High density lipoprotein-induced cardiac prostacyclin synthesis in vitro: relationship to cardiac arachidonate mobilization

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Abstract The objectives of this study were to characterize the effects of plasma lipoproteins on prostacyclin (PGI_2) production by the Langendorff-perfused rabbit heart, and to determine the mechanism of lipoprotein-induced cardiac PGI_2 production. PGI_2 production by perfused rabbit hearts was stimulated by injections of rabbit very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). HDL was much more effective than equivalent doses of VLDL or LDL. Infusion of HDL at a physiological concentration stimulated cardiac PGI_2 output by 417%, but infusion of VLDL or LDL was ineffective. Cardiac PGI_2 production increased from 47% to 340% with increasing doses of HDL. The release of cardiac PGI_2 in response to injections or infusions of HDL occurred rapidly; maximal release of PGI_2 was reached within 2 min after exposure to HDL. Injections of HDL stimulated the production of [^{3}H]arachidonic acid, [^{3}H]prostaglandin E_2, [^{3}H]-prostaglandin $F_{2\alpha}$, and [^{3}H]6-keto-prostaglandin $F_{1\alpha}$ from hearts after prelabeling of cardiac lipids with [^{3}H]arachidonic acid. These results indicate that plasma lipoproteins, specifically HDL, stimulate PGI_2 production by the isolated rabbit heart. The mechanism by which HDL increases cardiac PGI_2 production may involve the mobilization of cardiac arachidonic acid for PGI_2 synthesis. —**Van Sickle, W. A., H. G. Wilcox, K. U. Malik, and A. Nasjletti.** High density lipoprotein-induced cardiac prostacyclin synthesis in vitro: relationship to cardiac arachidonate mobilization. *J. Lipid Res.* 1986. **27:** 517–522.

Supplementary key words plasma lipoproteins • cardiac prostaglandins

Plasma lipoproteins have the potential of influencing a variety of cellular events concerned with lipid metabolism by virtue of their ability to deliver lipids to cells. Recently, it has been suggested that HDL delivers arachidonic acid to cultured vascular smooth muscle cells, and thereby stimulates prostaglandin synthesis (1). The ability of HDL and other lipoproteins to stimulate vascular prostaglandin synthesis has been documented with cultured vascular cells from a variety of sources (1–3), but there is no information concerning the effects of lipoproteins on prostaglandin synthesis by an intact vasculature. The present studies were conducted, therefore, to investigate the effect of HDL and other plasma lipoproteins on prostaglandin synthesis by the Langendorff-perfused rabbit heart preparation. We report that HDL stimulates cardiac PGI_2 synthesis, and that this stimulation is at least partly due to the activation of a cardiac phospholipase that increases the amount of endogenous arachidonic acid available for PGI_2 synthesis.

MATERIALS AND METHODS

Materials

Male New Zealand White (NZW) rabbits (2.0–2.5 kg) were purchased from Myrtle's Rabbitry, Nashville, TN. [^{3}H]Arachidonic acid (5,6,8,9,11,12,14,15-^{3}H; sp act 87.4 Ci/mmol, [^{14}C]phosphatidylcholine (1-palmitoyl, 2-arachidonyl-[^{14}C]glycerophosphocholine, sp act 52.0 mCi/mmol, and [^{3}H]6-keto-$PGF_{1\alpha}$ (sp act 170.0 Ci/mmol) were purchased from New England Nuclear, Boston, MA. Unlabeled 6-keto-$PGF_{1\alpha}$ was purchased from Upjohn Pharmaceutical Co., Kalamazoo, MI. The 6-keto-$PGF_{1\alpha}$ antibody was a gift from Dr. Lawrence Levine (Dept. of Biochemistry, Brandeis University, Waltham, MA). Porcine pancreatic phospholipase A_2, fatty acid-free bovine serum albumin, and the cholesterol reagents for lipoprotein total cholesterol analysis were from Sigma, St. Louis, MO. Spectropor 3 membrane tubing for lipoprotein dialysis was purchased from Fisher, Memphis, TN. Millipor Millex-GV 0.22-μm filters and Sep Pak C_{18} cartridges were purchased from Millipore Corp., Bedford, MA.

