Transfer of very low density lipoprotein-associated phospholipids to activated human platelets

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Abstract LDL-associated phospholipids (PLs) may be transferred into platelets. In this work, we characterized the role of VLDLs as PL donors. VLDL transferred radiolabeled PLs to platelets in a temperature- and concentration-dependent manner. LPL stimulated this process through its action on VLDL lipolysis, because it was abolished by tetrahydrolipstatin. LPL also stimulated the platelet production of thromboxane B_2 (TXB_2). Both LPL actions were inhibited in the presence of fatty acid-free albumin, suggesting that they were attributable to fatty acids generated during VLDL lipolysis. To study the relationship between PL transfers and platelet activation, we performed incubations in the presence of HDL, a physiological acceptor of PL released from VLDL. HDL antagonized the transfer of PL from VLDL to platelets but had no effect on the production of TXB_2, suggesting that PL transfers were driven by platelet activation. Confirming this idea, thrombin stimulated both the production of TXB_2 and the transfers of PL. In conclusion, VLDL can transfer PL to platelets. These transfers are stimulated by LPL and thrombin through their action on platelet activation. They might be enhanced in pathologies characterized by increased VLDL concentrations.—Ibrahim, S., A. Djimet-Baboun, V. Pruneta-Deloche, C. Calzada, M. Lagarde, and G. Ponsin. **Transfer of very low density lipoprotein-associated phospholipids to activated human platelets. *J. Lipid Res.* 2006.** 47: **341–348.**

Supplementary key words lipoprotein lipase • thromboxane production • high density lipoprotein • tetrahydrolipstatin

Phospholipids (PLs) are involved in a variety of cellular events. In platelets, they play important roles in signal transduction processes resulting from cell activation (1, 2). The generation of diacylglycerols through the action of phospholipase C activates several metabolic cascades leading to various effects, including protein phosphorylation, granule secretion, and release of fatty acids by diacylglycerol and monoacylglycerol lipases (3, 4). In addition, platelet activation stimulates the activity of phospholipase A_2 (PLA_2) enzymes that cleave fatty acids from the *sn2* position of PL. In particular, the cytosolic PLA_2 reaction favors the liberation of arachidonic acid, which is the precursor of prostaglandins and leukotrienes that are generated through the actions of cyclooxygenase and lipoxygenase, respectively (5, 6). Thus, in activated platelets, membrane PLs may be actively degraded, which necessitates their regeneration to maintain their cellular functions. Although PL may be resynthesized in platelets (7), a substantial part has been shown to be imported from extracellular sources (8–10). In vivo, platelets are in contact with circulating lipoproteins that constitute a major source of PL. Previous in vitro work has shown that phosphatidylcholine (PC), phosphatidylethanolamine (PE), and sphingomyelin can be transferred from LDLs and HDLs to human platelets (8). However, the underlying mechanisms of these transfers appeared to be complex.

Although platelets possess high-affinity binding sites for LDL, PL transfers were independent of LDL binding or internalization (9). In agreement with this finding, the scavenger receptor class B type I, which can mediate the specific import of PL into various cells, was shown to be absent in platelets (10). In addition, the uptake of PC and PE species by platelets appeared to be regulated by different mechanisms. The transfer of LDL- or HDL-derived PE into platelets, but not that of PC or sphingomyelin, was stimulated by platelet activators, including thrombin, collagen, and ADP, and was dependent on the secretion of an unidentified cellular protein factor (11). Although LDL and HDL transport the major part of lipoprotein PL, they do not necessarily represent the sole source of PL for

Manuscript received 24 May 2005 and in revised form 13 October 2005 and in re-revised form 15 November 2005.

Published, JLR Papers in Press, November 17, 2005.
DOI 10.1194/jlr.M500209-JLR200

Abbreviations: [^{3}H]DPPC, 1,2-dipalmitoyl-([^{3}H]methyl-choline)-phosphatidylcholine; FAF, fatty acid-free; [^{14}C]PAPC, 1-palmitoyl-2-[1-^{14}C]arachidonyl-phosphatidylcholine; [^{14}C]PAPE, 1-palmitoyl-2-[1-^{14}C]arachidonyl-phosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PL, phospholipid; PLA_2, phospholipase A_2; TG, triglyceride; THL, tetrahydrolipstatin; TXB_2, thromboxane B_2.

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platelets. Indeed, because the import of PL by platelets is independent of lipoprotein internalization, a putative specific role of VLDLs may be considered. VLDLs are lipoproteins secreted by the liver in the circulation, where they undergo hydrolysis of their core triglyceride (TG) content through the successive actions of LPL and hepatic lipase, which ultimately results in the formation of LDL (12, 13). During this process, the excess VLDL surface components, including apolipoproteins, cholesterol, and PL, are released from the particles. A large part of cholesterol and PL is transferred to HDL, serving as substrates for the lecithin:cholesterol acyltransferase-mediated generation of cholesteryl esters (14, 15).

From a mechanistic viewpoint, numerous works have clearly established that the transfers of PL from VLDL to HDL occur either as spontaneous transfers (16) or as PL transfer protein-facilitated transfers (17, 18). On the basis of these mechanisms, it is clear that not all of the PLs released from VLDL are necessarily transferred to HDL and that a part of them may be taken up by other acceptors. To date, the possibility that a transfer of PLs from VLDL to platelets may occur has never been considered. In this work, we present in vitro evidence showing that this transfer may occur under physiological conditions, that it is facilitated by the action of LPL, and that it is dependent on the activation of platelets.

