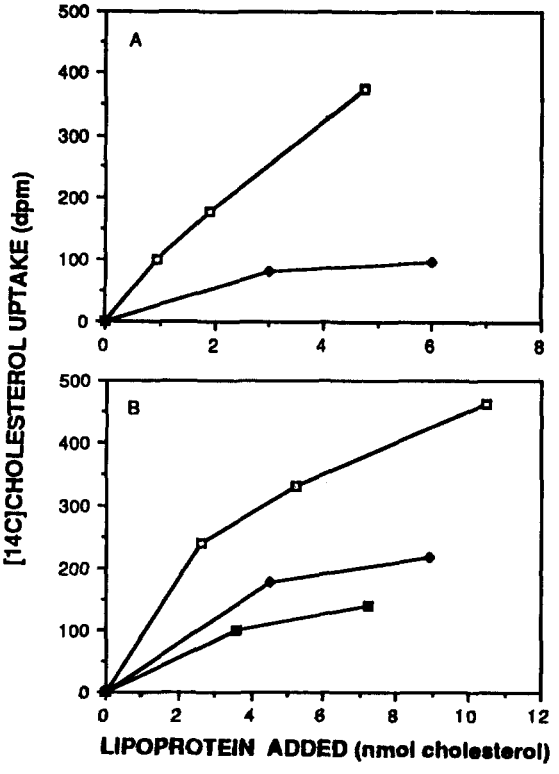

Fig. 3. Uptake of [^{14}C]cholesterol from labeled mitochondria by rat (A) and human (B) lipoproteins as a function of the amount of free cholesterol in the lipoproteins. Lipoprotein fractions containing the indicated amount of free cholesterol were incubated with [^{14}C]cholesterol-labeled mitochondria (1 µmol of phospholipid; 2500 dpm of [^{14}C]cholesterol) for 60 min at 37°C, and the uptake of [^{14}C]cholesterol by the lipoprotein fractions was measured after sedimentation by centrifugation. A: Rat lipoprotein fractions: (□) HDL, fraction 42, see Fig. 1A; (♦) LDL, fraction 33. B: Human lipoprotein fractions: (□) HDL, fraction 45, see Fig. 1B; (♦) LDL, fraction 33; (■) VLDL, fraction 24.

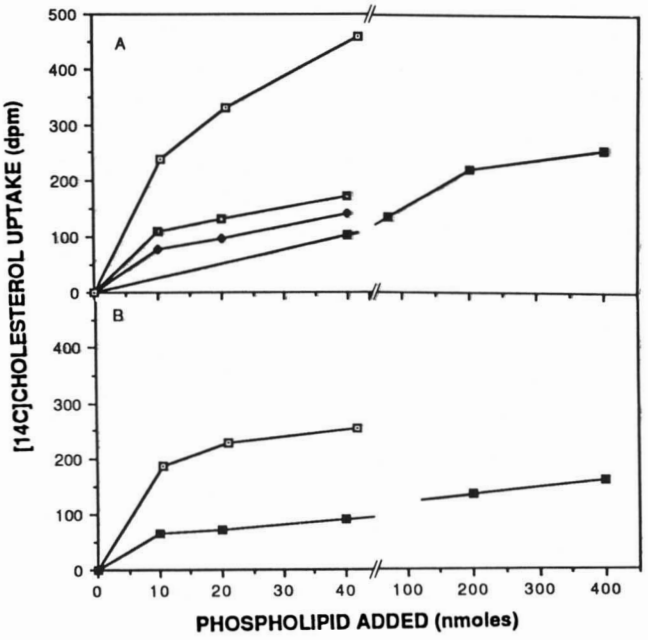

Fig. 4. Comparison of uptake of [^{14}C]cholesterol from bovine heart mitochondria (A) or from Friend erythroleukemic cells (B) by human HDL and sonicated vesicles. Various amounts of human HDL (fraction 45, agarose, column, Fig. 1; ⊡) or of sonicated vesicles of egg PC (■), of egg PC–phosphatidic acid–sphingomyelin 88:2:10 (mol %) (♦), and of egg PC–phosphatidic acid–sphingomyelin–cholesterol 58:2:10:30 (mol %) (■), containing the indicated amounts of phospholipid, were incubated with [^{14}C]cholesterol-labeled mitochondria (A) or Friend erythroleukemic cells (B) for 60 min at 37°C, and the uptake of [^{14}C]cholesterol was measured after sedimentation of the cells and mitochondria. The labeled mitochondria and Friend erythroleukemic cells added to each incubation contained 2,500 and 14,000 dpm of [^{14}C]cholesterol, respectively.

