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Phospholipid and cholesteryl ester transfer activities in plasma from 14 vertebrate species. Relation to atherogenesis susceptibility

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Abstract

Cholesteryl ester and phospholipid transfer activities were determined in plasmas from 14 vertebrates, and lipid transfer values were analyzed in the light of the known atherogenesis susceptibility of studied species. Whereas cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) activities among vertebrate species were only measured in lipoprotein-deficient fractions in previous studies, both endogenous lipoprotein-dependent and endogenous lipoprotein-independent assays were used in the present work. In agreement with previous studies, a few species (chicken, man, rabbit and trout) displayed substantial CETP activity, whereas CETP activity was not detectable in other species (cow, dog, horse, mouse, pig, and rat). Additional species that were not studied before, i.e. cat, goat, and sheep, were shown to be deficient in plasma cholesteryl ester transfer activity, while duck was shown to constitute a new member of the high activity group. Unlike CETP activity, PLTP activity was detected in plasmas from all studied species, most of them being assayed here for the first time (cat, chicken, cow, duck, goat, horse, sheep, and trout). While dog, trout, mouse, and pig displayed the highest phospholipid transfer activity levels, the remarkable preservation of facilitated phospholipid transfers in plasma from all vertebrates might indicate an essential role of PLTP *in vivo*. Interestingly, animals with well-documented atherogenesis susceptibility (chicken, pig, rabbit, and man) displayed significantly higher mean CETP activity, but lower mean PLTP activity than known 'resistant' animals (cat, dog, mouse, and rat). In conclusion, the present study revealed marked differences in plasma lipid transfer activities between vertebrate species, and interspecies comparisons indicated that both CETP and PLTP may constitute two determinants of the atherogenicity of the plasma lipoprotein profile. © 1998 Elsevier Science Inc. All rights reserved.

Keywords: Cholesteryl ester transfer protein; Phospholipid transfer protein; Plasma; Animal; Atherosclerosis

1. Introduction

In human plasma, lipoprotein particles can exchange lipid species through the action of two distinct lipid transfer proteins, i.e. the cholesteryl ester transfer protein (CETP) and the phospholipid transfer protein (PLTP). CETP catalyzes the transfer of cholesteryl esters and triglycerides between various lipoprotein

fractions, and it also accounts for some of the plasma phospholipid exchange activity [43]. PLTP promotes the net transfer of phospholipids from the triglyceride-rich lipoproteins towards high density lipoproteins (HDL) [43], and it is also involved in the transfer of α -tocopherol [24], unesterified cholesterol [38], and lipopolysaccharide [14]. Recently, CETP and PLTP were shown to constitute two important factors in the reverse cholesterol transport pathway by modifying the size distribution and the metabolic properties of HDL. Whereas both CETP and PLTP may improve cellular cholesterol efflux as well as plasma cholesterol esterifi-

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cation through the generation of pre- β HDL [11,22,35,47], the formation of large, cholesteryl ester-rich α HDL, associated with a low risk for coronary artery disease [3,5,23,36], was shown to be favored by PLTP, but reduced by CETP [18,26,46]. The latter observations suggest that CETP and PLTP might exert different effects on the atherogenicity of the plasma lipoprotein profile.

Human CETP [9] and PLTP [8] genes have been cloned, and sequence analysis revealed a 20% homology between the two genes [8]. In fact, CETP and PLTP belong to the same gene family, named lipid transfer/lipopolysaccharide binding protein (*LT/LBP*) gene family, which also includes the bactericidal permeability increasing protein (BPI) and the lipopolysaccharide binding protein (LBP) [21]. In addition to man, homologous *CETP* and *PLTP* genes have been found in other mammalian species, including hamster [19], cynomolgus monkey [39], and rabbit [37] for *CETP*, and mouse for *PLTP* [20]. Previous studies suggested that the plasma level and the tissue expression of CETP can vary widely [42], and plasma cholesteryl ester transfer activity measurements reported marked differences from one species to another [13]. Thus, in earlier studies by Ha and Barter [13], 16 vertebrate species could be arbitrarily subdivided into three distinct groups with low, intermediate or high cholesteryl ester transfer activity, and observed differences were shown to relate to variations in the level of some plasma lipoprotein components. Unlike CETP, that has been extensively studied during the last decade, PLTP is a newly emerging plasma factor that has been only recently characterized [45]. To date, cholesteryl ester and phospholipid transfer activity values have been obtained only in diluted plasma samples from several species [6,13,20,41], and data have not been analyzed in terms of atherosclerosis susceptibility.