MATERIALS AND METHODS

Materials

1-Palmitoyl-2-[1-^{14}C]arachidonyl-phosphatidylcholine ([^{14}C]PAPC; 40–60 mCi/mmol), 1-palmitoyl-2-[1-^{14}C]arachidonyl-phosphatidylethanolamine ([^{14}C]PAPE; 40–60 mCi/mmol), and 1,2-dipalmitoyl-([^{3}H]methyl-choline)-phosphatidylcholine ([^{3}H]DPPC; 40–85 Ci/mmol) were purchased from Perkin-Elmer (Boston, MA). Thrombin, bovine milk LPL (EC 3.1.1.34), BSA, fraction V, essentially fatty acid-free (FAF) albumin, and polyoxyethylen-9-laurylether were obtained from Sigma Chemical (St. Louis, MO). Tetrahydrolipstatin (THL; Orlistat™) was from Hoffman-La Roche (Basel, Switzerland). A commercial kit from Oxoid (Dardilly, France) was used to measure NEFA concentrations. PLs and TGs were assayed using enzymatic kits from Biomérieux (Marcy l'Etoile, France). Thromboxane B_2 (TXB_2) concentrations were determined using the enzyme immunoassay Biotrak system from Amersham Biosciences (Orsay, France).

Isolation and labeling of lipoproteins

VLDL ($d < 1.006$ g/ml), LDL ($1.006 < d < 1.063$ g/ml), and HDL ($1.063 < d < 1.21$ g/ml) were isolated from human plasma by sequential preparative ultracentrifugation (19). Depending upon the volume of plasma, the ultracentrifugations were performed either in a Beckman LE 80K using a 50.2 fixed-angle rotor or in a Beckman TL-100 tabletop ultracentrifuge using a TLA 100.3 fixed-angle rotor. The resulting lipoprotein-deprived plasma and the lipoprotein fractions were then extensively dialyzed against a buffer containing 150 mM NaCl, 1 mM EDTA, 10 mM Tris-HCl, and 10 mM NaN_3, pH 7.4. The lipoprotein fractions were labeled as needed with [^{14}C]PAPC, [^{14}C]PAPE, or [^{3}H]DPPC (1 μCi/10 μmol lipoprotein PL) as reported previously (20). The desired amount of radioactive label was dried under nitrogen, solubilized in ethanol, and added to lipoproteins under vortexing. To avoid destruction of the lipoprotein structure, the final proportion of ethanol in the samples was maintained at <1% (v/v). The samples were then incubated for 3 h at 37°C, and finally the labeled lipoproteins were reisolated by ultracentrifugation and dialyzed.

Platelet isolation

Fresh blood was collected at the local blood bank (Etablissement Français du Sang) from healthy volunteers. Blood was drawn into a one-seventh volume of a solution containing 19.6 mM citric acid, 89.4 mM sodium citrate, 16.1 mM NaH_2PO_4, and 128.7 mM dextrose, pH 5.6. The platelet isolation procedure was essentially based on that described previously (21). Briefly, platelet-rich plasma was obtained after blood centrifugation at 200 *g* for 17 min at 20°C and acidified to pH 6.4 with 0.15 M citric acid. Platelets were immediately pelleted by centrifugation at 900 *g* for 12 min and washed in acidified lipoprotein-deprived plasma. After repelleting, the platelets were finally washed and resuspended in a Tyrode-HEPES buffer solution containing 137 mM NaCl, 2.7 mM KCl, 0.41 mM NaH_2PO_4, 11.9 mM $NaHCO_3$, 1 mM $MgCl_2$, 5.5 mM glucose, and 5 mM HEPES, pH 7.35. Platelet suspensions were left for 1 h at room temperature before experiments were started.

Platelet aggregation

To avoid abnormal experimental data that could result from unknown medical treatment taken by blood donors, each platelet preparation was controlled for its functional ability to aggregate before being used in our studies. Aggregations were induced by arachidonic acid and performed in a Chronolog dual-channel aggregometer (Coulter, Margency, France) according to the turbidimetric method of Born (22).

Transfers of labeled PLs from lipoprotein fractions to platelets

Labeled VLDL, LDL, or HDL (200 nmol/ml PL) were incubated with platelets (3×10^8 cells) in a final volume of 1.5 ml. Unless indicated otherwise, the incubations were performed at 37°C for 1 h, and for each given experiment the lipoprotein fractions and platelets were each isolated from a single donor. When desired, LPL (100 or 500 ng/ml), THL (55 nM), thrombin (0.1 or 0.2 U/ml), FAF albumin (20 mg/ml), or unlabeled HDL was added at the beginning of the incubations. At the end of the incubations, platelets were separated from the medium by centrifugation. The pelleted platelets were first washed in plasma to remove nonspecifically adsorbed labeled lipoproteins and then in Tyrode-HEPES buffer. The final pellets were dissolved by overnight incubation in 0.25 ml of 0.4% polyoxyethylen-9-laurylether and counted for radioactivity. The results were corrected for nonspecifically adsorbed radioactivity at time zero (nonincubated platelets). In some experiments, after incubation of platelets with [^{14}C]PAPC-labeled VLDL, lipid extracts were analyzed by thin-layer chromatography as described previously (23). This procedure permitted us to separate intact [^{14}C]PAPC from its putative metabolic products, including [^{14}C]arachidonic acid and [^{14}C]TXB_2, which were then counted for radioactivity.

