Enrichment of endothelial cell arachidonate by lipid transfer from high density lipoproteins: relationship to prostaglandin I_2 synthesis

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Abstract We have previously shown that plasma high density lipoproteins (HDL) stimulate release of prostacyclin, measured as its stable metabolite, 6-keto-$PGF_{1\alpha}$, by cultured porcine aortic endothelial cells. The present experiments were designed to elucidate the contribution of HDL lipids to endothelial cellular phospholipid pools and to prostacyclin synthesis. In experiments with reconstituted HDL, both the lipid and protein moieties were required to stimulate prostacyclin release in amounts equivalent to the native HDL particle. Endothelial cells incorporated label from reconstituted HDL containing cholesteryl [1-^{14}C]arachidonate into the cellular neutral and phospholipid pools as well as into 6-keto-$PGF_{1\alpha}$ and PGE_2. Labeled arachidonate incorporated into endothelial cell lipids from reconstituted HDL containing cholesteryl [1-^{14}C]arachidonate was also metabolized to prostaglandins after the cells were exposed to the calcium ionophore, A-23187. Both rat and human HDL which stimulated 6-keto-$PGF_{1\alpha}$ release (rat > human) increased the weight percentage of arachidonate in endothelial cell phospholipids; phospholipid arachidonate in the enriched cells fell after exposure to the phospholipase activator, A-23187, with release of 6-keto-$PGF_{1\alpha}$ which was greater than in control cells. Rat HDL that was depleted of cholesteryl arachidonate (achieved by incubation with human low density lipoproteins (LDL) in the presence of cholesteryl ester transfer protein) stimulated 6-keto-$PGF_{1\alpha}$ release less than native rat HDL. LDL enriched in cholesteryl arachidonate stimulated 6-keto-$PGF_{1\alpha}$ release more than native LDL. ApoE-depleted HDL also stimulated 6-keto-$PGF_{1\alpha}$ release more than apoE-rich HDL suggesting the apoE receptor was not involved in the response. Unlabeled HDL also caused a slight increase in the incorporation of radioactivity into 6-keto-$PGF_{1\alpha}$ by cells preincubated with [1-^{14}C]arachidonate suggesting additional activation of phospholipases. In summary, the data indicate that arachidonate derived from lipoprotein cholesteryl esters can be incorporated into endothelial cell lipids, thereby contributing to cellular prostaglandin synthesis. —**Pomerantz, K. B., L. N. Fleisher, A. R. Tall, and P. J. Cannon.** Enrichment of endothelial cell arachidonate by lipid transfer from high density lipoproteins: relationship to prostaglandin I_2 synthesis. *J. Lipid Res.* 1985. **26:** 1269–1276.

Supplementary key words LDL • cholesteryl arachidonate • phospholipids • 6-keto-$PGF_{1\alpha}$ • prostacyclin

Prostacyclin (PGI_2), a vasodilator and an inhibitor of platelet aggregation, is synthesized by a number of tissues including heart, blood vessels, and endothelial or smooth muscle cells grown in tissue culture (1–4). Either arachidonic acid or prostaglandin endoperoxides can serve as substrate for PGI_2 synthesis by endothelial cells (3). In most tissues, after mechanical or hormonal stimulation, arachidonic acid is liberated from cell membrane phospholipids following the actions of phospholipase A_2 or C, and is made available to cyclooxygenase (5). Spector et al. (6) and Rosenthal and Whitehurst (7) reported that endothelial cells grown in medium containing 10% fetal bovine serum were deficient in the Δ^6-desaturase activity required for synthesis of arachidonic acid from linoleic acid. They suggested that endothelial cells might require an exogenous source of arachidonic acid (6, 7). In plasma, the concentrations of arachidonate present as the free fatty acid are quite low. Recently, however, an acyl coenzyme A synthetase specific for arachidonate and other eicosanoid precursors that enhances the uptake of arachidonate from plasma has been identified (8). In addition, considerable arachidonate is present in esterified form in the phospholipids and cholesteryl esters of the plasma lipoproteins.

In a previous study, we reported that human plasma high density lipoproteins (HDL) produced a dose-dependent increase in the synthesis of PGI_2 by aortic endothelial cells grown in tissue culture (9). LDL at equivalent doses did not stimulate PGI_2 synthesis. Rat HDL stimulated PGI_2 production to a greater extent than human HDL, whereas the apoproteins of both lipoprotein species had only slight effects. Since rat HDL contains more cholesteryl arachidonate than human HDL, it was suggested that HDL may provide arachidonate to the endothelial cells as substrate for PGI_2 synthesis (9). The present study was designed to investigate this hypothesis.

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; PGI_2, prostacyclin.

