

# Influence of prostaglandins on the lipid transfer between human high density and low density lipoproteins

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Prostaglandin (PG) E<sub>1</sub> was demonstrated to stimulate the transfer of phosphatidylcholine and cholesterol esters from human high density lipoproteins (HDL<sub>3</sub>) to low density lipoproteins (LDL). The enhancement effect of PGE<sub>1</sub> on the interlipoprotein lipid transfer was seen at low PG concentrations under conditions of spontaneous exchange as well as in the presence of lipoprotein-depleted plasma, or partly purified plasma lipid exchange protein. PGE<sub>2</sub> and PGF<sub>2α</sub> showed no significant influence on the interlipoprotein lipid transfer. Evidence is presented suggesting that the PGE<sub>1</sub>-induced stimulation of interlipoprotein lipid exchange results in enhancement of LCAT-catalyzed cholesterol esterification in plasma. It is proposed that the effect of PGE<sub>1</sub> is due to the previously described PGE<sub>1</sub>-induced reorganization of the HDL surface [(1984) FEBS Lett. 173, 291-293] and that PG-lipoprotein interaction may be a factor regulating cholesterol homeostasis.

Prostaglandin; Lipid transfer; Cholesterol esterification; LDL; HDL; (Human)

## 1. INTRODUCTION

Although PGs appear to play a role in atherosclerosis [1] little is known about their influence on the homeostasis of cholesterol. We have recently discovered that small amounts of PGs can substantially change the surface structure of serum lipoproteins and that these changes appear to be mediated by PG-apoprotein interaction [2]. Lipids, particularly phosphatidylcholine and cholesterol esters, are known to exchange between

lipoprotein classes. The exchange which occurs spontaneously and is considerably enhanced in the presence of plasma is thought to play an important role in lipid transfer and metabolism [3].

In human subjects the cholesterol esters of LDL are probably acquired from HDL via transfer involving specific plasma LEPs. A protein which simultaneously facilitates transfer of cholesterol esters and phosphatidylcholine has been detected in human plasma [4]. The purpose of the present work was to investigate whether the alterations of the lipoprotein surface structure brought about by PGs influence the exchange of phosphatidylcholine and cholesterol esters between HDL and LDL. It was found that PGE<sub>1</sub> significantly stimulated spontaneous and protein-mediated lipid transfer from HDL to LDL, whereas PGE<sub>2</sub> was inactive. These differences correlated with the results of our previous study [5], which demonstrated that PGE<sub>1</sub> at low concentration was able to induce a rearrangement of the HDL surface lipids, while PGE<sub>2</sub> was not.

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**Abbreviations:** PG, prostaglandins; DPPC, dipalmitoylphosphatidylcholine; HDL, high density lipoproteins; LDL, low density lipoproteins; LEP, lipid exchange protein; LDP, lipoprotein-depleted plasma; LCAT, lecithin:cholesterol acyltransferase (EC 2.3.1.43)

## 2. MATERIALS AND METHODS

1 $\alpha$ ,2 $\alpha$ -[ $^3$ H]Cholesterol (43 Ci/mmol) and [*N*-methyl- $^3$ H]DPPC (77 Ci/mmol) were purchased from Amersham (England). Protein was determined by the method of Lowry et al. [6] and lipid phosphorus according to Vaskovsky et al. [7].

LDL (1.019 g/ml < *d* < 1.065 g/ml) and HDL<sub>3</sub> (1.125 g/ml < *d* < 1.210 g/ml) were isolated from the blood of healthy donors according to [8]. Radioactive cholesterol and DPPC were incorporated into LDL and HDL<sub>3</sub> by coincubation of

5  $\mu$ l ethanolic solutions of the lipids with 1 ml lipoprotein suspensions for 4 h at 36°C. HDL<sub>3</sub> containing [ $^3$ H]cholesterol esters were prepared as in [9]. The [ $^3$ H]cholesterol esters introduced by that procedure into HDL<sub>3</sub> amounted to 97% of the total labeled cholesterol of these lipoproteins.

Partly purified LEP was isolated from the *d* > 1.21 g/ml infranant of human plasma by sequential chromatography on phenyl-Sepharose and DEAE-cellulose as described in [10].