Lipoprotein isolation

Blood was obtained by cardiac puncture of overnight-fasted, male NZW rabbits anesthetized with sodium pento-

Abbreviations: HDL, high density lipoproteins; PGI_2, prostaglandin I_2 or prostacyclin; 6-keto-$PGF_{1\alpha}$, 6-keto derivative of prostaglandin $F_{1\alpha}$; VLDL, very low density lipoproteins; LDL, low density lipoproteins; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGH_2, prostaglandin H_2; TLC, thin-layer chromatography.

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barbital (60 mg/kg). Na_2EDTA (1 mg/ml) with NaN_3 (0.1 mg/ml) was used as the anticoagulant. Plasma from three to five rabbits was pooled for each preparation, and lipoprotein isolation was begun immediately. Lipoproteins were isolated by sequential, preparative ultracentrifugation of the plasma at 12°C using a 50.2 Ti rotor, a Beckman L8-80 centrifuge, and solid NaBr to make density adjustments. VLDL (d < 1.006 g/ml) and LDL (d 1.019 to 1.063 g/ml) were isolated after 18 hr at 39,000 rpm, and HDL (d 1.063 to 1.21 g/ml) after 24 hr of centrifugation at the same speed. Each lipoprotein fraction was washed once by ultracentrifugation at the upper density limit, and dialyzed at 4°C for 24 hr in Spectropor 3 membrane tubing against 3 × 1.0 liters of 0.15 M NaCl. They were filtered through 0.22-μm filters and stored at 4°C. The lipoproteins were used within 2 weeks of isolation.

Heart perfusion

Male NZW rabbits were anesthetized with sodium pentobarbital (60 mg/kg). Heparin (1000 units) was injected into the inferior vena cava after laparotomy. Hearts were excised rapidly and rinsed in gassed Krebs buffer. They were suspended from a cannula tied into the aortic arch, placed in a humidified perfusion chamber, and perfused at 19 ml/min without recirculation at 37°C with gassed (95% O_2 + 5% CO_2) Krebs buffer, using a Harvard pump.

In preliminary studies the basal output of the PGI_2 metabolite, 6-keto-$PGF_{1\alpha}$, from hearts was found to be high initially but gradually declined over the first 15 to 20 min of perfusion. It was stable after 30 min, and remained stable for at least 90 min thereafter. Experiments were conducted during this latter period. The wet weight of the hearts varied between 7 and 11 g. The correlation coefficient of the regression of heart weight on basal 6-keto-$PGF_{1\alpha}$ output was 0.288 for 14 hearts; the heart weight was, therefore, disregarded for the purposes of normalizing the data. The hearts used in these studies continued to beat throughout the experimental period.

All injections and infusions were made by way of the aortic cannula. Lipoproteins or vehicle (0.15 M NaCl) were given in random order as either bolus injections (1.0 ml) or constant infusions (1.0 ml/min) added to the perfusate. A single heart received no more than four injections spaced at least 12 min apart. Samples of cardiac effluent were collected in 20-ml plastic scintillation vials for 1.0-min periods (19-ml samples) before and after a bolus injection, and before, during, and after an infusion. The samples were stored at −20°C.

[^{3}H]Arachidonic acid labeling of hearts and release of [^{3}H]prostaglandins

Cardiac lipids were labeled with [^{3}H]arachidonic acid according to a published procedure (4). Each heart received 50 μCi of [^{3}H]arachidonic acid, initially dissolved in 1.0 ml of 50 mM Na_2CO_3, and brought to 5.0 ml with Krebs buffer immediately before infusion. The labeled arachidonic acid was infused at 2.5 μCi/min over a 20-min period during perfusion with Krebs buffer. Following the period of labeling, the hearts were perfused for 5.0 min with Krebs buffer, followed by 10 min of perfusion with Krebs buffer containing 1.0 mg/ml fatty acid-free bovine serum albumin to remove unbound arachidonic acid. The radiolabeled hearts were then perfused for an additional 10 min prior to the start of an experiment. In one heart, 92% of the [^{3}H]arachidonic acid was recovered in cardiac phospholipids, 6% in triglycerides, and 1% each in unesterified fatty acids and cholesteryl ester, in agreement with other reports (4, 5).