METHODS

Isolation and culture of porcine aortic endothelial cells

Thoracic aortae from suckling pigs were rinsed with serum-free Dulbecco's Modified Eagle's Medium (DME, Grand Island Biological Company (GIBCO), Grand Island, NY) containing 1% (v/v) penicillin–streptomycin–fungizone–glutamine (GIBCO). The vessel was split longtitudinally; intimal scrapings were placed into DME containing 10% fetal bovine serum (Hyclone, Sterile Systems), centrifuged, and resuspended into DME. The cells were inoculated into 25-cm^2 COSTAR flasks (Costar Packaging Co.) and incubated at 37°C under 5% CO_2 in air at 90% humidity. Cells were subcultured into their third passage and frozen. The endothelial cells displayed characteristic monolayered cobblestone morphology at confluence.

Incubations

For each experiment, the endothelial cells were thawed and plated into petri dishes (16 × 35 mm, Falcon) at 8 × 10^4 cells/dish in DME containing 10% (v/v) fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 1% L-glutamine for 48–54 hr. The control cultures (triplicate dishes) were then washed three times, and incubated with 2 ml of DME for 24 hr. The experimental cultures (triplicates) were also washed and incubated with 2 ml of serum-free DME containing the test agents. The media were then stored at −70°C until they were assayed for arachidonic acid metabolites.

Preparation of plasma lipoproteins, reconstituted lipoproteins, and the cholesteryl ester transfer protein

Plasma lipoproteins were isolated by preparative ultracentrifugation of human and rat plasma between densities 1.019–1.063 g/ml (LDL) and 1.063–1.210 g/ml (HDL) and 1.075–1.210 g/ml (rat HDL) (10). The lipoproteins were recentrifuged once at their upper density limit and dialyzed against DME containing penicillin (100 U/ml), and streptomycin (100 μg/ml). For preparation of apoHDL, HDL was dialyzed against distilled water, lyophilized, and delipidated in chloroform–methanol 2:1 (v/v), and washed with diethyl ether. ApoHDL was then dried under argon, solubilized in 3.0 M guanidine HCl and dialyzed against 0.9% NaCl, 100 μM Tris (pH 8.0) (saline-Tris buffer). To prepare HDL–lipid microemulsions, the dried HDL lipids were agitated in saline-Tris buffer at 25°C, then sonicated at 37°C in a water-jacketed cell flushed with nitrogen using a Branson #185 cell disrupter. Reconstituted HDL was prepared by co-sonication of HDL lipids and apoHDL in saline-Tris buffer, and was reisolated by ultracentrifugation between densities 1.063–1.210 g/ml (11, 12). Reconstituted HDL containing cholesteryl [1-^{14}C]arachidonate was prepared by co-sonication of apoHDL with HDL lipids containing synthetic cholesteryl [1-^{14}C]arachidonate prepared according to the method of Grover and Cushley (13). HDL containing 1-palmitoyl, 2-[^{3}H]arachidonyl phosphatidylcholine (New England Nuclear) was prepared by injecting the labeled phospholipid into stirred plasma d > 1.10 g/ml fraction, followed by reisolation by ultracentrifugation between densities 1.063–1.210 g/ml.

The plasma cholesteryl ester transfer protein was purified from human plasma d > 1.21 g/ml fraction through the carboxymethylcellulose step as previously described (14). Based on specific activity, this material was purified about 500-fold relative to the d > 1.19 g/ml fraction; the activity of the transfer protein used in these experiments was approximately 10% of that of the most purified preparation (14). Cholesteryl arachidonate-depleted HDL and cholesteryl arachidonate-enriched LDL were prepared by co-incubation of rat HDL with human LDL in the presence of the transfer protein at LDL/HDL ratio of 2.5 at 37°C for 3 hr. The modified LDL was subsequently separated from the modified HDL by ultracentrifugation at density 1.063 g/ml.

Assays

For studies of prostaglandin synthesis from endogenous arachidonate, endothelial cells were prelabeled with 2.0 μCi of [1-^{14}C]arachidonate (40–60 mCi/mmol) (Amersham/Searle) by previously described methods (15). For studies with reconstituted HDL containing labeled cholesteryl arachidonate or phosphatidylcholine, endothelial cells were not prelabeled prior to incubation. Media from these incubations were analyzed for prostaglandins by thin-layer chromatography (15). Briefly, the media was acidified to pH 2.5, extracted into ethyl acetate, washed against water, and dried under N_2. The samples were developed on silica gel G plates in the organic phase of ethyl acetate–isooctane–acetic acid–water 11:5:2:10 (v/v), after 30 min of humidification. The radioimmunoassay for 6-keto-$PGF_{1\alpha}$ was performed as previously described (9).