Assay of the phosphatidylcholine and cholesterol ester exchange activity was carried out by monitoring the transfer of radioactive lipids from HDL<sub>3</sub> to LDL [10]. After coincubation of the

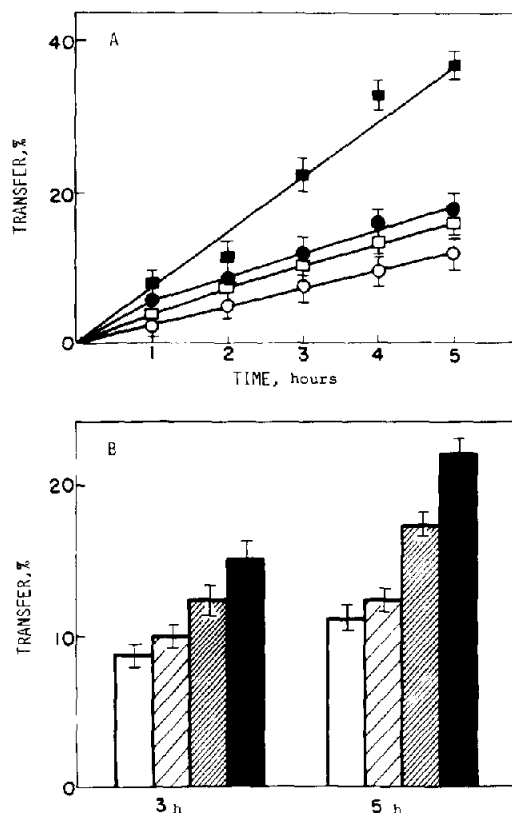


Fig.1. Influence of PGE<sub>1</sub> on the kinetics of lipid transfer from HDL<sub>3</sub> to LDL. (A) Transfer of [ $^3$ H]DPPC: (○—○) spontaneous transfer; (□—□) transfer in the presence of LEP; (●—●) spontaneous transfer + PGE<sub>1</sub>; (■—■) transfer in the presence of LEP and PGE<sub>1</sub>. (B) Transfer of cholesterol esters: (□) spontaneous transfer; (▤) transfer in the presence of LEP; (▨) spontaneous transfer + PGE<sub>1</sub>; (■) transfer in the presence of LEP and PGE<sub>1</sub>. PG concentration 10<sup>-8</sup> M. Concentrations of HDL<sub>3</sub> and LDL: 0.3 and 0.6 mg protein/ml, respectively. Samples were incubated at 36°C.

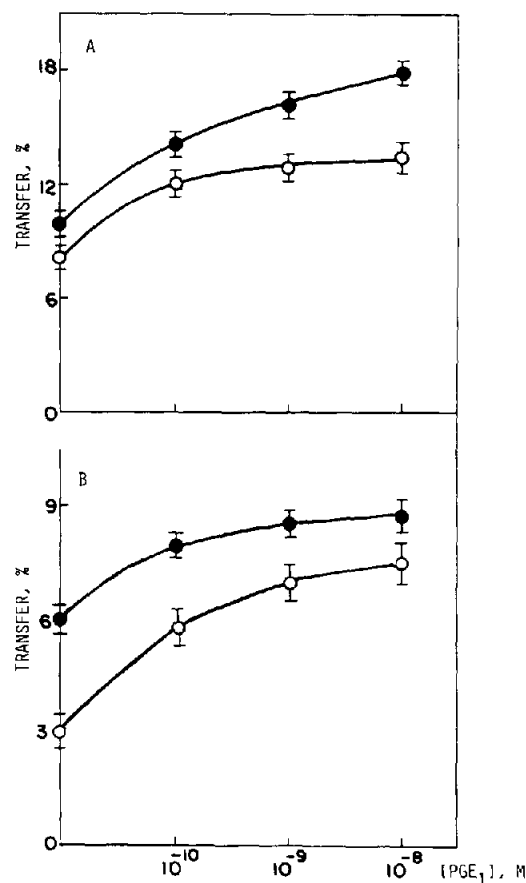


Fig.2. Dependence of rate of HDL<sub>3</sub> to LDL lipid transfer on the PGE<sub>1</sub> concentration in the medium. (A) Transfer of [ $^3$ H]DPPC. (B) Transfer of the [ $^3$ H]cholesterol ester. (○—○) Spontaneous transfer; (●—●) transfer in the presence of LEP. Concentrations of HDL<sub>3</sub> and LDL as in fig.1. Samples were incubated for 5 h at 36°C.