To determine whether HDL or LDL increased the production of [^{3}H]prostaglandins by these hearts, the cardiac effluent was collected for 2.0 min immediately before, and for 2.0 min immediately after an injection of lipoprotein (2.0 mg of cholesterol). A third sample was collected beginning 11.0 min later. Bradykinin (5 μg) was injected through the same hearts to provide a positive control (5). [^{3}H]Prostaglandins in the cardiac effluent were extracted as previously described (6). The samples were acidified with formic acid to pH 3.0 and passed through Sep Pak C_{18} columns prewashed with methanol and water. Prostaglandins were eluted by successively washing the columns with water (20 ml), petroleum ether (10 ml), and methyl formate (10 ml). The methyl formate fractions containing the prostaglandins were dried under N_2 gas, dissolved in methanol, and the prostaglandins were separated by TLC using silica gel G plates developed in isooctane–ethyl acetate–acetic acid–water 25:55:10:50 (by volume). Authentic prostaglandin standards were chromatographed concurrently. The samples and standards were identified with iodine vapor after development, and the zones corresponding to arachidonic acid, 6-keto-$PGF_{1\alpha}$, PGE_2, and $PGF_{2\alpha}$ were scraped off of the plates and counted in Insta-Gel scintillation fluid. The recoveries of tritiated 6-keto-$PGF_{1\alpha}$, PGE_2, $PGF_{2\alpha}$, and arachidonic acid were 88 ± 2%, 88 ± 3%, 92 ± 4%, and 73 ± 3%, respectively.

Analytical procedures

Levels of the stable prostacyclin metabolite, 6-keto-$PGF_{1\alpha}$, were measured directly in 100-μl aliquots of cardiac effluent by radioimmunoassay using 6-keto-$PGF_{1\alpha}$ antisera and tracer as previously described (7), except that standard curves were constructed using unlabeled 6-keto-$PGF_{1\alpha}$ diluted in gassed Krebs buffer. The maximum inhibition of antigen–antibody binding by lipoproteins (0.1 mg cholesterol/ml) in this radioimmunoassay was 12.3%, which corresponded to 0.012 ng of 6-keto-$PGF_{1\alpha}$ immunoactivity. Lipoprotein total cholesterol was measured enzymatically using a commercial kit. Phospholipase

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activity of HDL preparations was determined according to Russo-Marie and Duval (8), using phosphatidylcholine labeled with [^{14}C]arachidonic acid in the *sn*-2 position as substrate.

Cardiac PGI_2 production was expressed as ng of 6-keto-$PGF_{1\alpha}$ per min of perfusion. Basal (unstimulated) production and either lipoprotein- or vehicle-induced production were designated as "before" and "after", respectively, except for the data concerning [^{3}H]prostaglandin release where release during a recovery period was also measured. The data were summarized as mean ± standard error for each experiment. Paired *t*-tests were used to analyze the data for some experiments. For others, the analysis of variance, randomized block design, was used following log transformation of the data to achieve homogeneity of variances. Multiple comparisons were made by the Student-Newman-Kuels test with $P < 0.05$ chosen as the minimal acceptable level of significance. The type of data analysis is given in the figure legends.

RESULTS

Lipoprotein-induced cardiac 6-keto-$PGF_{1\alpha}$ production

HDL stimulated production of 6-keto-$PGF_{1\alpha}$ by the perfused hearts. The time course of the HDL effect is shown in **Fig. 1**. Single bolus injections of HDL caused 6-keto-$PGF_{1\alpha}$ release to increase rapidly; a maximal output was reached within 2 min of the dose. The production of 6-keto-$PGF_{1\alpha}$ in response to bolus injections of HDL remained elevated for at least 3 min, after which it returned to basal levels. **Fig. 2** shows that the production of cardiac 6-keto-$PGF_{1\alpha}$ was dependent on the dose of HDL. The levels of 6-keto-$PGF_{1\alpha}$ in the cardiac effluent increased from 47% to 340% with increasing doses of HDL. A maximal enhancement of cardiac 6-keto-$PGF_{1\alpha}$ output was not reached within the dose range studied. The magnitude of cardiac 6-keto-$PGF_{1\alpha}$ output in response to HDL was markedly greater than that in response to equivalent doses (as cholesterol) of VLDL or LDL (**Fig. 3**). Both VLDL and LDL caused statistically significant increases in the production of cardiac 6-keto-$PGF_{1\alpha}$, but their effect was quantitatively minor in comparison to HDL. The effects of free arachidonic acid and of arachidonic acid complexed to albumin on cardiac 6-keto-$PGF_{1\alpha}$ release are given in **Table 1**. One ml of a 20 μM solution of free arachidonate caused a 72-fold increase in the release of 6-keto-$PGF_{1\alpha}$. However, no significant stimulation occurred when the same amount of arachidonic acid complexed to albumin was injected. The arachidonic acid–albumin complex stimulated cardiac 6-keto-$PGF_{1\alpha}$ release when the molar ratio of fatty acid to albumin was greatly increased.

