Phosphatidylinositol promotes cholesterol transport in vivo

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Abstract **To examine the role that lipoprotein charge plays in cholesterol metabolism in vivo, we characterized the effects of an intravenous injection of 40 μmol of an uncharged phospholipid (phosphatidylcholine, PC) or an anionic phospholipid (phosphatidylinositol, PI) into fasted rabbits. PC injection had a negligible effect on lipoprotein charge and composition, similar to that observed in a saline-injected animal. In contrast, PI injection caused a significant increase in the net negative surface charge of all lipoproteins after only 10 min, followed by a gradual return to normal by 24 h. Lipoprotein compositional analysis showed that PI caused a significant increase of cholesteryl ester (CE) and cholesterol (FC) in the VLDL pool by 3 h, with no changes in VLDL-triglyceride content. While the bulk of the plasma CE was located in the HDL pool in the PC-injected animals, in the PI animals, VLDL became the major CE storage compartment. No major changes in the levels or composition of HDL or LDL were evident over the 24-h turnover period. Co-injection of [^{3}H]FC revealed a 30-fold greater rate of clearance of the labeled cholesterol from the PI-injected rabbit plasma. In addition, the rate of cholesterol esterification by lecithin:cholesterol acyltransferase was almost completely inhibited in the PI animals. In summary, a bolus injection of PI into rabbits appears to enhance the mobilization of cellular sterol and promote a rapid clearance of both FC and CE from the plasma compartment. The data show that lipoprotein charge can affect cholesterol transport and that this process can be selectively manipulated.**—Stamler, C. J., D. Breznan, T. A-M. Neville, F. J. Viau, E. Camlioglu, and D. L. Sparks. **Phosphatidylinositol promotes cholesterol transport in vivo.** ***J. Lipid Res.*** **2000.** 41: **1214–1221.**

Supplementary key words caveolae • lipid clearance • cholesteryl ester • transfer protein • lipoprotein charge • scavenger receptor

The factors that regulate cholesterol flux to the liver are as yet poorly understood but are thought to involve two distinct systems: a cellular sterol regulatory system and an intravascular transport system. The classic view has been that excess extrahepatic cholesterol can be transported in high density lipoprotein (HDL) particles to the liver for excretion (1). HDL has been shown to be able to adsorb cholesterol and cholesteryl esters (CE) from cell membranes (2–4). Studies suggest that this efflux of sterol may be closely regulated by the cell and may involve specific cell surface domains called caveolae (5). HDL is thought to transport sterol either directly or indirectly to the liver and clearance appears to involve specific cell-surface receptors called scavenger receptor-BI (SR-BI) (6). The direct pathway describes the undeviating delivery of this lipid to the liver, which is thought to account for approximately 30% of hepatic uptake of CE in rabbits (7). The second, indirect, pathway constitutes the major hepatic sterol uptake route in rabbits (7). The sterol is initially transferred from HDL to the rapidly turning over VLDL remnant lipoprotein pool, from which it is then cleared by the liver (8, 9).

Intravascular sterol transport is thought to primarily involve the actions of two different plasma proteins: lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP). The consensus view has been that LCAT may form a concentration gradient to move sterol into and through the plasma compartment by promoting the conversion of free cholesterol (FC) to CE on HDL particles (10). CETP may then promote this lipid flux by moving the newly formed CE from HDL to the apoB-containing lipoprotein pool (11, 12). Studies have shown that both the rate of FC esterification and the transfer of these CE into VLDL are enhanced, both in a postprandial lipemic state (13, 14) and also in some hyperlipidemic states (12, 15, 16). This CETP-mediated enhanced transfer of CE into VLDL was originally thought to be a result of a reciprocal mass exchange of CE and triglyceride between HDL and VLDL (11). More recent work has shown, however, that both the presence of preferred triglyceride (TG)-enriched substrates and end products of

Abbreviations: CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; FC, free cholesterol; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; LPL, lipoprotein lipase; NEFA, non-esterified fatty acid; PC, palmitoyl-oleoyl phosphatidylcholine; PBS, phosphate-buffered saline; PI, phosphatidylinositol; PL, phospholipid; PLTP, phospholipid transfer protein; SR-BI, scavenger receptor-BI; TG, triglyceride; VLDL, very low density lipoproteins.

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lipolysis can influence the actions of CETP and enhance transfer into VLDL (12). Other work has revealed that the electrostatic properties of plasma lipoproteins can also affect the interlipoprotein transfer of lipid by CETP (17, 18). Investigations with LCAT have shown that this enzyme is also sensitive to charge properties of HDL (19, 20). While an increase in the protein-dependent negative charge on HDL was shown to be associated with a stimulation of LCAT, increased anionic lipid content in HDL had an inhibitory effect on the enzyme (20).