The lipoprotein and cellular protein content were determined by the method of Lowry et al. (16). Lipoprotein lipids were extracted by the method of Bligh and Dyer (17). Endothelial cell lipids were extracted by the method of Folch, Lees, and Sloane Stanley (18) after scraping the cells into phosphate-buffered saline. The free and total cholesterol contents of HDL and LDL were determined by gas-liquid chromatography (3% OV-17 on 100–120 mesh WHP, Supelco), using coprostanol as internal standard (19). Lipoprotein and cellular lipid separation was accomplished by thin-layer chromatography on silica gel G in hexane–diethyl ether–acetic acid–methanol 85:20:2:4 (v/v). The fatty acid composition of the relevant lipid species (cellular phospholipids and lipoprotein cholesteryl

esters) was determined by derivitization to their corresponding fatty acid methyl esters, followed by separation by gas-liquid chromatography using 10% Silar CP on Gas-Chrom-Q, 100-200 mesh (Supelco). The distribution of [1-^{14}C]arachidonate in endothelial cell phospholipids was determined by subjecting an aliquot of the total cell lipid extract to two-dimensional chromatography on 20 × 20 cm silica gel H in chloroform-methanol-ammonium hydroxide 65:25:5 (first dimension) followed by development in chloroform-acetone-methanol-acetic acid-water 3:4:1:1:0.5 (v/v) (second dimension). Standards were visualized by exposure to phosphomolybdic acid or to iodine vapor.

Statistical analyses

Results of the experiments were analyzed by analysis of variance after logarithmic transformation of the data (20, 21). Differences in treatments were tested using Duncan's multiple range test (22). Data are reported as mean ± standard error.

RESULTS

Effects of native HDL, apoHDL, HDL-lipids, and reconstituted HDL on prostaglandin release by endothelial cells

We first investigated which HDL determinant (either the lipid or protein moiety) might be required for the ability of HDL to stimulate prostacyclin synthesis. Endothelial cells were incubated with native HDL, a sonicated microemulsion of HDL-lipids, apoHDL, and with reconstituted HDL. Reconstituted HDL and native HDL induced a similar, pronounced stimulation of 6-keto-$PGF_{1\alpha}$ release (**Fig. 1**). Although both apoHDL and the sonicated microemulsion of HDL-lipids stimulated 6-keto-$PGF_{1\alpha}$ release, such stimulation was significantly less than native or reconstituted HDL. These data suggested that both the lipid and protein moiety of HDL are required for stimulation of 6-keto-$PGF_{1\alpha}$ release by endothelial cells.

Transfer of [1-^{14}C]arachidonate from reconstituted HDL containing cholesteryl [1-^{14}]arachidonate into prostaglandins and endothelial cell lipids

To determine directly whether HDL provides arachidonate to endothelial cells as substrate for prostacyclin synthesis, endothelial cells were incubated with reconstituted HDL containing cholesteryl [1-^{14}C]arachidonate. In four experiments, reconstituted HDL containing cholesteryl [1-^{14}C]arachidonate stimulated endothelial cell release of [1-^{14}C]6-keto-$PGF_{1\alpha}$ and [1-^{14}C]$PGF_{2\alpha}$. A typical experiment is illustrated in **Fig. 2**. Analysis of the cell lipids in these experiments (n = 6) indicated that 1.11 ± 0.27% of the original radioactivity associated with the reconstituted HDL (2.8 μCi/experiment) was incorporated into cellular lipids (phospholipids, 35.35%; free fatty acids, 2.16%; monoglycerides, 2.07%; diglycerides, 3.91%; and triglycerides, 8.32%). The distribution of radioactivity incorporated into cellular phospholipids was as follows: phosphatidylcholine, 17.16%; phosphatidylethanolamine, 35.12%; phosphatidylinositol, 27.82%; sphingomyelin, 9.97%; and phosphatidylserine, 18.29%. Analysis of reconstituted HDL lipids prior to the experiment showed 100% of radioactivity in cholesteryl esters; following the experiment, the media also showed small amounts of radioactivity in phospholipids and fatty acids.

In some experiments, endothelial cells previously incubated with reconstituted HDL containing cholesteryl [1-^{14}C]arachidonate were exposed to media alone or media containing A-23187 (5 μM). A representative experiment is illustrated in **Fig. 3**. The calcium ionophore stimulated release of radiolabeled 6-keto-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$, and free arachidonate, whereas cells exposed to media alone did not release any prostaglandin, and no significant amounts of radioactivity were associated with free arachidonate. Cell lipid analysis of these experiments revealed that A-23187 reduced the percent radioactivity associated with the cellular phospholipid fraction, with corresponding increases in the percent radioactivity associated with triglycerides, diglycerides, free fatty acid, and no change in other lipid class profiles (**Table 1**).