Determination of TXB_2 production

The production of TXB_2 by platelets was determined at the beginning and end of the incubations carried out under various conditions as described above. However, platelets were not sepa-

rated from their media before the TXB_2 assay, thereby permitting the measurement of total TXB_2.

Determination of lipoprotein lipase activity

The time course of LPL-mediated hydrolysis of VLDL TG was monitored by measuring the release of NEFA as described previously (24). These determinations were performed during specifically designed incubations carried out either in the presence or in the absence of LPL.

RESULTS

The transfers of [^{14}C]PAPC from VLDL, LDL, or HDL to platelets were compared after 1 h incubations at 37°C (**Fig. 1A**). At identical concentrations of PL, VLDL and LDL elicited comparable transfers, whereas HDL was a rather poor PL donor. The transfers of VLDL-derived [^{14}C]PAPC depended upon temperature and VLDL concentrations (Fig. 1B). At 0°C, no transfer occurred, whereas that observed at 25°C reached >70% of the value observed at 37°C. When a 5-fold increased concentration of VLDL was used, the fraction of [^{14}C]PAPC transferred to platelets decreased by only 50%, which corresponded to a 2.5-fold increase in the absolute amount of transferred PL.

Because the normal metabolic fate of VLDLs is to undergo lipolysis of their TG content through the action of LPL, we examined the putative effect of this enzyme on the magnitude of the transfer of VLDL-associated [^{14}C]PAPC to platelets and on the putative occurrence of molecular species that could derive from [^{14}C]PAPC metabolism (**Table 1**). The transfer of VLDL-associated [^{14}C]PAPC to platelets was clearly increased by LPL in a concentration-dependent manner (**Fig. 2**). Analysis of the radioactive molecular species after incubations performed in the presence or absence of LPL revealed no

TABLE 1. Labeled molecular species in platelets

Species	Controls	LPL
[^{14}C]PAPC (%)	93.9 ± 2.0	94.1 ± 3.3
[^{14}C]arachidonic acid (%)	Traces	Traces
[^{14}C]TXB_2 (%)	4.1 ± 0.2	4.4 ± 1.0

[^{14}C]PAPC, 1-palmitoyl-2-[1-^{14}C]arachidonyl-phosphatidylcholine; TXB_2, thromboxane B_2. Values are shown as means ± SEM. Platelets were incubated with [^{14}C]PAPC-labeled VLDL (200 nmol phospholipid/ml) for 1 h at 37°C in a total volume of 1.5 ml in the presence or absence of LPL (100 ng/ml). Their intracellular contents in intact [^{14}C]PAPC, [^{14}C]TXB_2, and [^{14}C]arachidonic acid were determined after thin-layer chromatography, as described in Materials and Methods. Data are expressed as percentages of intracellular radioactivity.

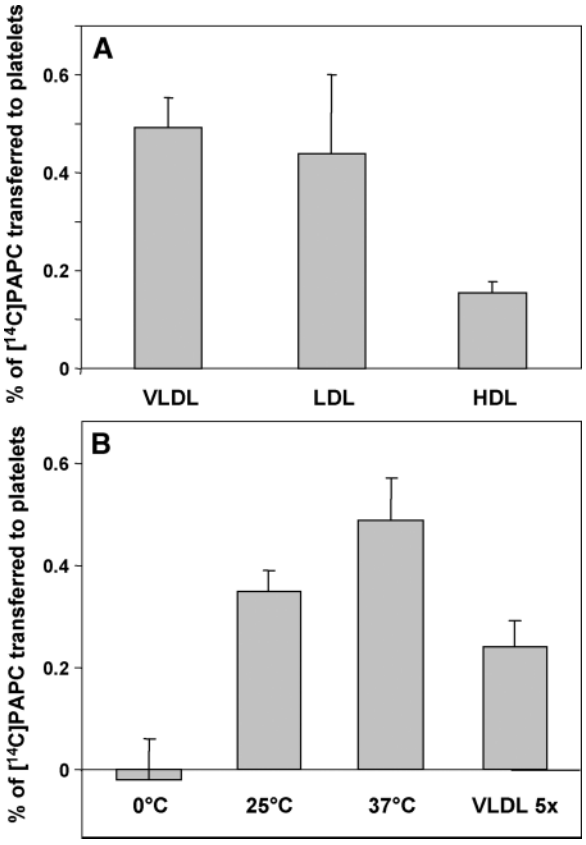

Fig. 1. Characterization of the transfers of 1-palmitoyl-2-[1-^{14}C]arachidonyl-phosphatidylcholine ([^{14}C]PAPC) from lipoproteins to platelets. A: Incubations of platelets (2×10^8 cells/ml) were performed in the presence of [^{14}C]PAPC-labeled VLDL, LDL, or HDL [200 nmol phospholipid (PL)/ml] for 1 h at 37°C in a total volume of 1.5 ml. B: The effects of temperature and VLDL concentrations on the transfers of [^{14}C]PAPC from VLDL to platelets. In addition, to assess the effect of VLDL concentration, platelets were incubated at 37°C with [^{14}C]PAPC-labeled VLDL at a concentration of 1 μmol PL/ml (VLDL 5×). Transfers are expressed as the percentage of radioactivity incorporated by 3×10^8 platelets. Values shown are means ± SEM from three to four independent experiments.