TABLE 1. Effect of trypsin treatment on uptake of [^{14}C]cholesterol from [^{14}C]cholesterol-labeled mitochondria by rat and human HDL

Addition to Incubation	[^{14}C]Cholesterol Uptake		
	Expt. 1 (Rat HDL)	Expt. 2 (Rat HDL)	Expt. 3 (Human HDL)
		dpm	
None	203	185	383
Trypsin (50 μg) + inhibitor	200	nd	nd
Trypsin (20 μg)	nd	142	299
Trypsin (50 μg)	113	92	243

nd, Not determined.

other than the dissociation of cholesterol monomers from the membrane interface are involved.

The involvement of proteins in the cholesterol uptake was suggested by the fact that trypsin treatment of HDL reduced the transfer of [^{14}C]cholesterol by 40–50% (Table 1). Such a role was further supported by the observation that vesicles prepared from lipids extracted from HDL were as active in extracting cholesterol from mitochondria as egg PC vesicles (data not shown). What role the proteins play in the cholesterol uptake by HDL is still unclear. DeLamatre et al. (40) proposed a model in which the primary effect of apolipoproteins is the dispersion of phospholipid into small particles (i.e., HDL), which are better acceptors for the free cholesterol monomer. However, this proposal does not explain why the relatively large rat HDL particle (36)

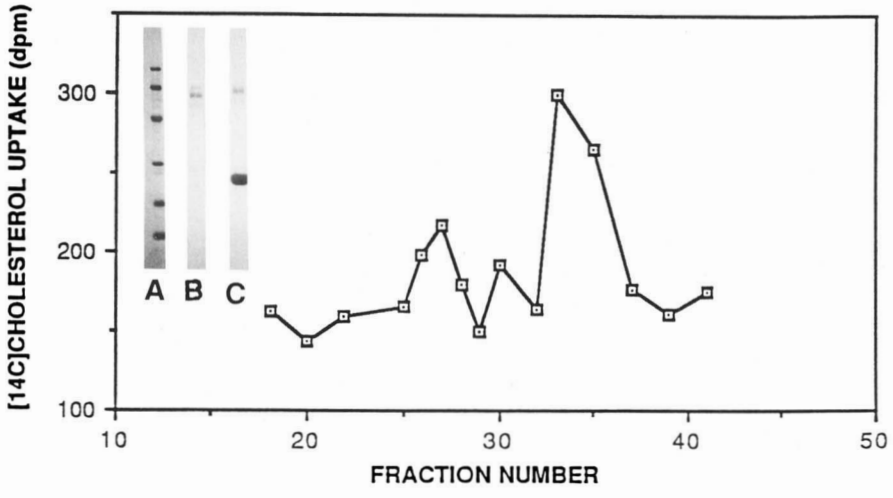

Fig. 5. Effect of apolipoproteins isolated from human HDL by chromatofocusing, on the uptake of [^{14}C]cholesterol by PC vesicles. Human HDL (fractions 44–47; see Fig. 1B) was delipidated, and the apolipoproteins were dissolved in 10 ml of 25 mM imidazole, pH 7.4–1 mM dithioerythritol–7.2 M urea. The mixture was applied onto a PBE-94 column (1 × 30 cm) and the proteins were eluted with Polybuffer 74 (1:8, pH 4.0) containing 7.2 M urea. The fractions (6 ml each) were dialyzed to remove the urea, and 0.2-ml aliquots were incubated with [^{14}C]cholesterol-labeled mitochondria (1 μmol of phospholipid; 2500 dpm of [^{14}C]cholesterol) in the presence of vesicles of egg PC (80 nmol of lipid) for 60 min at 37°C. The radioactivity in the vesicles was measured after sedimentation of the mitochondria by centrifugation. Insert: SDS-PAGE gels (7.5–25% gradient) stained with Coomassie brilliant blue. A: Molecular weight markers: phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400) (Bio-Rad, Richmond, CA). B: Fraction 27 (1 ml). C: Fraction 33 (1 ml).