A study was conducted to determine whether lipoproteins could stimulate cardiac 6-keto-$PGF_{1\alpha}$ production

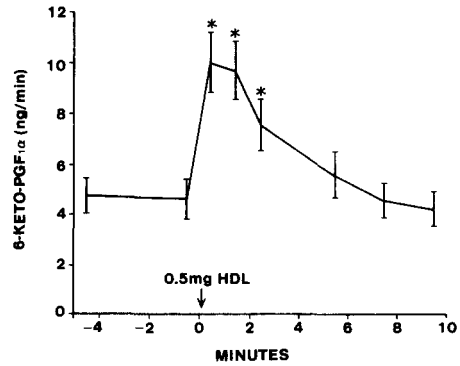

Fig. 1. Release of 6-keto-$PGF_{1\alpha}$ from rabbit hearts as a function of time before and after bolus injections of HDL. HDL (0.5 mg of cholesterol) was injected into the cardiac perfusate at the indicated time, and immunoreactive 6-keto-$PGF_{1\alpha}$ was measured in samples of cardiac effluent collected at 1-min intervals. Points are mean ± SEM plotted at the midpoint of the collection interval; n = 8; *, $P < 0.05$.

when administered as constant infusions. The concentrations of VLDL, LDL, and HDL cholesterol in whole blood of male NZW rabbits are approximately 0.02, 0.09, and 0.12 mg/ml, respectively (9, 10) (Van Sickle, W. A., unpublished data), considering that these animals have a hematocrit of 0.38. The effects on cardiac 6-keto-$PGF_{1\alpha}$ production of adding these lipoproteins to the perfusate to achieve the above concentrations are shown in **Fig. 4**. Infusion of HDL caused cardiac effluent 6-keto-$PGF_{1\alpha}$ levels to increase by 417% above basal levels. This increase occurred rapidly after the start of infusion and was maintained at a constant level until the infusion was stopped. There was no evidence of tachyphylaxis to HDL. Both VLDL and LDL failed to appreciably affect cardiac 6-keto-$PGF_{1\alpha}$ production when infused at the above concentrations.

HDL-induced release of [^{3}H]prostaglandins from hearts prelabeled with [^{3}H]arachidonic acid

To examine the possibility that HDL stimulates cardiac prostaglandin production by mobilizing arachidonic acid from cardiac lipids, hearts were prelabeled with [^{3}H]-arachidonic acid and the release of [^{3}H]prostaglandins was measured before and after injections of HDL, LDL, and bradykinin, a known activator of rabbit heart phospholipases (5). HDL did, indeed, augment the release of radiolabeled prostaglandins from the prelabeled hearts (**Fig. 5**). An injection of HDL caused the output of [^{3}H]-arachidonate to increase by an average of 79%, [^{3}H]PGE_2 by 46%, [^{3}H]$PGF_{2\alpha}$ by 57%, and [^{3}H]6-keto-$PGF_{1\alpha}$ by 151%. The output of these [^{3}H]prostaglandins returned to basal levels within 11.0 min. The effects of LDL and bradykinin on the release of cardiac [^{3}H]prostaglandins are given in **Table 2**. LDL, at a cholesterol concentration equivalent to HDL, produced an appreciable increase in [^{3}H]6-keto-$PGF_{1\alpha}$ release in only one of the hearts tested;