All lipoprotein classes exhibit a net negative charge and studies have shown that this charge is due to both the apolipoprotein composition of the lipoprotein and its content of anionic lipids (specifically phosphatidylinositol (PI)) (21, 22). Experiments in this laboratory have shown that interfacially active enzymes are affected by lipoprotein charge and that HDL metabolism in vivo is directly affected by the charge on HDL particles (23). The data suggest that lipoprotein charge may affect cholesterol metabolism in vivo. The present study examines the effects of the anionic lipid, PI, on lipoprotein charge and cholesterol metabolism in a rabbit. We show that this lipid: *1*) increases the negative surface charge of all the plasma lipoproteins, *2*) increases VLDL cholesterol content, and *3*) stimulates a sterol flux through the plasma compartment. The study shows that cholesterol metabolism in vivo can be manipulated by altering the charge characteristics of plasma lipoproteins.

EXPERIMENTAL PROCEDURES

Materials

[^{3}H]cholesterol was purchased from Mandel NEN Life Science Producers (Guelph, ON). 1-Palmitoyl-2-oleoyl phosphatidylcholine (PC) and phosphatidylinositol (PI) were obtained from Avanti Polar Lipids (Birmingham, AL). Silica Gel Impregnated Glass Fiber Sheets (ITLC™ SG) were obtained from Gelman Sciences (Ann Arbor, MI) and silica gel 60 plates were obtained from EM Science (Gibbstown, NJ). All other reagents were of analytical grade.

Preparation of phospholipid vesicles

PI and PC vesicles were prepared by drying 40 μmol of each lipid to completion in a 12 × 75 mm culture tube under N_2. The lipids were solubilized in 3 mL of 50 mM sodium phosphate, pH 7.2, 150 mM sodium chloride (PBS) by sonication for 1 min at constant duty cycle. The vesicles were incubated at 37°C for 10 min and then sonicated at a high output for 4 min in 10°C water bath under N_2. Samples were centrifuged for 5 min at 3,000 rpm to remove any particulate titanium.

Lipid injection into rabbits

Male New Zealand white rabbits (3.5–4.0 kg) were fasted for 12 h prior to the experiment and remained fasted until after the final time point sample was taken. Rabbits had free access to water during this time. A catheter was inserted into the marginal ear vein and blood samples were collected into tubes containing 7.5% (K_3) EDTA solution at the desired time points. A pre-injection blood sample was taken and the vesicle solution of either PI (n = 4), or PC (n = 2) or saline (n = 1) was injected into the marginal ear vein. A sample of blood was removed at 10 and 30 min, 1, 3, 6, and 24 h after the injection of the lipid vesicles. All blood samples were placed on ice and then centrifuged at 3,000 rpm for 15 min at 4°C to separate the plasma. In order to ensure that LCAT was inhibited in the stored plasma, iodoacetamide (150 mM) was added to plasma samples (24).

Characterization of [^{3}H]FC clearance

In order to determine the rate of clearance of cholesterol from the rabbit, a radioactive tracer was added to the vesicle preparations prior to injection into the rabbit. Two hundred μCi [^{3}H]FC was dried in a 12 × 75 mm culture tube with 40 μmol of PI or POPC. Three ml of PBS was added to the dried lipids and the mixture was sonicated as described above. In some studies, to verify that PI did not affect the incorporation/clearance of the tracer, the tracer was combined with 1 mg of PC and 3 mL of PBS and vesicles were prepared as described above. This tracer/vesicle preparation was injected and then, 5 min later, the PI or PC vesicles were injected and blood was sampled as described.

Characterization of lipoproteins

Lipoprotein fractions were isolated by sequential ultracentrifugation (VLDL+IDL, d < 1.019 g/mL; LDL, d 1.019–1.063 g/mL; and HDL, d 1.063–1.21 g/mL) and lipoprotein lipid composition (total cholesterol, FC, and TG concentrations) was determined enzymatically using kits from Roche Diagnostic (Laval, PQ). An aliquot of each lipoprotein was dialyzed into PBS and surface charge characteristics were determined by electrophoresis on pre-cast 0.5% agarose gels (Beckman, Paragon Lipo Kit) (21). Protein concentrations were determined using the Lowry method as modified by Markwell et al. (25). PI concentration was determined after lipid extraction in chloroform and methanol by high performance thin-layer chromatography on silica gel 60 plates using a solvent system composed of chloroform–methanol–ammonia, 65:35:5 (v/v/v). Lipids were charred with a 10% copper sulfate (w/v), 8% phosphoric acid (w/w) solution and PI concentration was quantified from a standard curve. To determine the amount of ^{3}H associated with FC and CE, a fraction of each lipoprotein (20 μL) and whole plasma (40 μL) was extracted in ethanol and hexane and the lipids were separated by thin-layer chromatography on ITLC™ SG plates using a solvent system composed of hexane–diethyl ether–acetic acid, 90:10:1 (v/v/v).