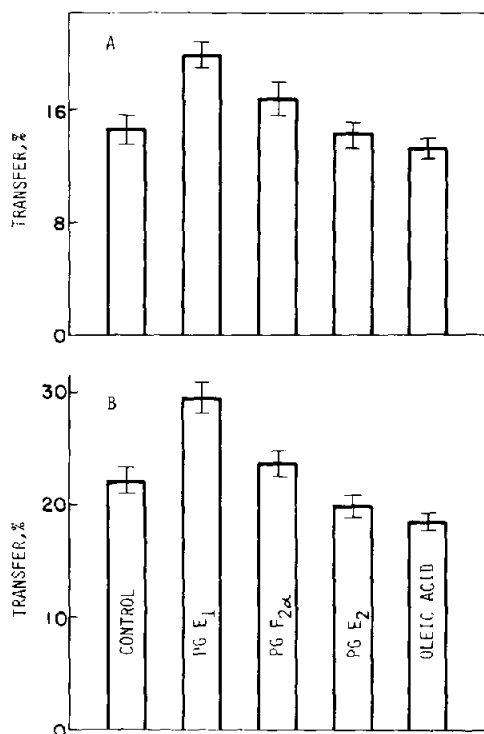


Fig.3. Influence of PGE<sub>1</sub>, PGE<sub>2</sub>, PGF<sub>2</sub> and oleic acid on the rate of cholesterol ester transfer from HDL<sub>3</sub> to LDL. (A) Spontaneous transfer. (B) Transfer in the presence of LEP. Concentrations of PG and oleic acid: 10<sup>-8</sup> M. For experimental conditions see figs 1,2.

donor and acceptor lipoprotein the latter was separated from the donor by Mn<sup>2+</sup> sedimentation in phosphate buffer solution. Under these conditions cosedimentation of HDL<sub>3</sub> did not exceed 6%. This value was taken as 'per cent of transfer at time zero' in fig.1.

Cholesterol esterification assays were carried out by incubation of [<sup>3</sup>H]cholesterol labeled LDL with LDP and unlabeled HDL<sub>3</sub> as described [11].

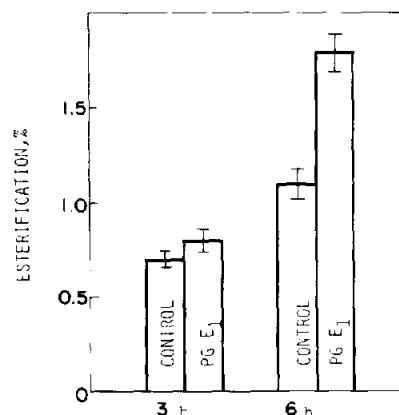


Fig.4. Influence of PGE<sub>1</sub> on the rate of LDL cholesterol esterification in the presence of LDP and unlabeled HDL<sub>3</sub>. Concentration of PGE<sub>1</sub> in the medium: 10<sup>-8</sup> M; 36°C.

### 3. RESULTS

The time course of [<sup>3</sup>H]DPPC and [<sup>3</sup>H]cholesterol ester transfer from HDL<sub>3</sub> to LDL in the absence and presence of PGE<sub>1</sub> is presented in fig.1. At 10<sup>-8</sup> M PGE<sub>1</sub> the prostaglandin enhanced the spontaneous transfer of [<sup>3</sup>H]DPPC about 2-fold and considerably facilitated the transfer of cholesterol esters. These effects were markedly augmented in the presence of partly purified LEP.

The lipid-transfer stimulating effect of PGE<sub>1</sub> was concentration-dependent (fig.2). Even at concentrations as low as 10<sup>-10</sup> M PGE<sub>1</sub> caused more than 50% enhancement of DPPC transfer in both the absence and presence of partly purified plasma LEP.

The influence of PGE<sub>1</sub>, PGE<sub>2</sub> and PGF<sub>2α</sub> on the cholesterol ester transfer from HDL<sub>3</sub> to LDL is shown in fig.3. At 10<sup>-8</sup> M only PGE<sub>1</sub> was able to enhance the transfer significantly. PGE<sub>2</sub> and oleic

Table 1

Influence of PGE<sub>1</sub> on the cholesterol ester transfer from HDL<sub>3</sub> to LDL in the absence and presence of LDP

| [PGE <sub>1</sub> ] (M):        | % transfer |                   |                  |                  |
|---------------------------------|------------|-------------------|------------------|------------------|
|                                 | 0          | 10 <sup>-10</sup> | 10 <sup>-9</sup> | 10 <sup>-8</sup> |
| Spontaneous transfer            | 2.0 ± 0.1  | 3.5 ± 0.1         | 3.7 ± 0.1        | 5.6 ± 0.2        |
| Transfer in the presence of LDP | 2.2 ± 0.1  | 5.7 ± 0.2         | 5.9 ± 0.2        | 6.2 ± 0.2        |

Concentrations of HDL<sub>3</sub> and LDL were 0.3 and 0.4 mg protein/ml, respectively. Samples were incubated for 3 h at 36°C

acid did not influence the transfer whereas the effect of  $\text{PGF}_2$  was not significant.