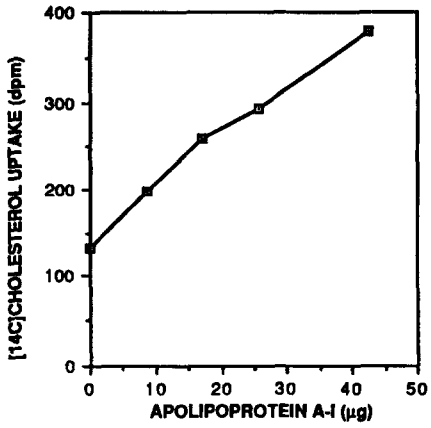

Fig. 6. Effect of apoA-I on the efflux of [^{14}C]cholesterol from mitochondria to vesicles of egg PC. Various amounts of apoA-I were incubated with [^{14}C]cholesterol-labeled mitochondria (1 μmol of phospholipid, 2500 dpm of [^{14}C]cholesterol) in the presence of vesicles of egg PC (80 nmol of lipid) for 60 min at 37°C. [^{14}C]Cholesterol uptake was measured after sedimentation of the mitochondria by centrifugation.

is as active as the smaller human HDL particle in cholesterol uptake. This indicates that the size of the particle is not necessarily critical. Therefore, our results strongly suggest that protein components of HDL directly affect the donor membrane, thereby making cholesterol better available for uptake by HDL. In line with this observation, it has been shown that intact HDL can disorganize lipid vesicles (19).

In this study we have shown that the major apolipoprotein in HDL, apoA-I, promotes the uptake of [^{14}C]cholesterol from mitochondria or cells by lipid vesicles (Fig. 6). In agreement with this observation, Swaney (41) found that reconstituted complexes of human apoA-I and phosphatidylcholine or sphingomyelin were able to extract cholesterol from erythrocytes. Using immunoaffinity chromatography. Fielding and Fielding (2) showed that sterol efflux from cultured fibroblasts was highly dependent on a minor lipoprotein fraction containing apoA-I. Recently, Castro and Fielding (38) observed that incubation of [^{3}H]cholesterol-labeled fibroblasts with human plasma initially resulted in the labeling of an apoA-I-containing lipoprotein fraction with a molecular weight of 70,000. On the other hand, Jackson et al. (20) showed that reconstituted complexes of PC with apoA-II, C-I, or C-III were more active in releasing cholesterol from ascites cells than similar complexes with apoA-I. Stein et al. (42) incubated [^{14}C]cholesterol-labeled fibroblasts overnight with complexes of the ether analog dioleylphosphatidylcholine and apolipoproteins A-I, A-IV, or E; all three apolipoproteins were reported to be equally active in promoting cholesterol efflux from these cells. However, in this latter study rates of cholesterol efflux were not measured. Using a different approach, Demel et al. (43) showed that apoE was able to transfer cholesterol from a monolayer of [^{14}C]cholesterol to sonicated vesicles present in the subphase. Because of these conflicting results, additional studies are required to clarify whether, in addition to apoA-I, other apolipoproteins can directly influence the uptake of cholesterol from cell membranes. In mammalian tissues a protein has been identified that in vitro stimulates the transfer of cholesterol between membranes (28,44–46). This protein, known as nonspecific lipid transfer protein or sterol-carrier protein 2, also stimulates cholesterol transfer between membranes and serum lipoproteins (G. P. H. van Heusden, unpublished observations). It was suggested that the nonspecific lipid transfer protein could be secreted in the serum (47). So far we have not been able to identify this protein with any certainty in human and rat serum.

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