Measurement of cholesterol esterification

The effect of PI on LCAT activity was examined in incubations of plasma samples that were not treated with iodoacetamide. Four hundred μL of plasma at each time point was incubated for 30 min with 10 μCi of [^{3}H]FC on filter paper discs (16) at 37°C and the reaction was terminated with the addition of 2 mL ethanol. Reaction products were extracted in hexane and the amount of ^{3}H associated with CE and FC was determined by thin-layer chromatography as described above.

RESULTS

Effects of lipid vesicles on lipoprotein surface charge

Figure 1 illustrates the effect of injection of PI and PC vesicles on the estimated surface potential of HDL, LDL, and VLDL. While injection of PC or saline (not shown) had no significant effect on lipoprotein charge, injection of PI vesicles caused all lipoprotein fractions to migrate further on the agarose gel, indicative of an increased negative surface charge. This increased negative charge in

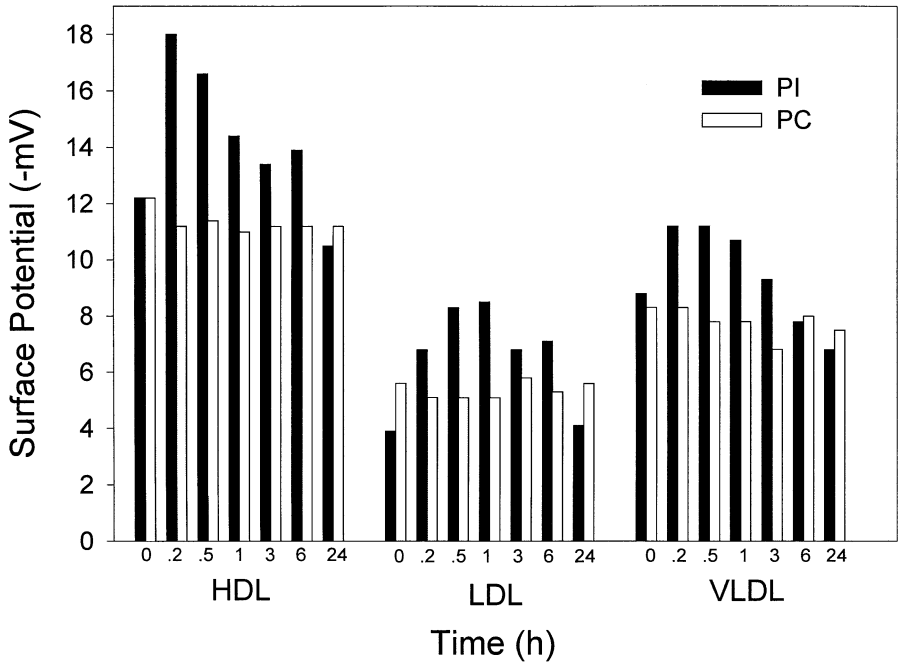

Fig. 1. Lipoprotein surface charge in rabbits injected with phosphatidylinositol (PI) or phosphatidylcholine (PC). Rabbits were injected with 40 μmol of either PI or PC vesicles and then plasma samples were taken at various time points. Lipoproteins were separated ultracentrifugally and lipoprotein surface potentials were determined as described in Experimental Procedures. Values are the average of duplicate determinations and are representative of two (PC) and four (PI) different injections.

lipoproteins from the PI-injected rabbit reached a peak early in the time course and then returned to normal by 24 h. The HDL fraction exhibited a background surface potential of −12.2 mV, prior to the PI injection and then reached a peak negative charge of −18.0 mV 10 min after the PI injection. Similarly, the VLDL fraction had an initial surface potential of −8.8 mV, which increased to −11.2 mV after 10 min. LDL charge peaked somewhat later; LDL had an initial surface potential of −3.9 mV, and peaked 60 min after the PI injection at −8.0 mV.

Effects of lipid vesicles on lipoprotein composition

Plasma lipoprotein compositions of ultracentrifugally isolated lipoproteins sampled before and after the bolus injection of phospholipid were determined. The protein concentrations of the HDL fractions remained relatively constant after injection of the different lipids, while the protein concentrations in the LDL and VLDL fractions increased slightly in both the PC and PI injected animals over time (**Table 1**). The HDL and LDL fractions exhibited no significant change in either TG/protein, CE/protein, or FC/protein ratios after injection of PI. However, injection of PC or saline was associated with a small reduction in HDL CE and FC and a concomitant increase in these lipids in LDL (Table 1). In PI-injected animals, HDL and LDL-PI concentrations increased from being below detection levels to 33.3 and 3.6 nmol/ml plasma, respectively, by 10 min post injection. The PI concentration in VLDL increased to 1.4 nmol/ml by 30 min and then all PI concentrations decreased to undetectable background levels by 3 h. VLDL fractions from the PI-injected rabbit showed a significant change in FC/protein and CE/protein ratios (**Fig. 2**). While the FC/protein ratios in VLDL increased after both PC and PI injections, the magnitude of increase was 3-fold greater in the PI-injected animal and peaked 3 h post injection (Fig. 2A). Figure 2B shows a similar 3-fold increase in VLDL-CE, which also peaked 3 h after the injection. These increases in VLDL-CE shifted the relative plasma CE storage location from the HDL pool to the VLDL pool (65–30% and 10–50% of total plasma CE, respectively). This was followed by a subsequent return to near normal VLDL compositional values by 24 h. No other changes in VLDL composition were observed in PI-injected rabbits and there was only a slight increase in CE and TG in the VLDL pools after injection of PC or saline.