In similar experiments, incubation of endothelial cells with HDL containing 1-palmitoyl, 2-[^{3}H]arachidonyl-

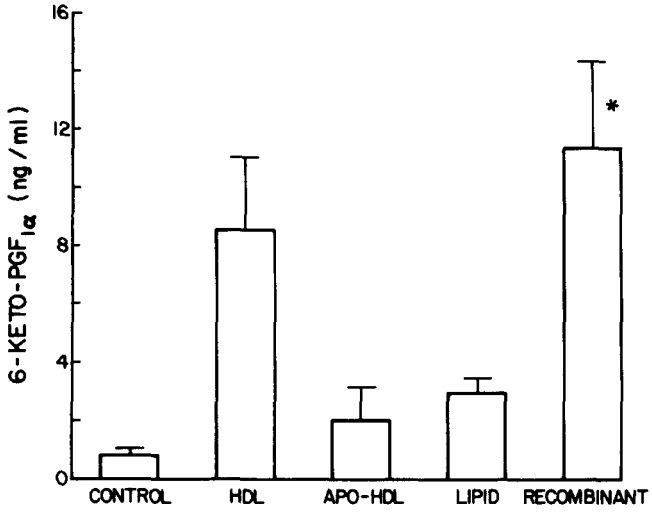

Fig. 1. Effect of lipid and protein moiety of HDL on 6-keto-$PGF_{1\alpha}$ release by porcine aortic endothelial cells. Subconfluent cultures (35-mm plates in triplicate) were exposed to serum-free DME (control) or DME containing native rat HDL, apoHDL, a sonicated microemulsion of HDL lipids, or reconstituted HDL for 24 hr at 37°C. HDL was used at a concentration of 0.1 mg of protein/ml. HDL lipids were derived from extraction of HDL at equivalent protein concentrations. Media were removed and assayed for 6-keto-$PGF_{1\alpha}$ by RIA (n = 3, mean ± SE, * = $P < 0.05$ vs control).

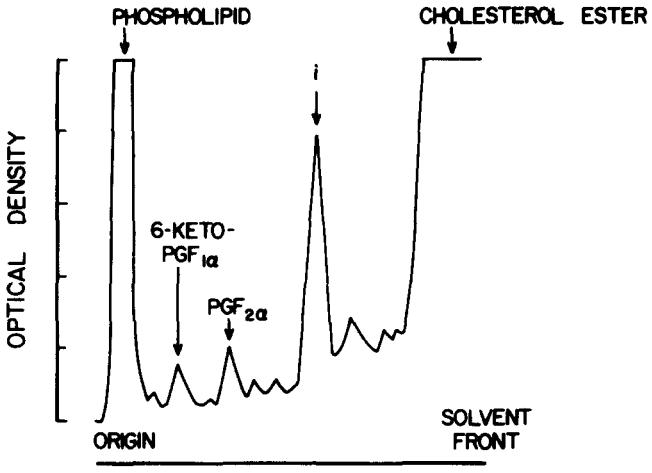

Fig. 2. Transfer of [1-^{14}C]arachidonate from reconstituted HDL containing cholesteryl [1-^{14}C]arachidonate into prostacyclin, $PGF_{2\alpha}$, and phospholipid pools by porcine aortic endothelial cells. Endothelial cells grown to subconfluent density in 25-cm^2 flasks in DME containing 10% fetal bovine serum were exposed to reconstituted HDL (0.4 mg of protein/ml) containing cholesteryl [1-^{14}C]arachidonate (2.8 μCi/plate) for 24 hr at 37°C. Media were then removed and subjected to thin-layer chromatography. Radioactive zones on the TLC plate were visualized by radioautography and analyzed by densitometry. Areas of radioactivity were identified by co-migration with unlabeled internal standards (i = unknown compound).

phosphatidylcholine did not stimulate release of labeled prostaglandins. However, 0.5% of the phospholipid radioactivity was incorporated into cellular lipids, with 95% in phospholipids, and 5% neutral lipids.

HDL-induced alterations in endothelial cell phospholipid fatty acid composition

The ability of HDL to enrich endothelial phospholipase-sensitive pools with arachidonic acid was also investigated by gas–liquid chromatographic measurements of cellular phospholipid fatty acid content after exposure to rat and human HDL as well as human LDL. The media were removed for measurement of 6-keto-$PGF_{1\alpha}$, and the cells were then incubated with media alone or with media containing A-23187. Cell phospholipid-fatty acids were then analyzed by gas–liquid chromatography. During the 24-hr incubation, there was greater stimulation of 6-keto-$PGF_{1\alpha}$ release by rat HDL (7.26 ± 1.29 ng/ml), compared to human HDL (3.83 ± 1.44, $P < 0.05$). The percent arachidonate in phospholipids of endothelial cells incubated with rat HDL increased twofold (5.7 ± 1.8 to 14.7 ± 1.5, $P < 0.05$); there was also a significant but smaller increase in percent arachidonate in the phospholipids of cells incubated with human HDL (10.5 ± 0.7; **Table 2**). There were no significant changes in the percent arachidonate in cells incubated with control media or LDL. In cells enriched in arachidonate, there was also an increase in the percent linoleate and a corresponding reduction in the percent oleate, but not significant changes in the percent of other fatty acids (Table 2).