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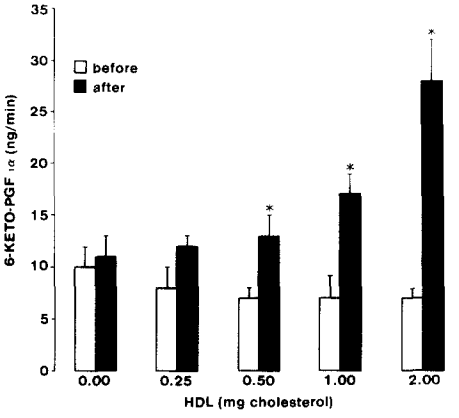

Fig. 2. Dose–response relationship of HDL versus cardiac 6-keto-$PGF_{1\alpha}$ production. Single samples of cardiac effluent were collected for 1.0 min each immediately before HDL, and two separate samples were collected for 1.0 min each immediately after doses of HDL. The post-HDL peak 6-keto-$PGF_{1\alpha}$ response is shown on the figure. Bars are mean ± SEM; n = 4; *, $P < 0.05$, paired *t*-tests.

it failed to affect the release of other [^{3}H]prostaglandins. Bradykinin, as expected, enhanced the release of [^{3}H]6-keto-$PGF_{1\alpha}$ from these hearts.

The ability of HDL to stimulate prostaglandin production by the hearts may have been due to a phospholipase that co-isolated with HDL. However, we found that the average phospholipase activity of HDL was 13.4 ± 1.3 pmol of arachidonic acid release per mg of protein per hr (n = 4) which was negligible. Injections of 5.8 times this amount of HDL-phospholipase activity as pancreatic phospholipase A_2 (in 1.0 ml of 0.15 M NaCl) through the hearts increased cardiac 6-keto-$PGF_{1\alpha}$ production by 1.0 ± 0.5 ng/min (n = 6). This value did not differ from the vehicle (2.6 ± 1.0 ng/ml), indicating that the residual lipase activity of HDL was not responsible for stimulating cardiac 6-keto-$PGF_{1\alpha}$ release.

DISCUSSION

Our data demonstrate that HDL stimulates the production of PGI_2 (measured as its metabolite, 6-keto-$PGF_{1\alpha}$) by the isolated, perfused rabbit heart. We cannot identify the type of cell that is affected by HDL, however this result is consistent with other reports of a stimulatory effect of HDL on PGI_2 synthesis by cultured vascular cells (1–3) and on the conversion of the prostaglandin endoperoxide, PGH_2, to PGI_2 by aortic microsomes (11).

We found that VLDL and LDL also stimulated cardiac PGI_2 synthesis, but at equivalent doses of cholesterol were less effective than HDL. Furthermore, we found that an arachidonic acid–albumin complex failed to stimulate cardiac PGI_2 synthesis unless an unusually high molar ratio of arachidonate to albumin was used. We used only one dose level of VLDL and LDL, and it is possible that higher doses could have stimulated cardiac PGI_2 production to a greater extent. That doses of LDL higher than HDL can indeed stimulate PGI_2 production to the same extent as HDL has been demonstrated in cultured endothelial cells (3). Nevertheless, our data are consistent with other reports suggesting a relative specificity of HDL among the plasma lipoproteins with regard to stimulation of vascular PGI_2 synthesis (1, 2).

The mechanism by which HDL stimulates cardiac prostaglandin production could involve the activation of a cardiac lipase with the consequent liberation of endogenous arachidonic acid from membrane phospholipids, the indirect delivery of arachidonic acid to the heart by the lipoprotein, or the activation of cyclooxygenase or PGI_2 synthetase by the lipoprotein. The latter possibility, suggested by the work of Beitz and Forster (11), seems unlikely because the availability of arachidonic acid, rather than the activity of these enzymes, is believed to be rate-limiting for prostaglandin synthesis (12). The second possibility, that HDL could deliver arachidonic acid to the heart, has received support from cell culture studies (1). In these studies there was a slow and continuous accumulation of PGI_2 in the culture media during incubation with HDL. This is consistent with a mechanism involving the continuous uptake and degradation of the lipoprotein and the conversion of lipoprotein arachidonate to PGI_2. Pomerantz et al. (1), in a direct test of this hypothesis, found that [^{14}C]prostaglandins were produced by cultured aortic smooth muscle cells during incubation with recombinant HDL containing cholesteryl [^{14}C]arachidonate. In this study the recombinant HDL was incubated with the cells for 24 hr before the culture media was removed and analyzed for radiolabeled prostaglandins. In our study, however, cardiac PGI_2 production reached a maxi-