Effect of PI on clearance of [^{3}H]FC and formation of [^{3}H]CE

Figure 3 compares the clearance of [^{3}H]FC at each time point for rabbits injected with PI or PC vesicles. It is evident that the radioactive FC in plasma rapidly fell to approximately 15% of the initial dose after only 1 h in the rabbit injected with PI. In comparison, the rabbit injected with PC still contained approximately 90% of the injected radioactivity after 1 h. The PC-injected rabbit took more than 6 h to clear [^{3}H]FC to a baseline level comparable to that of an animal injected with PI. The half-life of [^{3}H]FC in the PI-injected rabbits (0.3 h) was approximately 30-fold shorter than that for the PC-injected rabbits (8.4 h). To determine whether the tracer clearance was affected by co-injection of [^{3}H]FC with different lipids, experiments

TABLE 1. Effect of phospholipid injection on lipoprotein lipid and protein concentrations

	CE/Protein	FC/Protein	TG/Protein	Protein
		mg/mg protein		*mg/dl*
PC Injection				
HDL				
t = 0	0.23 ± 0.02	0.06 ± 0.01	0.12 ± 0.01	57.4 ± 2.2
t = 3	0.14 ± 0.02	0.04 ± 0.01	0.17 ± 0.02	43.9 ± 0.7
t = 24	0.11 ± 0.01	0.04 ± 0.01	0.20 ± 0.01	46.9 ± 1.7
LDL				
t = 0	3.1 ± 0.8	0.41 ± 0.05	2.7 ± 0.2	1.7 ± 0.1
t = 3	9.2 ± 0.5	0.87 ± 0.10	3.3 ± 0.3	1.4 ± 0.1
t = 24	5.8 ± 0.3	0.33 ± 0.07	2.6 ± 0.2	3.1 ± 0.1
VLDL				
t = 0	0.22 ± 0.02	0.03 ± 0.01	1.6 ± 0.1	5.7 ± 0.3
t = 3	0.44 ± 0.08	0.23 ± 0.02	3.4 ± 0.1	6.3 ± 0.1
t = 24	0.53 ± 0.05	0.32 ± 0.01	3.8 ± 0.2	9.4 ± 0.2
PI Injection				
HDL				
t = 0	0.63 ± 0.07	0.13 ± 0.01	0.29 ± 0.03	26.6 ± 2.4
t = 3	0.63 ± 0.12	0.15 ± 0.01	0.36 ± 0.05	31.8 ± 3.3
t = 24	0.70 ± 0.06	0.16 ± 0.03	0.42 ± 0.06	29.3 ± 2.6
LDL				
t = 0	1.8 ± 0.3	0.43 ± 0.02	1.6 ± 0.3	3.5 ± 0.1
t = 3	1.8 ± 0.3	0.47 ± 0.03	1.7 ± 0.2	5.3 ± 0.2
t = 24	2.0 ± 0.2	0.49 ± 0.03	2.0 ± 0.1	8.8 ± 0.5
VLDL				
t = 0	0.34 ± 0.04	0.03 ± 0.02	3.5 ± 0.2	2.9 ± 0.1
t = 3	1.50 ± 0.3	0.79 ± 0.06	4.4 ± 0.2	2.6 ± 0.1
t = 24	0.70 ± 0.2	0.50 ± 0.04	2.3 ± 0.2	5.4 ± 0.2

Phosphatidylinositol (PI) and phosphatidylcholine (PC) vesicles (40 μmol) were injected into fasting rabbits. Plasma was sampled at various times (t) and lipoproteins were isolated ultracentrifugally. Lipoprotein triglyceride (TG) cholesterol (FC), and cholesteryl ester (CE) concentrations (mg/mg protein) were determined as described and are representative of two injections of PC and four injections of PI in rabbits. Protein concentrations (mg/dl) were determined as described and values are representative of two injections of PC and four injections of PI in rabbits.