A-23187 induced 6-keto-$PGF_{1\alpha}$ release in all treatment groups; the amount of 6-keto-$PGF_{1\alpha}$ produced by cells that had been incubated with lipoproteins, however, was larger than that of the control cells ($P < 0.05$). A-23187 induced a greater release of 6-keto-$PGF_{1\alpha}$ and a greater reduction in phospholipid arachidonate in cells preincubated with rat HDL and human HDL compared with the control cultures where the reduction in the percent arachidonate following A-23187 was not significant (Table 2).

Comparison of human LDL and rat HDL; effects of modifying lipoprotein-cholesteryl arachidonate

To investigate further whether HDL-induced prostacyclin release by endothelial cells was dependent upon the content of cholesteryl arachidonate in HDL, endothelial cells were exposed to HDL whose cholesteryl arachidonate content had been reduced by co-incubation with LDL in the presence of the cholesteryl ester exchange protein (14). Fatty acid analysis of lipoprotein cholesteryl esters revealed that modified rat HDL contained significantly less (11.71 ± 0.28%, n = 13) cholesteryl arachidonate, compared to native rat HDL (27.72 ± 3.43% cholesteryl arachidonate, n = 3, $P < 0.01$). In the modified LDL there was an increase in cholesteryl arachidonate (10.0 ± 0.8%; n = 6) relative to unmodified LDL (4.9 ± 0.6%; n = 2; $P < 0.05$) and a corresponding reduction of cholesteryl linoleate. At equivalent cholesterol concentrations, rat HDL depleted of cholesteryl arachidonate induced significantly less 6-keto-$PGF_{1\alpha}$ release (10.70 ± 0.28 ng/ml) than did native HDL (17.40 ± 0.67 ng/ml, n = 17, $P < 0.01$). The modified LDL stimulated 6-keto-$PGF_{1\alpha}$ release more than native LDL (1.07 vs 0.59 ng/ml) when cholesteryl arachidonate was increased from 8.89 to 27.72% in the modified LDL particle.

Role of apoE in HDL-induced 6-keto-$PGF_{1\alpha}$ release

To investigate a possible role of apoE in the greater stimulation of 6-keto-$PGF_{1\alpha}$ release by rat HDL, rat HDL was fractionated into apoE-rich and apoE-poor fractions by heparin affinity chromatography. When incubated with endothelial cells, the apoE-depleted fraction of HDL induced a greater stimulation of 6-keto-$PGF_{1\alpha}$ release than the apoE-rich fraction (8.96 ± 3.01 vs 2.82 ± 1.01 ng/ml, n = 6, $P < 0.05$). Since the apoE-rich fraction represented less than 10% of the total cholesterol mass of rat HDL, the data indicate that the stimulation of PGI_2 synthesis by rat HDL is produced primarily by the fraction of HDL that is rich in apoA-I and poor in apoE. Thus, the greater stimulation of 6-keto-$PGF_{1\alpha}$ by rat HDL is probably due to its high content of cholesteryl arachidonate and not to its high amount of apoE.