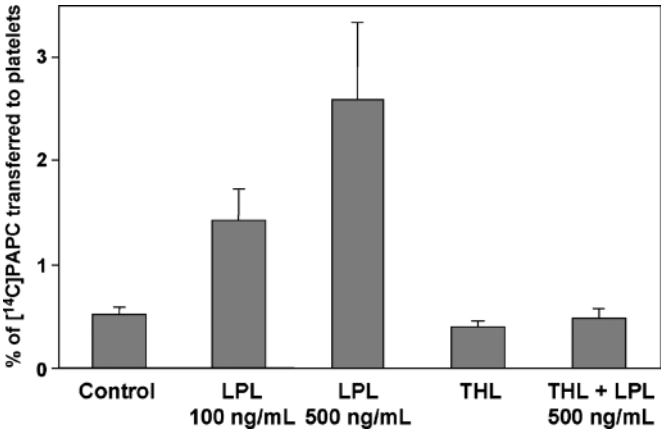

Fig. 2. Effects of LPL on the transfers of [^{14}C]PAPC from VLDL to platelets. Platelets were incubated with [^{14}C]PAPC-labeled VLDL (200 nmol PL/ml) for 1 h at 37°C in a total volume of 1.5 ml in the presence or absence of LPL (100 or 500 ng/ml). When desired, LPL lipolytic activity was inhibited by 55 nM tetrahydrolipstatin (THL). Transfers are expressed as the percentage of radioactivity incorporated by 3×10^8 platelets. Values shown are means ± SEM from four to seven independent experiments.

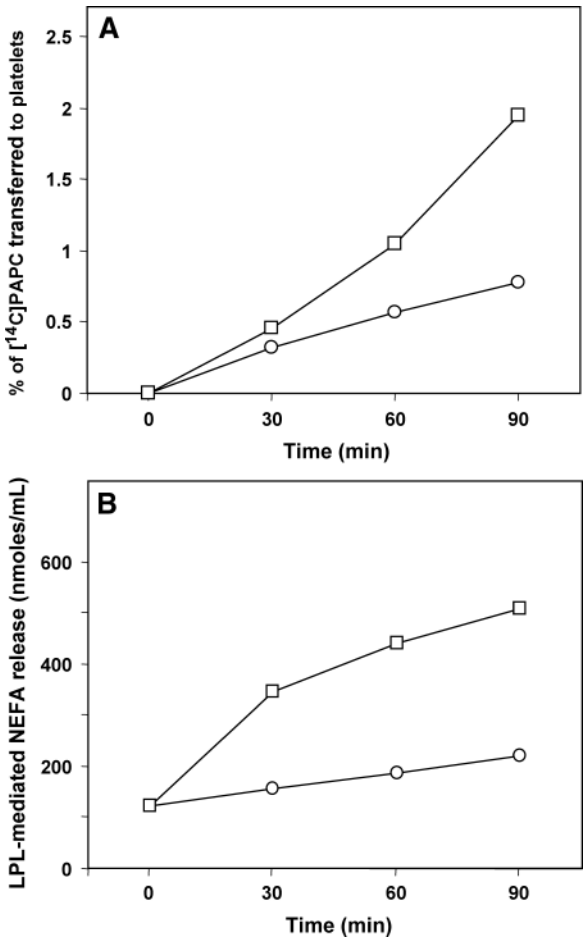

Fig. 3. Time courses of LPL actions on the transfers of [^{14}C]PAPC from VLDL to platelets (A) and on the lipolysis of VLDL triglycerides (B). Platelets were incubated with [^{14}C]PAPC-labeled VLDL (200 nmol PL/ml) at 37°C in a total volume of 1.5 ml in the presence (open squares) or absence (open circles) of LPL (100 ng/ml). Transfers are expressed as the percentage of radioactivity incorporated by 3×10^8 platelets. NEFA release was measured using a commercial kit, as described in Materials and Methods. The values are taken from one experiment representative of three experiments.

other species than [^{14}C]PAPC in the extracellular compartment (data not shown). However, in the intracellular compartment, although >90% of radioactivity was recovered as [^{14}C]PAPC, traces of unesterified [^{14}C]arachidonic acid and small but measurable amounts of [^{14}C]TXB_2 were recovered, showing that [^{14}C]PAPC could be metabolized by platelets. Similar data were obtained in the presence of LPL (Table 1). When the incubations were performed in the presence of THL, an inhibitor of LPL-dependent lipolysis, the stimulating action of LPL on the PL transfers was inhibited, suggesting that it resulted from the LPL-dependent lipolysis of VLDL (Fig. 2). In agreement with this idea, we observed that the time course of action of LPL was faster on VLDL lipolysis than on VLDL-derived [^{14}C]PAPC transfers (**Fig. 3**). For example, after 30 min of incubation, LPL-stimulated lipolysis reached a value of ~240% of that of controls, whereas [^{14}C]PAPC transfers only increased to a value of ~150% of controls.