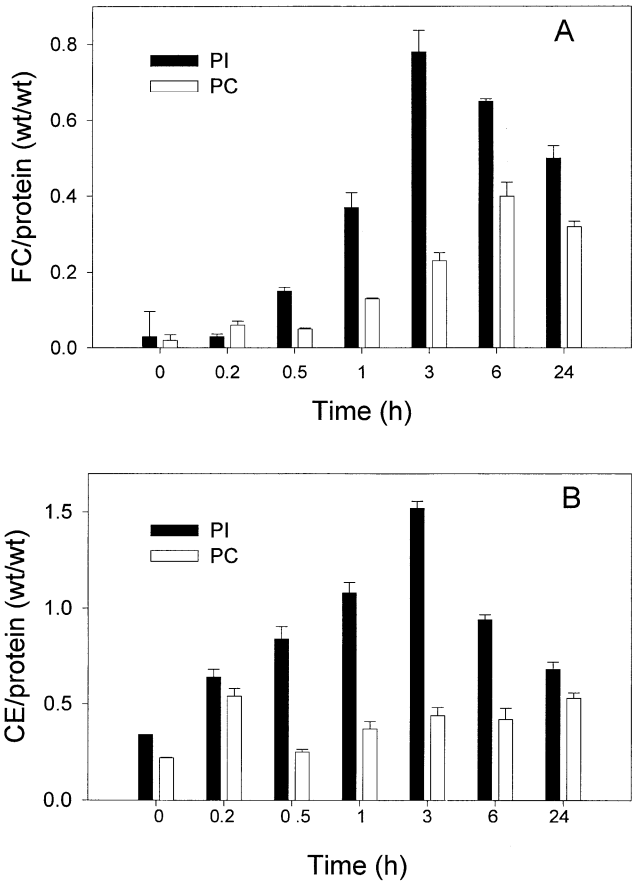

Fig. 2. Effects of phospholipid injections on VLDL composition. Rabbits were injected with equivalent amounts of either PI or PC vesicles and blood samples were taken. VLDL was isolated ultracentrifugally (d < 1.019 g/ml) and the FC (panel A) and CE (panel B) compositions were analyzed. Values are presented relative to VLDL protein and are the means of triplicate determinations. The data are representative of two (PC) and four (PI) different injections.

were undertaken where [^{3}H]FC was complexed with a small amount (1 mg) of PC, injected into the rabbit, and then PI vesicles were injected 5 min later. This resulted in the same rapid clearance of the tracer as was seen with the co-injection of PI and [^{3}H]FC (data not shown).

Figure 4 shows the amount of newly formed [^{3}H]CE remaining in the plasma after the injection of [^{3}H]FC incorporated into either PC or PI vesicles. Initial 10-min levels of [^{3}H]CE were >6 times lower in the PI-injected rabbit, as compared to the PC-injected rabbit. The levels of [^{3}H]CE in the PI-injected rabbit began to rise after 1 h and eventually rose 4-fold by 24 h. In the PC-injected rabbit, the level of [^{3}H]CE fell to approximately 10% of the injected dose by 6 h and remained constant until 24 h. It is notable that after 24 h, there was almost twice the amount of [^{3}H]CE in the plasma of the PI-injected rabbit, relative to that of the PC-injected animal.

Figure 5A and **B** illustrates the distribution of [^{3}H]FC and [^{3}H]CE in each lipoprotein fraction. In the PC-injected rabbit, [^{3}H]FC was predominantly found in the LDL and HDL fractions at the early time points (Fig. 5A). By 6 h, the amount of [^{3}H]FC in LDL and HDL fell and paralleled a major increase in the VLDL [^{3}H]FC content. In contrast, in the PI-injected animal, most of the [^{3}H]FC was found in the VLDL fraction at the early time points, while by 24 h, [^{3}H]FC was approximately equally distributed through all the lipoprotein fractions. Newly synthesized [^{3}H]CE distribution in the different lipoprotein fractions showed quite different patterns in the PI- and PC-injected animals (Fig. 5B). In the PC-injected rabbits, [^{3}H]CE was enriched in the HDL pool and depleted in the VLDL pool. However, in the PI-injected rabbits, [^{3}H]CE levels in HDL were reduced by almost 50% relative to the PC animals, and paralleled an equivalent increase of [^{3}H]CE in the VLDL pool.

Effect of the presence of PI on LCAT activity

The effect of PC or PI injections on the rate of cholesterol esterification by LCAT was measured in individual plasma samples from the various time points. No major change in LCAT activity was observed after injection of PC vesicles (**Fig. 6**). In contrast, Fig. 6 shows that endogenous LCAT activity was significantly inhibited (18% of normal) 10 min after injection of PI. PI injection reduced the fractional rates of cholesterol esterification from 45% to 8%