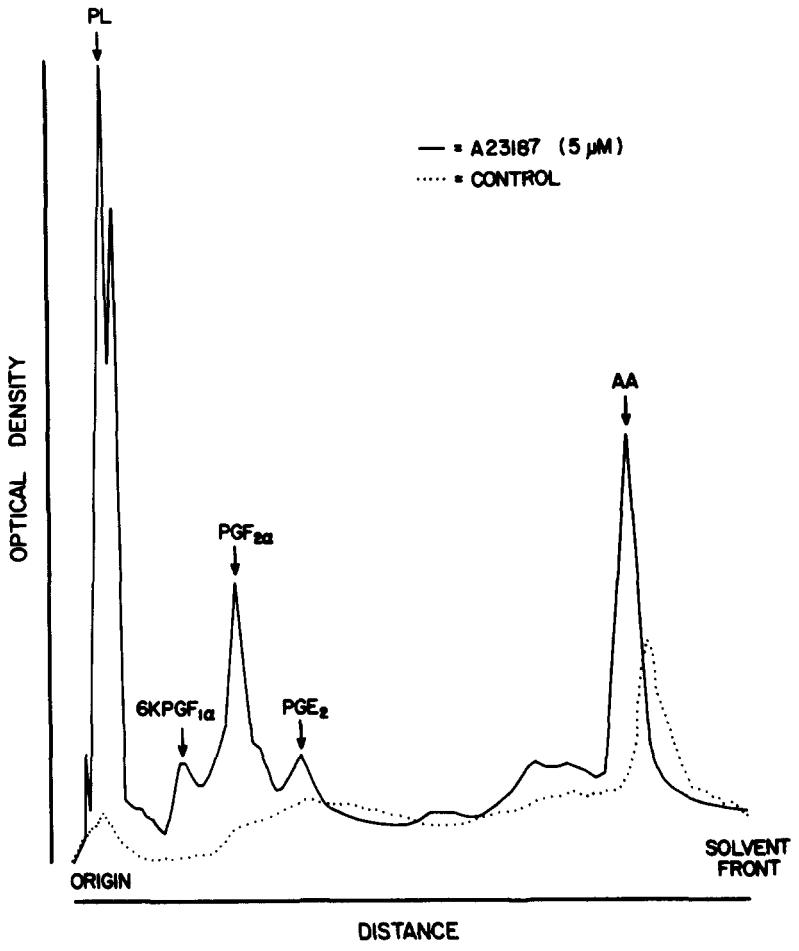

Fig. 3. Calcium ionophore stimulation of [1-^{14}C]arachidonate release and production of [1-^{14}C]prostaglandins by cells preincubated with reconstituted HDL containing cholesteryl [1-^{14}C]arachidonate. Endothelial cells grown into subconfluent density in duplicate 25-cm^2 flasks in DME containing 10% fetal bovine serum were exposed to reconstituted HDL (0.4 mg of protein/ml) containing cholesteryl [1-^{14}C]arachidonate (2.8 μCi/plate). The media were removed and the cells were washed three times in PBS containing 0.1% BSA, then three times in albumin-free PBS. One flask was then incubated in DME alone, while the parallel flask was exposed to DME containing A-23187. The supernatants of each flask were then removed, and analyzed by thin-layer chromatography, radioautography, and densitometry as described in Fig. 2 (solid line = DME + A-23187, dotted line = DME alone).

Because apo-HDL stimulated 6-keto-PGF$_{1\alpha}$ release, we investigated the possibility that phospholipase activity was enhanced by HDL. Endothelial cells were prelabeled with [1-^{14}C]arachidonate prior to incubation with unlabeled HDL. In these experiments, HDL increased the amount of radioactivity incorporated into 6-keto-PGF$_{1\alpha}$, PGE$_2$, and free arachidonate relative to control incubations. However, these amounts of radioactivity were significantly less than that produced by the calcium ionophore, A-23187 (**Table 3**).

DISCUSSION

The results of these experiments indicate that arachidonate is transferred from the lipid moiety of HDL to cultured endothelial cells where it acts as substrate for cyclooxygenase, resulting in PGI$_2$ synthesis. Direct evidence for a role of HDL cholesteryl arachidonate in the stimulation of endothelial cell PGI$_2$ biosynthesis is provided by the incorporation of label from cholesteryl [1-^{14}C]arachidonate of reconstituted HDL into 6-keto-PGF$_{1\alpha}$ (Figs. 2 and 3). Analysis of cell lipids in these experiments showed incorporation of radiolabel into cellular phospholipids and neutral lipids. The results imply that arachidonate in HDL cholesteryl esters is made available to the cyclooxygenase pathway of endothelial cells following hydrolysis and incorporation into cellular lipids.

HDL can enrich the phospholipids of endothelial cells with arachidonate (Table 2). The greater enrichment of cell phospholipid arachidonate by rat HDL, which contains more cholesteryl arachidonate than human HDL, is consistent with the hypothesis that HDL cholesteryl arachidonate undergoes hydrolysis and incorporation into

TABLE 1.

Cell Lipid Class	Treatment	
	Control	A-23187
Phospholipids	39.16[a]	29.33
Free fatty acids	1.08	5.37
Triglycerides	1.94	16.46
Diglycerides	6.05	9.47
Monoglycerides	1.48	0.96

A-23187 stimulates transfer of arachidonate in cellular phospholipid pools to free fatty acid and triglycerides by endothelial cells incubated with reconstituted HDL containing cholesteryl [1-^{14}C]arachidonate. Endothelial cells as described in Fig. 2 were extracted by the method of Folch et al. (18). The cell lipids were separated by TLC. Radioactivity of each lipid was determined by scintillation counting of the TLC plate scrapings.