These results prompted us to examine whether the magnitude of the observed PL transfers might be related to platelet activation. To this aim, we determined the transfers of [^{14}C]PAPC from VLDLs to platelets during incubations performed in the presence of thrombin (**Fig. 4**). The results clearly exhibited a concentration-dependent stimulating effect of thrombin. Therefore, we compared the effects of thrombin and LPL on platelet TXB_2 production (**Fig. 5**). Although VLDL alone were unable to stimulate the production of TXB_2 by platelets, the latter was strongly enhanced by either LPL or thrombin. Interestingly, when the incubations were performed in the absence of VLDL, the stimulating effect of thrombin was maintained, whereas that of LPL was totally inhibited, showing that the LPL-dependent activation of platelets resulted from its ability to stimulate VLDL lipolysis. Because the LPL-mediated hydrolysis of VLDL TG generates the release of free fatty acids, we performed incubations in the presence of FAF albumin. Under these conditions, the stimulating effects of LPL on both the transfer of [^{14}C]PAPC to platelets (**Fig. 6A**) and the production of TXB_2 by platelets (Fig. 6B) were abolished.

In vivo, the major part of PL released during VLDL lipolysis is believed to be taken up by HDL. Therefore, we tested the possibility that HDL could antagonize the transfer of [^{14}C]PAPC from VLDL to platelets. At a physiological concentration, HDL abolished the effect of LPL on the PL transfer to platelets (**Fig. 7A**), whereas it had no effect on the LPL-stimulated production of TXB_2 by platelets (Fig. 7B). To mimic the pathophysiological conditions

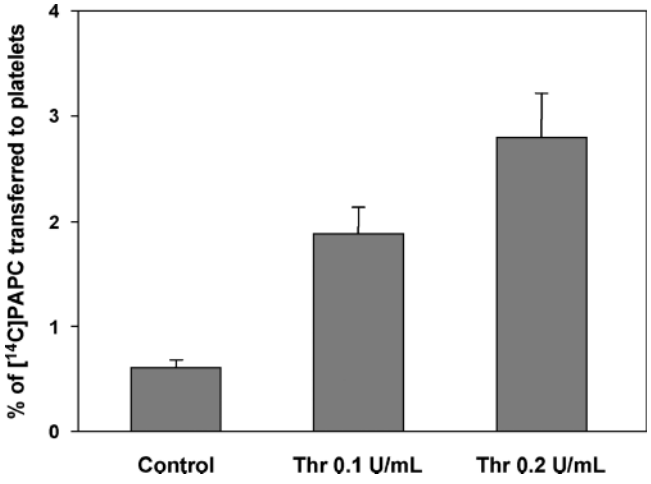

Fig. 4. Effects of thrombin (Thr) on the transfers of [^{14}C]PAPC from VLDL to platelets. Platelets were incubated with [^{14}C]PAPC-labeled VLDL (200 nmol PL/ml) for 1 h at 37°C in a total volume of 1.5 ml in the presence or absence of thrombin (0.1 or 0.2 U/ml). Transfers are expressed as the percentage of radioactivity incorporated by 3×10^8 platelets. Values shown are means ± SEM from four independent experiments.

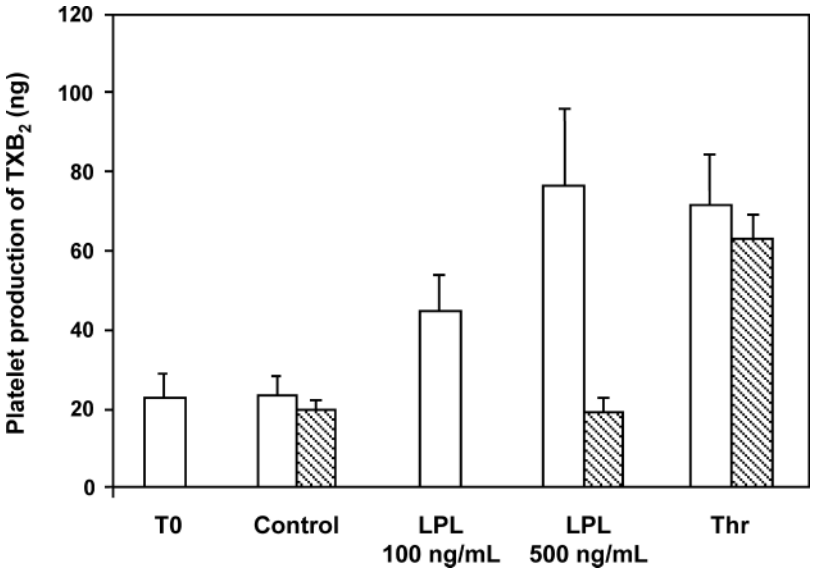

Fig. 5. Effects of LPL (100 or 500 ng/ml) and thrombin (Thr; 0.1 U/ml) on the production of thromboxane B_2 (TXB_2) by platelets. Platelets were incubated for 1 h at 37°C in a total volume of 1.5 ml in the presence (open bars) or absence (hatched bars) of VLDL (200 nmol PL/ml). TXB_2 concentrations were measured using a commercial kit, as described in Materials and Methods. The results are expressed as the amounts of TXB_2 produced by 3×10^8 platelets. Values shown are means ± SEM from six to nine independent experiments.

of hypertriglyceridemia, we measured LPL-stimulated transfers of [^{14}C]PAPC from an increased concentration of VLDL (1 μmol/ml PL) to platelets in the presence of various concentrations of HDL (**Fig. 8**). Although increasing concentrations of HDL were able to progressively antagonize the transfers observed, the latter remained significantly higher than those of controls, even at the highest concentration of HDL tested.