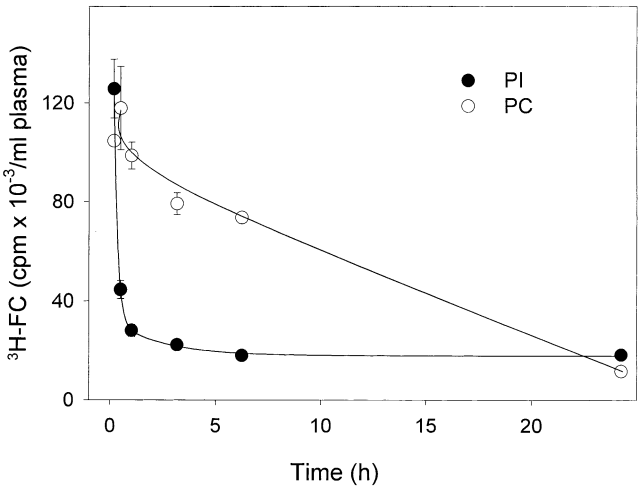

Fig. 3. Effect of phospholipid injections on the clearance of [^{3}H] free cholesterol from the plasma. Rabbits were injected with PI or PC vesicles containing [^{3}H]free cholesterol (FC). Blood samples were taken at specific intervals and [^{3}H]FC was separated from [^{3}H]CE by TLC. The plasma clearance of [^{3}H]FC is illustrated as the plasma radioactivity decay. Values are means of triplicate determinations and are representative of two (PC) and three (PI) different injections.

per hour. Six hours after the PI vesicle injection, LCAT activity returned to approximately 75% of normal.

DISCUSSION

Studies have shown that alterations in the composition of lipoprotein particles can affect their charge and structural properties and that these characteristics will influence lipoprotein remodelling and plasma lipid metabolism (26–28). In general, lipoprotein charge is primarily due to the specific apolipoprotein composition of the lipoprotein class (22, 29, 30). Our studies have shown that this protein-dependent charge is governed by the conformation of the apolipoprotein and therefore directly affected by different uncharged lipids that are able to modify the protein conformation (31, 32). In addition, lipoprotein charge is also affected by its content of charged molecules, predominantly anionic lipids such as PI and nonesterified free fatty acids (NEFA) (22). PI is an anionic lipid found in all classes of lipoproteins and accounts for approximately 4% of the total phospholipid (PL) in HDL (22). Little is known of what affects or regulates the amount of PI in the different lipoprotein classes. In contrast, numerous factors can affect the level of NEFA in plasma lipoproteins (33). Higher levels of NEFA have been

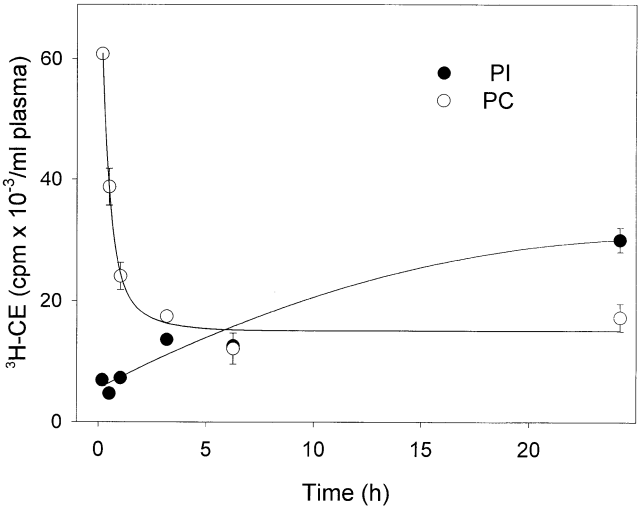

Fig. 4. Effect of phospholipid injections on the clearance of [^{3}H]cholesteryl ester from the plasma. Rabbits were injected with PI or PC vesicles containing [^{3}H]FC and plasma samples were taken. [^{3}H]FC was separated from [^{3}H]CE by TLC and the plasma clearance of [^{3}H]CE is illustrated. Values are the means of triplicate determinations and are representative of two (PC) and three (PI) different injections.