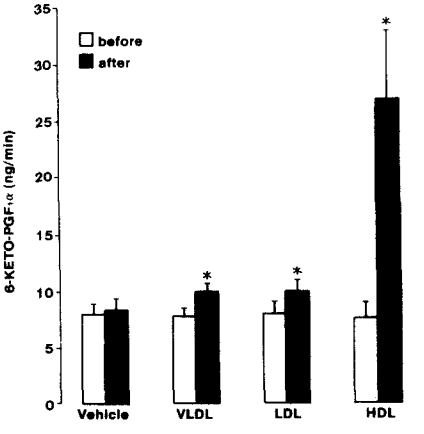

Fig. 3. Comparison of the effects of VLDL, LDL, HDL, and vehicle on cardiac 6-keto-$PGF_{1\alpha}$ production. One mg of lipoprotein cholesterol in 1.0 ml of 0.15 M NaCl or 1.0 ml of 0.15 M NaCl (vehicle) was injected into the cardiac perfusate. Samples of cardiac effluent were collected as indicated in the legend of Fig. 2, and assayed for 6-keto-$PGF_{1\alpha}$ by RIA. Bars are mean ± SEM; n = 6; *, $P < 0.05$.

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TABLE 1. Effect of arachidonic acid and of arachidonic acid bound to albumin on rabbit heart 6-keto-$PGF_{1\alpha}$ release[a]

	6-keto-$PGF_{1\alpha}$ (ng/min)		
	Before	After	Difference
20 μM Arachidonic acid	10 ± 2	730 ± 107	719 ± 108[b]
20 μM Arachidonic acid plus 100 μM BSA	10 ± 1	13 ± 2	3 ± 1
200 μM Arachidonic acid plus 100 μM BSA	9 ± 1	239 ± 43	229 ± 42[b]
100 μM BSA	9 ± 1	9 ± 1	0 ± 1

[a]One ml of 0.15 M NaCl containing the given concentrations of arachidonic acid, arachidonic acid complexed to bovine serum albumin (BSA), or BSA alone was administered as a bolus injection into the cardiac perfusate. Samples of cardiac effluent were collected for 1.0-min periods immediately before and immediately after an injection. Data are mean ± SEM; n = 3.

[b]Significant ($P < 0.05$) differences as determined by paired *t*-tests.

mum within 2 min of exposure to HDL. The velocity of this response in the rabbit heart appears to be incompatible with a mechanism involving uptake and degradation of HDL, followed by the activation of the prostaglandin cascade. Rather, our results support a mechanism involving activation of a cardiac lipase. We found that injections of HDL caused the release of radiolabeled prostaglandins from hearts that were prelabeled with [^{3}H]arachidonic acid. Since most of the [^{3}H]arachidonic acid was in phospholipids, our data suggest that HDL activated a cardiac phospholipase that made endogenous arachidonic acid available for prostaglandin synthesis. HDL could activate a phospholipase by increasing intracellular Ca^{2+} levels and by changing the structure of cell membranes, resulting in an increased access of phospholipid substrate to the enzyme. Regardless, our results do not rule out the possibility that HDL also stimulates vascular prostaglandin synthesis by delivering arachidonic acid to the heart. An additional mechanism of HDL-induced vascular prostaglandin synthesis, however, is indicated by our data.

We found that a concentration of HDL, physiologically appropriate for rabbits, infused through the rabbit heart, caused a marked and sustained enhancement of PGI_2 production. This effect, rather than that of bolus injections, may have more relevance to the in vivo setting where plasma HDL levels do not fluctuate rapidly. However, our data do not allow for conclusions to be made about the physiological importance of HDL as a stimulus for cardiac PGI_2 synthesis. This must await further evaluation. In this regard, it is known that PGI_2 has potent platelet antiaggregatory effects, dilates coronary, pulmonary, and splanchnic circulations, and may modulate the actions of a number of vasoactive hormones (see references 13 and 14 for reviews) and drugs (15, 16). PGI_2 may also play a role in HDL-mediated cholesterol efflux from cells (17), and thereby could be involved in the process of atherogenesis. Thus, an effect of HDL on vascular PGI_2 synthesis has important implications for cardiovascular homeostasis.