Table 1 presents data obtained on measuring the influence of  $\text{PGE}_1$  on the HDL-LDL cholesterol ester transfer in the presence of LDP.  $\text{PGE}_1$  ( $10^{-8}$  M) caused an about 200% increase of the transfer and the enhancement effect of the prostaglandin was even higher than in the absence of LDP.

Fig.4 illustrates the influence of  $\text{PGE}_1$  on esterification of LDL cholesterol in the presence of LDP and unlabeled  $\text{HDL}_3$ . Under such conditions,  $10^{-8}$  M  $\text{PGE}_1$  caused a 50% increase of cholesterol esterification over 6 h incubation. In the absence of  $\text{HDL}_3$   $\text{PGE}_1$  had no effect on the esterification of cholesterol.

#### 4. DISCUSSION

The stimulation of phosphatidylcholine and cholesterol ester transfer from  $\text{HDL}_3$  to LDL by PGs depends in a highly specific manner on the structure of the PGs. Among the PGs tested only  $\text{PGE}_1$  was able to cause significant enhancement of the lipid transfer. The efficiency of the action of  $\text{PGE}_1$  is astonishingly high. Even at  $10^{-10}$  M,  $\text{PGE}_1$  increased the transfer of cholesterol ester by about 150% in the absence and about 3-fold in the presence of LDP. Such concentrations correspond to 1  $\text{PGE}_1$  molecule/approx.  $10^4$  lipoprotein globulae.

The kinetics of spontaneous transfer of phospholipids between lipoproteins are consistent with a mechanism involving a rate-limiting dissociation of monomers from the lipoprotein surface which is then followed by rapid diffusion of the lipid monomer through the aqueous phase [12]. In contrast, kinetic studies of protein-mediated interlipoprotein cholesterol ester transfer suggest that the transfer takes place via formation of a ternary collision complex involving donor and acceptor lipoproteins and the LEP [13]. Since  $\text{PGE}_1$  enhances both the spontaneous and protein-mediated transfer in a similar manner, it seems likely that the effect is due to reorganization of the lipoprotein surface by the prostaglandin rather than to interaction of  $\text{PGE}_1$  with the LEP. That  $\text{PGE}_1$  induces a rearrangement of the HDL surface has been demonstrated previously using fluorescence measurements [5]. The results of that fluorescence

study correlate with those of the present work because in both studies  $\text{PGE}_1$  was active, whereas the closely related  $\text{PGE}_2$  was not. Moreover, the very low  $\text{PGE}_1$  concentrations inducing HDL surface rearrangements on the one hand and changing the HDL to LDL lipid transfer rate on the other were approximately equal.

We have previously demonstrated that  $\text{PGE}_1$  stimulates cholesterol esterification in whole human plasma [14]. However, esterification experiments carried out with purified LCAT and cholesterol incorporated into lipoproteins, apo-A1 recombinants or  $\text{HDL}_3$  did not reveal any effect of the prostaglandin on the cholesterol esterification rate (not shown). This indicated that the  $\text{PGE}_1$ -induced stimulation of cholesterol esterification in whole plasma [14] might be due to enhancement of the interlipoprotein flux of LCAT substrates and products. To check such a possibility we studied the effect of  $\text{PGE}_1$  on the esterification of LDL-cholesterol in LDP in the presence or absence of  $\text{HDL}_3$ . The results (fig.4) revealed that only in the presence of  $\text{HDL}_3$  did  $\text{PGE}_1$  cause a considerable increase in cholesterol esterification. Thus, the combined evidence of our studies suggests that the  $\text{PGE}_1$ -induced rearrangement of the HDL surface may be sufficient to cause an enhancement of interlipoprotein lipid exchange, which in turn facilitates the cholesterol esterification in human plasma by augmenting fluxes of the substrate and product of the LCAT reaction. Since  $\text{PGE}_1$  is an anti-atherogenic prostaglandin whereas  $\text{PGE}_2$  and  $\text{PGF}_2$  are not, the present results suggest further that the influence of  $\text{PGE}_1$  on cholesterol homeostasis may be mediated by interaction of the prostaglandin with lipoproteins. This possibility is currently being examined in our laboratory.

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