[a]The data are expressed as the percent of total cell lipid radioactivity per treatment.

cellular phospholipid pools. The incorporation of label from cholesteryl [1-^{14}C]arachidonate in reconstituted HDL into endothelial phospholipids (Table 1) is also consistent with this hypothesis. The subsequent fall in cellular phospholipid arachidonate (Table 2) and the release of label from the cells with incorporation into 6-keto-$PGF_{1\alpha}$ by A-23187 (Table 1; Figs. 1 and 2) indicate that lipoprotein arachidonate is provided to a cellular phospholipid pool that is sensitive to phospholipases and accessible to cyclooxygenase.

It is of interest to note that the radiolabel of HDL containing 1-palmitoyl 2-[^{3}H]arachidonyl phosphatidylcholine was not incorporated into prostaglandins, even though it was incorporated into cellular phospholipids in amounts similar to the radiolabel of HDL containing cholesteryl [1-^{14}C]arachidonate. This suggests that the arachidonate from cholesteryl esters enters a different pool of cellular lipids that is more available for prostaglandin synthesis.

Indirect evidence that HDL arachidonate promotes PGI_2 biosynthesis was also obtained from the experiments with the cholesteryl ester transfer protein. By co-incubating arachidonate-rich rat HDL with linoleate-rich human LDL in the presence of the cholesteryl ester transfer protein, cholesteryl arachidonate in HDL was partially replaced with cholesteryl linoleate from LDL. The stimulation of 6-keto-$PGF_{1\alpha}$ release by the modified rat HDL was lower than that produced by native rat HDL. The increased stimulation of 6-keto-$PGF_{1\alpha}$ release by rat HDL and its reversal with depletion of cholesteryl arachidonate points to the quantitative importance of lipoprotein cholesteryl arachidonate in promoting prostaglandin biosynthesis in this tissue.

The importance of lipoprotein cholesteryl arachidonate to endothelial cell PGI_2 biosynthesis is further suggested by the enhancement of 6-keto-$PGF_{1\alpha}$ release by cells incubated with modified LDL that had become enriched in cholesteryl arachidonate. However, at approximately the same cholesteryl arachidonate content, native rat HDL was threefold more effective than modified LDL in stimulating 6-keto-$PGF_{1\alpha}$ release. Thus, there is relative but not absolute specificity of HDL in producing 6-keto-$PGF_{1\alpha}$ release. This could be related to the greater surface area to volume of HDL compared to LDL. Thus, a particle of HDL has about 3.2 times as much lipid surface area per cholesteryl ester molecule as an LDL particle (23), possibly endowing the HDL with a greater capacity to exchange neutral lipids with cells.

The molecular mechanism of entry of HDL arachidonate into cells is not known at this time. In our experiments, the release of 6-keto-$PGF_{1\alpha}$ upon incubation with HDL did not depend on receptor-mediated endocytosis involving the apoB, E receptor since HDL depleted of apoE produced a larger stimulation of 6-keto-$PGF_{1\alpha}$ release than the apoE-rich fraction. Also, we have shown that co-incubation of HDL with an excess of nonstimulatory LDL did not reduce the 6-keto-$PGF_{1\alpha}$ release produced by HDL (9) and that the prostaglandin response to HDL

TABLE 2. Effect of HDL_3, rat HDL, and LDL on endothelial cell phospholipid fatty acid content and modification by A-23187

Fatty Acid	Control		HDL_3		Rat HDL		LDL	
	DME	A-23187	DME	A-23187	DME	A-23187	DME	A-23187
16:0	29.7 ± 2.9	30.7 ± 2.1	33.2 ± 2.2	36.3 ± 2.5	31.1 ± 4.2	34.1 ± 4.9	29.4 ± 1.8	31.6 ± 2.4
16:1	6.4 ± 1.2	6.6 ± 0.3	3.7 ± 0.9	3.5 ± 0.9	5.2 ± 1.5	2.9 ± 0.5	5.5 ± 1.4	5.3 ± 1.9
18:0	20.9 ± 2.4	20.3 ± 1.9	19.3 ± 2.3	19.5 ± 0.9	19.2 ± 1.3	22.8 ± 1.5	19.9 ± 1.8	21.1 ± 1.9
18:1	34.8 ± 2.1	31.2 ± 5.8	23.4 ± 2.37[a]	23.9 ± 0.9	21.5 ± 0.8[a]	22.5 ± 0.9	26.0 ± 4.9[a]	32.0 ± 1.1
18:2	1.9 ± 0.7	1.4 ± 0.5	8.1 ± 1.1[a]	8.1 ± 0.7	7.9 ± 1.1[a]	8.0 ± 1.0	4.5 ± 1.4	3.6 ± 0.5
20:4	5.7 ± 1.8	4.8 ± 1.5	10.5 ± 0.7[a]	7.4 ± 1.2[b]	14.7 ± 1.5[a]	9.7 ± 2.1[b]	6.6 ± 1.2	6.5 ± 1.4
6k$PGF_{1\alpha}$ (ng/ml)	0.2 ± 0.03	1.1 ± 0.1[b]	0.2 ± 0.1	1.8 ± 0.2[a,b]	0.2 ± 0.1	1.6 ± 0.4[a,b]	0.2 ± 0.1	1.6 ± 0.2[a,b]