In summary, our results demonstrate a stimulatory action of HDL on rabbit heart PGI_2 production, and a relative specificity of this effect for HDL. The mechanism by

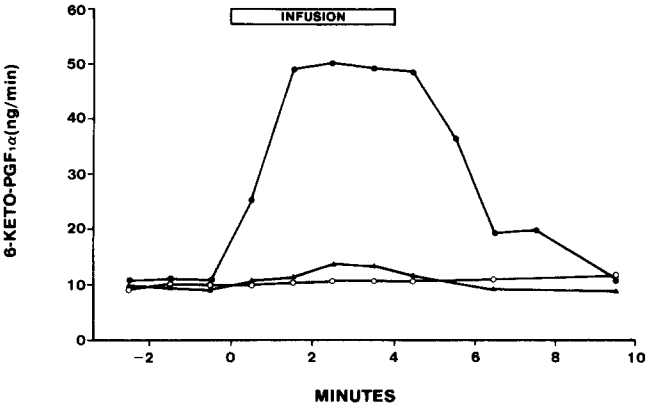

Fig. 4. Effects on cardiac 6-keto-$PGF_{1\alpha}$ production of constant infusions of HDL (●, 2.28 mg/min), VLDL (▲, 0.38 mg/min), and LDL (○, 1.71 mg/min). Infusions were begun at the zero time mark, and continued for 4 min. Data for VLDL and LDL are from single experiments; those for HDL are the average of two experiments.

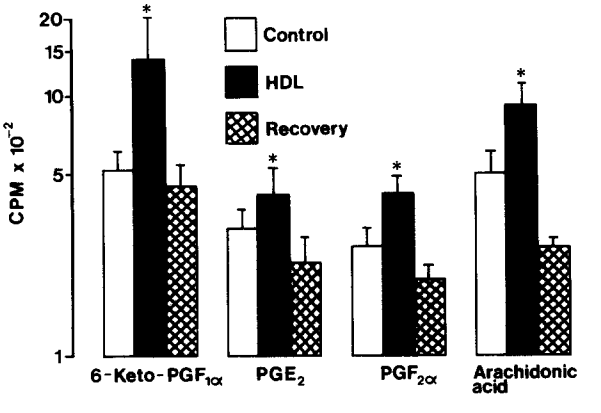

Fig. 5. HDL-induced release of ^{3}H-labeled cardiac prostaglandins. Cardiac effluent was collected for 2.0 min immediately before a bolus injection of HDL (control), and for 2.0 min immediately after HDL (2.0 mg of cholesterol). A third sample was collected between 11.0 and 13.0 min after HDL (recovery). Data are given as mean ± SEM, (n = 7) on a logarithmic scale. The asterisks refer to significant ($P < 0.05$) differences between control and HDL-induced ^{3}H-prostaglandin release as determined by Student-Newman-Kuels tests after analysis of variance.

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TABLE 2. Effects of LDL and bradykinin on the release of [^{3}H]prostaglandins by [^{3}H]arachidonate-labeled rabbit hearts

		Radioactivity (cpm)			
		6-keto-$PGF_{1\alpha}$	PGE_2	$PGF_{2\alpha}$	AA[a]
LDL	Before	593 ± 147	365 ± 78	311 ± 67	730 ± 425
LDL	After	915 ± 519	359 ± 66	354 ± 80	452 ± 132
LDL	Recovery	595 ± 235	327 ± 44	299 ± 67	570 ± 238
BK	Before	343 ± 88	168 ± 53	160 ± 44	216 ± 45
BK	After	912 ± 181[b]	198 ± 54	166 ± 26	235 ± 39
BK	Recovery	374 ± 109	122 ± 43	129 ± 34	217 ± 43

[a] An experiment identical to that described in the legend to Fig. 5 was conducted except that LDL (2.0 mg of cholesterol; n = 3) or bradykinin (BK; 5 μg; n = 4) was substituted for HDL. Data are mean ± SEM; analyzed by paired *t*-tests.