In the present study, cholesteryl ester transfer and phospholipid transfer activities were simultaneously determined in plasma from 14 vertebrate species. In contrast to previous interspecies comparisons that were only based on the evaluation of the activity of either CETP or PLTP independently of endogenous lipoprotein substrates [6,13,20,41], we chose in the present study to measure CETP and PLTP activities in parallel by making use of two distinct experimental approaches for each lipid transfer protein. Thus, lipid transfer activities were evaluated by using either lipoprotein-independent assays or lipoprotein-dependent assays, reflecting mainly the lipid transfer protein mass concentration, and the activity of the lipid transfer protein as modulated by endogenous plasma factors, respectively [25]. Subsequently, lipid transfer activity values were compared with plasma lipid parameters, and they were analyzed in the light of the known atherogenesis susceptibility of some of the studied species.

2. Materials and methods

2.1. Blood samples

Normolipidemic subjects were fasted volunteers from the laboratory staff. Blood from rat, rabbit, and mouse was obtained from the laboratory animal house, and blood samples from cow, sheep, pig and horse were provided by the Institut National de la Recherche Agronomique (INRA, Dijon, France). Blood from chicken, duck and goat was obtained from farm animals, and blood from cat and dog was from a veterinary surgery. Plasma from trout was a generous gift of Dr P. Babin (Centre National de la Recherche Scientifique (CNRS), Orsay).

Blood was collected into EDTA-containing glass tubes either by veno-puncture in most cases or by heart-puncture in the case of trout. Plasma was separated by a 5-min centrifugation at $3000 \times g$ at 4°C and was immediately stored at -70°C for further analysis.

2.2. Plasma lipoprotein preparation

Lipoproteins were isolated from normolipidemic human plasma by sequential ultracentrifugation at a speed of 100000 rpm ($386000 \times g$) in a TLA-100.2 rotor in a Beckman TL100 ultracentrifuge (Beckman, Palo Alto, CA). Densities were adjusted with solid potassium bromide. Total lipoproteins, HDL₃, and LDL were isolated as the $d < 1.21$, $1.13 < d < 1.21$, and $1.019 < d < 1.063 \text{ g ml}^{-1}$ plasma fractions, respectively.

Recovered lipoproteins were dialyzed against a 10-mmol l^{-1} Tris, 150-mmol l^{-1} NaCl, pH 7.4 buffer containing 0.2 g l^{-1} NaN₃ and 1 mmol l^{-1} EDTA (TBS buffer).

2.3. Plasma CETP activity

Plasma cholesteryl ester transfer activity was determined as the rate of radiolabeled cholesteryl esters transferred from radiolabeled HDL₃ containing tritiated cholesteryl esters ($[^3\text{H}]$ CE-HDL₃) that were biosynthetically labelled according to the general procedure previously described [29].

Two distinct assays that were dependent (lipoprotein-dependent assay) or not (lipoprotein-independent assay) on endogenous plasma lipoprotein substrates were set up.

In the lipoprotein-dependent assay, CETP activity was measured as the capacity of the plasma sample to promote the transfer of radiolabeled cholesteryl esters from a tracer dose of radiolabeled HDL₃ towards endogenous apo B-containing lipoproteins as described before [27]. Resulting cholesteryl ester transfer rates reflect the transfer of cholesteryl esters as modulated by intrinsic plasma components, including the concentra-