Finally, we carried out experiments to compare the transfers of radiolabels from VLDL containing [^{14}C]PAPC, [^{14}C]PAPE, or [^{3}H]DPPC to platelets (**Fig. 9**). In all cases, the stimulating effects of both LPL and thrombin had similar magnitudes.

DISCUSSION

Previous works have shown that LDL and HDL were able to transfer PL to platelets (8, 9). Our data demonstrate that VLDL-associated PL can also be transferred to platelets and that these transfers are favored by LPL. It is important to note that, with the exception of specifically designed experiments (see below), both the concentrations of VLDL and platelets used in the incubations were in the normal physiological range. With regard to LPL, the concentrations of 100 and 500 ng/ml corresponded to the basal and postheparinic plasma concentrations, respectively (25). Because [^{14}C]PAPC was used to measure the transfers of PL from VLDL to platelets, we considered the possibility that this marker could be lipolyzed during the incubations, resulting in the production of [^{14}C]arachidonic acid. Thin-layer chromatography analysis showed that only intact [^{14}C]PAPC was found in the extracellular compartment, irrespective of the presence of LPL. Thus, although the latter can in principle somewhat hydrolyze PLs (26), this process appears to be too slow to permit measurable extracellular lipolysis of [^{14}C]PAPC. In contrast, traces of [^{14}C]arachidonic acid and small proportions of [^{14}C]TXB_2 were detected in the intracellular compartment, strongly suggesting that imported [^{14}C]PAPC could be lipolyzed by the cytosolic PLA_2, thereby releasing [^{14}C]arachidonic acid that could enter the metabolic cascade leading to the formation of thromboxanes.

The effect of LPL on the transfers of [^{14}C]PAPC from VLDL to platelets was attributable to its ability to stimulate VLDL TG lipolysis, because it was inhibited in the presence of THL, an agent known to block LPL lipolytic activity (27). In principle, LPL could favor the transfer of PL by two nonexclusive mechanisms. First, it has been clearly established that the hydrolysis of VLDL TGs, which are packed in the core of the particles, induces a destabilization of the particle surface, which results in the release of PL in excess (14, 15). Second, one could hypothesize that products of TG hydrolysis could favor the transfers of PL by activating platelets. To study this point, we measured the production of TXB_2 by platelets as an index of their degree of activation (1, 2). LPL clearly stimulated the production of TXB_2 by platelets, but this was true only in the presence of VLDL, ruling out the possibility of a direct effect of LPL on platelet activation. LPL-mediated TG hydrolysis directly generates several products, including fatty acids as well as monoacylglycerols and diacylglycerols, that could be taken up by platelets. Because in our experiments TGs were not labeled, no direct information concerning the putative silent transfers of these products can be drawn from our data. However, when the incubations were performed in the presence of FAF albumin to adsorb the fatty acids generated during VLDL lipolysis, the production of TXB_2 was no more stimulated by LPL. Thus, albeit not excluding the possibility of transfers of other molecular species, these data suggest that the fatty acids released during LPL-mediated VLDL lipolysis might favor platelet activation, in particular through the action of arachidonic acid (28, 29). The stim-

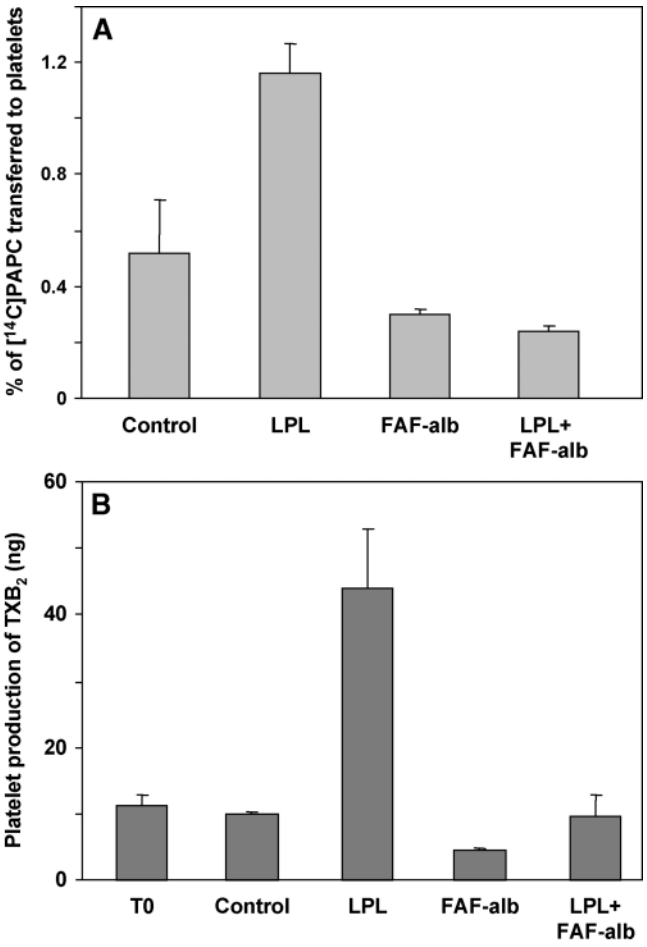

Fig. 6. Effects of fatty acid-free albumin (FAF-alb) on LPL-mediated transfers of [^{14}C]PAPC from VLDL to platelets (A) and platelet activation (B). Platelets were incubated with [^{14}C]PAPC-labeled VLDL (200 nmol PL/ml) for 1 h at 37°C in a total volume of 1.5 ml in the presence or absence of 2% FAF-alb. Transfers are expressed as the percentage of radioactivity incorporated by 3×10^8 platelets. The concentrations of TXB_2 were measured using a commercial kit, as described in Materials and Methods. The results are expressed as the amounts of TXB_2 produced by 3×10^8 platelets. Values shown are means ± SEM from four independent experiments.