[b] $P < 0.05$; no other differences were significant.

which HDL exerts this effect is different from that proposed to account for HDL-induced prostaglandin production by cultured cells. In the isolated rabbit heart, HDL appears to act by causing the release of endogenous cardiac arachidonic acid, rather than by delivering arachidonic acid to the cardiac cells.

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REFERENCES

1. Pomerantz, K. B., A. R. Tall, S. J. Feinmark, and P. J. Cannon. 1984. Stimulation of vascular smooth muscle cell prostacyclin and prostaglandin E_2 synthesis by plasma high and low density lipoproteins. *Circ. Res.* **54:** 554–565.
2. Fleisher, L. N., A. R. Tall, L. D. Witte, R. W. Miller, and P. J. Cannon. 1982. Stimulation of arterial endothelial cell prostacyclin synthesis by high density lipoproteins. *J. Biol. Chem.* **257:** 6653–6655.
3. Spector, A. A., A. M. Scanu, T. L. Kaduce, P. H. Figard, G. M. Fless, and R. L. Czervionke. 1985. Effect of human plasma lipoproteins on prostacyclin production by cultured endothelial cells. *J. Lipid Res.* **26:** 288–297.
4. Isakson, P. C., A. Raz, and P. Needleman. 1976. Selective incorporation of ^{14}C-arachidonic acid into the phospholipids of intact tissues and subsequent metabolism to ^{14}C-prostaglandins. *Prostaglandins.* **12:** 739–748.
5. Hsueh, W., P. C. Isakson, and P. Needleman. 1977. Hormone selective lipase activation in the isolated rabbit heart. *Prostaglandins.* **13:** 1073–1091.
6. Powell, W. S. 1982. Rapid extraction of arachidonic acid metabolites from biological samples using octadecylsilyl silica. *Methods Enzymol.* **86:** 467–477.
7. Diz, D. I., P. G. Baer, and A. Nasjletti. 1983. Angiotensin II-induced hypertension in the rat. *J. Clin. Invest.* **72:** 466–477.
8. Russo-Marie, F., and D. Duval. 1982. Dexamethasone-induced inhibition of prostaglandin production does not result from a direct action on phospholipase activities but is mediated through a steroid-inducible factor. *Biochim. Biophys. Acta.* **712:** 177–185.
9. Brattsand, R. 1976. Distribution of cholesterol and triglycerides among lipoprotein fractions in fat-fed rabbits at different levels of serum cholesterol. *Atherosclerosis.* **23:** 97–110.
10. Havel, R. J., T. Kita, L. Kotite, J. P. Kane, R. L. Hamilton, J. L. Goldstein, and M. S. Brown. 1982. Concentration and composition of lipoproteins in blood plasma of the WHHL rabbit. *Arteriosclerosis.* **2:** 467–474.
11. Beitz, J., and W. Forster. 1980. Influence of human low density and high density lipoprotein cholesterol on the in vitro prostaglandin I_2 synthetase activity. *Biochim. Biophys. Acta.* **620:** 352–355.
12. Vogt, W. 1978. Role of phospholipase A_2 in prostaglandin formation. *Adv. Prostaglandin Thromboxane Res.* **3:** 89–95.
13. Dusting, G. J., S. Moncada, and J. R. Vane. 1979. Prostaglandins, their intermediates and precursors: cardiovascular actions and regulatory roles in normal and abnormal circulatory systems. *Prog. Cardiovasc. Dis.* **21:** 405–430.
14. Moncada, S., and J. R. Vane. 1979. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2, and prostacyclin. *Pharmacol. Rev.* **30:** 293–331.
15. Levin, R. I., E. A. Jaffe, B. B. Weksler, and K. Tack-Goldman. 1981. Nitroglycerin stimulates synthesis of prostacyclin by cultured human endothelial cells. *J. Clin. Invest.* **67:** 762–769.
16. Rubin, L. J., and J. D. Lazar. 1981. Influence of prostaglandin synthesis inhibitors on pulmonary vasodilatory effects of hydralazine in dogs with hypoxic pulmonary vasoconstriction. *J. Clin. Invest.* **67:** 193–200.
17. Hajjar, D. P. 1985. Prostaglandins and cyclic nucleotides. Modulators of arterial cholesterol metabolism. *Biochem. Pharmacol.* **34:** 295–300.

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