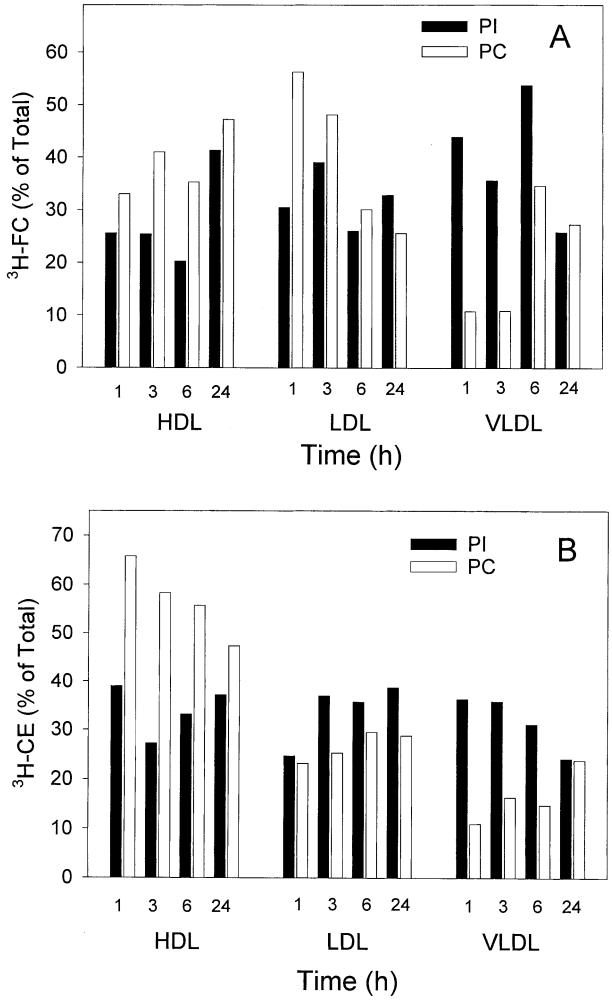

Fig. 5. Effect of phospholipid injection on the distribution of [^{3}H]free cholesterol and [^{3}H]cholesteryl ester in plasma lipoproteins. Rabbits were injected with [^{3}H]FC in either PI or PC vesicles. Lipoprotein fractions were isolated and the relative [^{3}H]FC (panel A) and [^{3}H]CE (panel B) distributions were determined after lipid extraction and TLC. Values are average of duplicate determinations and are representative of two (PC) and three (PI) different injections.

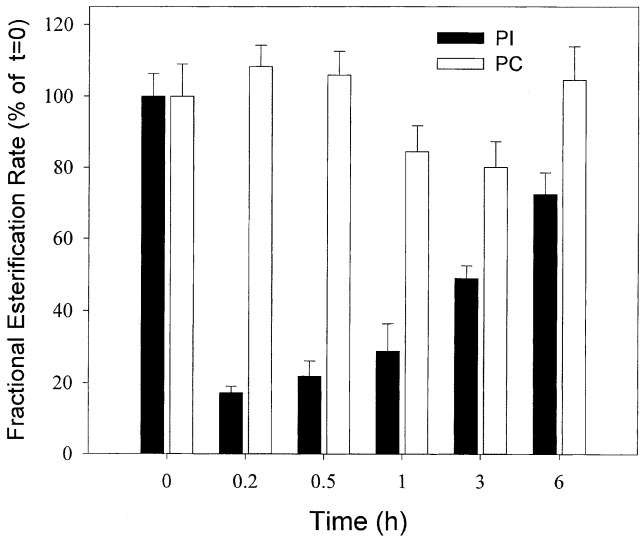

Fig. 6. Effect of phosphatidylinositol on the cholesterol esterification by LCAT. Rabbits were injected with PI or PC vesicles and then plasma samples were drawn at various times. Plasma samples were incubated in the presence of [^{3}H]FC and the rate of formation of CE by LCAT was measured after extraction and TLC. Values are the means of triplicate determinations.

found in some lipoprotein fractions of nephrotic syndrome patients and were shown to increase the negative charge of these lipoproteins (34). Similarly, alterations in the amount and function of lipoprotein lipase (LPL) in the postprandial lipemic state may directly affect lipoprotein charge (35).

Injection of PI vesicles into a rabbit caused a dramatic increase in negative surface potential of all classes of lipoprotein (Fig. 1). This was directly due to the incorporation of the anionic lipid into the lipoproteins. Incubation of PI with plasma or with ultracentrifugally isolated HDL, LDL, or VLDL in vitro has shown that all lipoproteins can spontaneously adsorb PI in a manner that appears phospholipid transfer protein (PLTP)-independent (ultracentrifuged lipoproteins are devoid of this protein). PI mass measurements showed that most of the PI was associated with the HDL particles and its level rose rapidly, from below detection levels, to up to 30% of the total HDL-PL, by 10 min. HDL-PI content then slowly decreased to about 5–10% of the HDL-PL by 3 h. The clearance of PI from the different lipoproteins may have been due to either a lipoprotein–cellular transfer or to a selective hydrolysis by an anionic phospholipid-specific phospholipase (36).

When the clearance of [^{3}H]FC from plasma was monitored, the data showed that after the injection of PI, the tracer was much more rapidly cleared from the plasma, as compared to the rabbit injected with the equivalent amount of PC (Fig. 3). Our data show that this was not the result of an increased conversion of FC to CE, as very little [^{3}H]CE was detectable and LCAT was almost completely inhibited at the early time points. Therefore, the results suggest that PI may stimulate a rapid flux and clearance of cholesterol from the plasma compartment. Several lines of evidence suggest that this clearance of FC may involve an increased hepatic uptake of this lipid. PI may directly enhance interactions with the HDL receptor, SR-BI, within caveolin-enriched domains, and promote a selective clearance of both FC and CE through this receptor. Early studies originally identified SR-BI as an anionic lipid-specific receptor that could directly bind PI and phosphatidylserine liposomes (37). Recent studies have shown that SR-BI may mediate alterations in the membrane FC domains and promote an enhanced bidirectional flux of FC between cells and plasma lipoproteins (3). As a preferred ligand, PI may increase interactions between the different plasma lipoproteins and SR-BI and thereby enhance the selective removal of specific lipids. Alternatively, PI may also promote a selective clearance of [^{3}H]FC through a CETP-mediated pathway. Several studies have shown that CETP may play a role in a selective uptake of HDL CE by hepatocytes (38, 39) and adipose tissues (40). CETP has also been shown to affect FC transfer from HDL to cell membranes (41, 42). As this protein is acutely regulated by lipoprotein charge (17), it seems likely that CETP could also affect the clearance of FC from the plasma compartment.