ulating effect of LPL on PL transfers was also inhibited in the presence of FAF albumin, favoring the concept that the VLDL-associated PL transfers might be stimulated by platelet activation. Interestingly, when LPL-stimulated PL transfers were antagonized by HDL, no decrease of TXB_2 production was observed, showing that the import of PL by platelets is not necessary to their short-term activation process. Moreover, thrombin stimulated both TXB_2 production and transfers of PL, confirming that platelet activation resulted in the enhancement of PL import from VLDL.

Two facts concerning the mechanism underlying the import of PL from VLDL to platelets can be drawn from a comparison of our data with previous reports. First, because VLDL has been shown to bind to the platelet receptor CD36 (30), one could hypothesize that PL transfer could occur during this interaction. If this were true, one would not expect an antagonizing effect of HDL. Indeed, the transfers of PL from VLDL to HDL occur either as spontaneous molecular transfers or as PL transfer protein-facilitated transfers through the aqueous phase (16–18). Thus, it appears likely that the transfers of PL from VLDL to platelets result from a similar mechanism. To further explore this point, we undertook a specific series of experiments designed to study the regulation of these transfers. Preliminary data suggest that they can be stimulated by PL transfer protein (data not shown). The second observation relates to the apparent nonspecificity of the PL species transferred from VLDL to platelets. Both LPL and thrombin stimulated the import by platelets of VLDL-derived PAPC, PAPE, and DPPC with similar efficiencies. These results are in marked contrast with those of a previously published work (11). Those authors compared the trans-

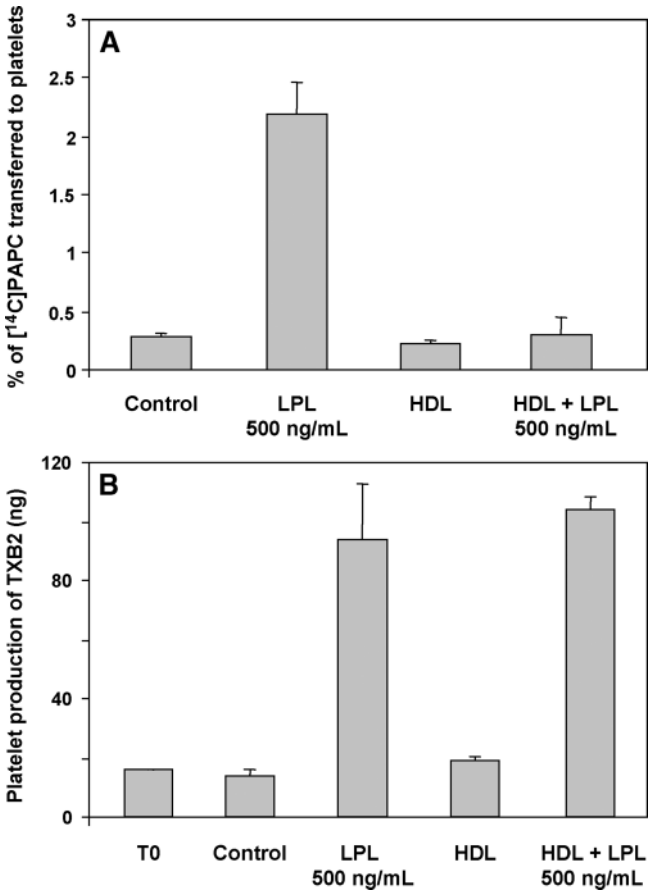

Fig. 7. Effects of HDL on LPL-mediated transfers of [^{14}C]PAPC from VLDL to platelets (A) and platelet activation (B). Platelets were incubated with [^{14}C]PAPC-labeled VLDL (200 nmol PL/ml) for 1 h at 37°C in a total volume of 1.5 ml in the presence or absence of HDL (1 μmol PL/ml). Transfers are expressed as the percentage of radioactivity incorporated by 3×10^8 platelets. The concentrations of TXB_2 were measured using a commercial kit, as described in Materials and Methods. The results are expressed as the amounts of TXB_2 produced by 3×10^8 platelets. Values shown are means ± SEM from four independent experiments.