Endothelial cells at subconfluent density were incubated in DME alone, or DME containing either human or rat HDL (0.4 mg of protein/ml) for 24 hr. The supernatant was assayed for immunoreactive 6-keto-$PGF_{1\alpha}$ by RIA. The cells were then exposed to media alone or media containing the calcium ionophore, A-23187, (10 μM). The media were then assayed for immunoreactive 6-keto-$PGF_{1\alpha}$. The cells were then extracted. The cellular phospholipids were separated from the other lipid classes by TLC, and transmethylated using BF_3-methanol; the resultant fatty acid methyl esters were then separated by gas-liquid chromatography. Data are expressed as percent of each fatty acid (n = 5, with three replicates per treatment; data are expressed as mean ± SE).

[a]$P < 0.05$ from control (DME).

[b]$P < 0.05$ from control (DME) within treatment group.

TABLE 3. Effect of HDL and A-23187 on [1-^{14}C]arachidonate and [1-^{14}C]prostaglandin release from endothelial cells prelabeled with [1-^{14}C]arachidonate

Treatment	6-keto-$PGF_{1\alpha}$	$PGF_{2\alpha}$	PGE_2	Arachidonate
Control	163 ± 42	373 ± 107	167 ± 27	841 ± 126
HDL_3	375 ± 39[a]	1070 ± 182[a]	307 ± 54[a]	4996 ± 714[a]
A-23187	1353 ± 203[a]	5391 ± 720[a]	888 ± 182[a]	15839 ± 3077[a]

Endothelial cells were incubated overnight with 2 μCi of [1-^{14}C]arachidonate/plate. The cells were then exposed to DME alone (control) or DME containing HDL_3 (0.4 mg of protein/ml) for 24 hr at 37°C. Cells were also exposed to A-23187 (5 μM) for 30 min. The media were then removed, acidified, extracted, and developed by TLC to separate prostaglandins as described. Data are expressed as DPM (n = 3, with three replicates of each treatment group per experiment; mean ± SE).

[a] $P < 0.05$ from control.

is not inhibited by chloroquine (Pomerantz, K. B., L. N. Fleisher, A. R. Tall, and P. J. Cannon, unpublished observation). Our data suggest that HDL cholesteryl esters are hydrolyzed with subsequent incorporation of free arachidonate into cellular phospholipids and into prostaglandins. Since very little HDL apoprotein is internalized in endothelial cells, hydrolysis of cholesteryl esters might occur at the cell surface. Alternatively, intact cholesteryl arachidonate may be enter cells prior to hydrolysis by mechanisms analagous to the transfer of intact cholesteryl ester molecules from HDL into a variety of tissues (24) or to the entry of chylomicron cholesteryl esters into endothelial cells (25). From these studies, there appears to be a mechanism of entry of HDL cholesteryl esters into cells independent of particle uptake. Such mechanisms of entry of HDL cholesteryl esters might involve diffusion through the aqueous phase, pinocytosis, HDL binding (which appears to be mediated by nonspecific lipid-lipid interactions in endothelial cells (26)), formation of a shuttle complex (for example, with small amounts of cholesteryl transfer protein present in the HDL preparations (27)), or specific transport into the cells.

The physiological significance of the transfer of arachidonate from HDL lipid to endothelial cells or of the stimulation of cellular prostacyclin synthesis by HDL is unknown at this time. Our results, which indicate that HDL can provide arachidonate as substrate for PGI_2 synthesis by endothelial cells, may explain in part the ability of endothelial cells to synthesize large amounts of PGI_2 despite deficiency of the enzyme required to elongate linoleic acid to arachidonic acid (6, 7). Whether HDL-derived arachidonate or arachidonate bound to albumin provides the quantitatively more important extracellular source of substrate for cyclooxygenase in endothelial cells will require additional investigation.

Other potential roles of HDL might include replenishment of cellular arachidonate stores in endothelium subsequent to activation of phospholipases and stimulation of prostaglandin synthesis by hormones or other stimuli. It is also conceivable that HDL might promote PGI_2 release at sites of endothelial injury or, alternatively, PGI_2 release in response to lipoprotein endothelial cell interaction may be involved in the action of HDL to mobilize cholesterol from peripheral tissues (28).

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