Injection of PI vesicles also promotes major lipid compositional changes in the VLDL pool (Fig. 2); there is an increase in the level of CE and FC by 3 h and then a return to normal by 24 h. This suggests that CE and FC may have been transported into the VLDL pool from other lipoprotein particles. CETP has been shown to regulate the interlipoprotein exchange of CE and TG (11, 43) and may also facilitate the transport of FC between different lipoprotein fractions (41, 42). An increased CETP-mediated lipid transport may therefore explain the increases in both CE and FC in VLDL in the PI-injected animal. The differential distribution of [^{3}H]CE in various lipoprotein fractions is consistent with this view and provides additional evidence that, in vivo, CETP-mediated lipid transfers into VLDL are promoted in the presence of PI. We observed between 30–40% more [^{3}H]CE in the VLDL fraction of the PI-injected rabbit, relative to the control, with an equivalent reduction in the amounts in HDL. The activity of CETP was shown to be directly affected by lipoprotein electrostatic properties (17, 18). The increased lipoprotein PI content may increase the binding of CETP to all classes of lipoprotein and thereby promote an increased lipid transfer (17). Interestingly, we observed no change in the TG content of any lipoprotein after PI injection, which suggests that CETP may utilize a unidirectional transport mechanism when lipoproteins are enriched with PI. Although CETP is normally thought to promote a neutral lipid mass transfer by exchanging TG for CE (bidirectional transfer) (11), studies have shown that CETP may also promote a unidirectional CE mass transfer in the presence of NEFA (44, 45). In vitro experiments have shown that an increase in NEFA levels can directly affect lipid transfers by CETP (17, 34, 46). As a result, LPL-generated NEFA appear to promote an enhanced transfer of CE into the VLDL pool (15, 47), which, in the postprandial lipemic state may promote a selective clearance of CE by the liver (8, 9). Therefore, PI may act similarly to NEFA and regulate the actions

of CETP by modifying lipoprotein charge. In this study, however, it is striking that increases in VLDL-FC and CE do not parallel a reduction in these lipids in HDL or LDL. This may suggest that either the lipids do not come from the HDL or LDL pools and/or that these sterol pools are able to maintain a cholesterol homeostasis in the plasma and can replenish lost CE and FC by uptake from a cellular sterol storage location.

To investigate the effect of PI on the production and transport of CE in the plasma, the amount of radioactive CE was measured at each time point (Fig. 4). The levels of [^{3}H]CE in the PI- and PC-injected rabbits were significantly different. Most notably, at the initial time points, there was 85% less [^{3}H]CE in the PI-injected rabbit than in the control. This suggested that the production of CE may be impaired in the PI-injected rabbits and so the endogenous activity of LCAT in plasma was measured (Fig. 6). The data show that enrichment of plasma lipoproteins with PI almost completely inhibits cholesterol esterification by LCAT. This observation is consistent with previous studies in this laboratory, which have shown that lipoprotein charge can affect LCAT and that high levels of PI in a reconstituted HDL particle will inhibit cholesterol esterification by LCAT (20). Therefore, it appears that the reduced levels of [^{3}H]CE in PI-injected animals were primarily due to a decreased production by LCAT. Because plasma CE levels actually rise in the presence of PI (and LCAT inhibition), the data suggest that LCAT is not solely responsible for the amount of CE in plasma lipoproteins. In contrast, it appears that PI may modify intravascular cholesterol levels by mobilizing a cellular cholesterol pool.

These experiments show that injection of the anionic lipid, PI, can dramatically affect cholesterol transport in vivo. The data suggest that PI-induced changes in lipoprotein cholesterol metabolism may be the result of altered interactions between the PI-enriched lipoproteins and specific cell surface microdomains, as well as a change in interactions with the plasma proteins involved in lipoprotein remodelling and metabolism. Almost complete inhibition of the enzyme that produces CE actually paralleled major increases in the amount of this lipid in VLDL particles. Therefore, this may suggest that the action of LCAT is not required for reverse cholesterol transport. Cholesterol transport into VLDL may instead be governed by the charge and structural characteristics of plasma lipoproteins, which may regulate interfacial interactions with specific receptors and cholesterol storage depots on cell surfaces. Control of lipoprotein charge by the administration of charged compounds may therefore allow for the manipulation of FC and CE influx/efflux from cell surfaces and the selective control of plasma cholesterol metabolism.

We would like to thank Drs. Y. Marcel and R. Milne for valuable discussions. This work was supported by a grant from the Medical Research Council of Canada.

Manuscript received 12 October 1999 and in revised form 9 March 2000.

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