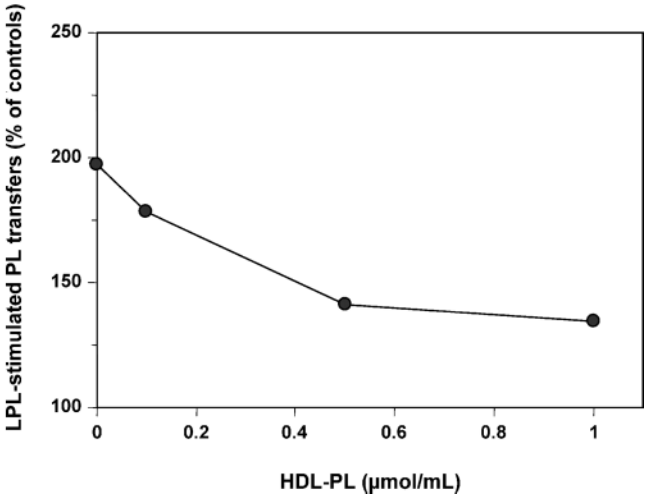

Fig. 8. Dose-response effects of HDL on LPL-stimulated transfers of [^{14}C]PAPC from VLDL to platelets. Platelets were incubated with [^{14}C]PAPC-labeled VLDL (1 μmol PL/ml) for 1 h at 37°C in a total volume of 1.5 ml in the presence of various concentrations of HDL in the range of 0 to 1 μmol PL/ml. LPL was used at a concentration of 100 ng/ml. Results are expressed as the percentages of LPL effects with respect to controls. Because of the elevated amounts of both VLDL and HDL necessary to perform this particular experiment, lipoproteins isolated from several donors were mixed before use.

fers of various PLs from LDL to platelets. The transfers of PC and sphingomyelin were unaffected by thrombin, whereas the latter accelerated that of PE species. In addition, this PE import was related to the activation of protein kinase C and to the secretion by platelets of an unknown proteinaceous factor. Thus, the regulation of PL uptake by platelets appears to depend dramatically upon the lipoprotein used as the donor. Although LDL might preferentially transfer certain PL species, namely PE, by a specific mechanism, VLDL could supply all types of PLs to platelets without consideration of their nature.

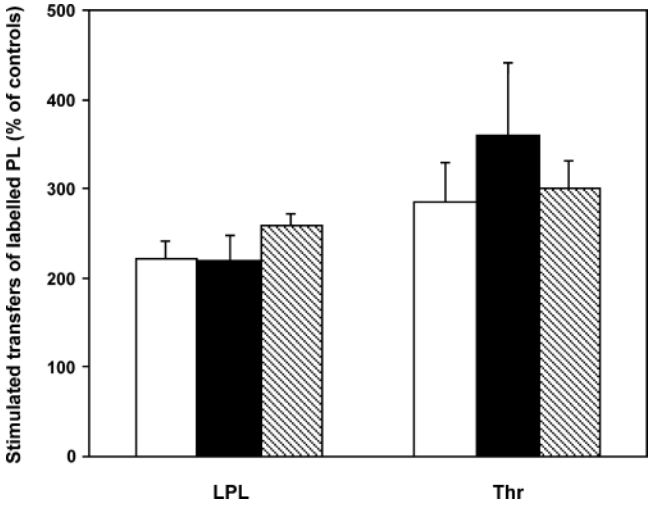

Fig. 9. Comparison of the transfers of various labeled PLs from VLDL to platelets. Platelets were incubated with VLDL (200 nmol PL/ml) labeled with [^{14}C]PAPC (open bars), 1-palmitoyl-2-[1-^{14}C]arachidonyl-phosphatidylethanolamine (closed bars), or 1,2-dipalmitoyl-([^{3}H]methyl-choline)-phosphatidylcholine (hatched bars) at 37°C for 1 h in the absence or presence of 100 ng/ml LPL or of 0.1 U/ml thrombin (Thr). For each of the labeled PLs used, the results of either LPL- or thrombin-stimulated transfers are expressed as percentages of basal transfer values. Data are presented as mean values obtained in two independent experiments.

The pathophysiological relevance of our data depends on several considerations. First, to avoid the uncontrolled actions of numerous factors on platelet activation, we carried out the platelet incubations in a serum-free medium. Thus, we cannot exclude the possibility that the effects of LPL observed in vitro might be somewhat different in vivo in the presence of albumin and other plasma factors. In particular, the binding of fatty acids and other lipids generated through the LPL reaction to albumin might decrease their ability to be taken up by platelets, which could attenuate the apparent effect of LPL. The second point to consider relates to lipoprotein concentrations. Humans are physiologically under postprandial rather than fasting conditions during a large proportion of the day. Thus, the average plasma concentration of VLDL is likely higher than that used in our experiments. In addition, postprandial chylomicrons might also act as PL donors during their LPL-mediated lipolysis. Finally, we have to consider certain pathological situations, such as primary hypertriglyceridemia or type 2 diabetes, that are accompanied by increased plasma concentrations of TG-rich lipoproteins together with decreased HDL concentrations (31–33). To mimic these situations, we incubated platelets with moderately increased concentrations of [^{14}C]PAPC-labeled VLDL (1 mM PL) in the presence of HDL at concentrations ranging from 0 to 1 mM. In these conditions, a nonnegligible proportion of VLDL-associated PL was transferred to platelets despite the presence of HDL. Thus, the occurrence of PL transfers from VLDL to platelets appears to be relevant, at least in certain pathophysiological situations in which hyperVLDLemia is associated with hypoHDLemia.

This work was supported by the Institut National de la Santé et de la Recherche Médicale. The authors gratefully thank Hoffman-La Roche (Basel, Switzerland) for providing THL (Orlistat™).

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