tion and composition of endogenous lipoproteins [12]. Briefly, each incubation mixture contained a 25- μ l aliquot of total plasma, radiolabeled human HDL₃ (2.5 nmol of cholesteryl ester), and iodoacetate (final concentration 1.5 mmol l⁻¹) in a final volume of 50 μ l. Incubations were performed in triplicate for 3 h at 37°C. A 45- μ l volume of incubated mixtures was then added to 2 ml of a $d=1.07$ g l⁻¹ KBr solution in Beckman centrifuge tubes. The tubes were sealed and centrifuged for 7 h at 50000 rpm (269000 $\times g$) in a 50.4 Ti rotor in a L7 ultracentrifuge (Beckman, Palo Alto, CA). The $d < 1.068$ and $d > 1.068$ g ml⁻¹ fractions were subsequently recovered in 1-ml volumes and transferred into counting vials. A volume of 2 ml of scintillation fluid (OptiScint Hisafe 3, Pharmacia, Uppsala, Sweden) was added to each vial and the radioactivity was assayed for 5 min in a Wallac 1410 liquid scintillation counter (Pharmacia). Results from triplicate determinations are expressed in percentage of radiolabeled cholesteryl esters transferred after deduction of non-incubated controls maintained at 4°C.

In the lipoprotein-independent assay, CETP activity was measured in diluted plasma samples as the transfer of radiolabeled cholesteryl esters from HDL₃ towards an excess of exogenous human LDL. In that particular case, resulting cholesteryl ester transfer rates reflect the transfer of cholesteryl esters independently of endogenous plasma components, and, as previously described [33], they are mainly a function of plasma CETP mass concentration. Briefly, 10- μ l volumes of plasma samples were incubated for 12 h at 37°C with radiolabeled HDL₃ (25 nmol of cholesteryl ester), unlabeled LDL (500 nmol of cholesteryl ester), and iodoacetate (final concentration, 1.5 mmol l⁻¹) in a final volume of 100 μ l. After incubation, the $d < 1.068$ and $d > 1.068$ g ml⁻¹ fractions were ultracentrifugally isolated and radioactivity was assayed as described above. Results from triplicate determinations were expressed as the percentage of radioactivity transferred to the $d < 1.068$ g ml⁻¹ fraction after deduction of non-incubated controls maintained at 4°C.

2.4. Plasma PLTP activity assay

Phospholipid transfer activity was measured in total plasma as the transfer of radiolabeled phosphatidylcholine from [¹⁴C]PC-liposomes to endogenous HDL (lipoprotein-dependent assay) [30] or to an excess of exogenous human HDL₃ (lipoprotein-independent assay) [7]. Radiolabeled phosphatidylcholine-containing liposomes were prepared according to the general procedure previously described [7,28]. This phospholipid transfer assay was proven to be specific for PLTP activity since CETP is unable to transfer phospholipids from liposomes to lipoproteins [6,28].

In the lipoprotein-dependent phospholipid transfer assay, total plasma (30 μ l) was incubated for 30 min at 37°C with [¹⁴C]PC-liposomes (10 μ l) in the presence of iodoacetate (final concentration, 1.5 mmol l⁻¹) in a final volume of 80 μ l [7]. At the end of incubation, sample mixtures were immediately placed on ice, and 320 μ l of TBS buffer were added to each tube. Phospholipid liposomes and apoB-containing lipoproteins were subsequently precipitated by the addition of 300 μ l of a 500-mmol l⁻¹ NaCl, 215-mmol l⁻¹ MnCl₂, 445-U ml⁻¹ heparin solution. 'Total Count' controls received 300 μ l of TBS buffer instead of the MnCl₂/heparin precipitating reagent. Tubes were then vortexed, left for 10 min at room temperature, and finally centrifuged at 10000 rpm for 10 min in an Eppendorf centrifuge to remove precipitate. The radioactivity in the supernatant was measured in 500- μ l aliquots supplemented with 2 ml of scintillation fluid (OptiScint Hisafe 3, Pharmacia, Uppsala, Sweden) by using a Wallac 1410 liquid scintillation counter. PLTP activity was expressed as the rate of total radiolabeled phospholipids ('Total Count' sample) transferred from radiolabeled liposomes to the endogenous HDL fraction after deduction of non-incubated controls maintained at 4°C.

In the lipoprotein-independent assay, 10 μ l of plasma were incubated for 1 h at 37°C with radiolabeled liposomes (10 μ l) and isolated human HDL₃ (250 μ g of protein) in the presence of iodoacetate (final concentration, 1.5 mmol l⁻¹) in a final volume of 400 μ l. Following incubation, phospholipid liposomes were selectively precipitated and radioactivity in the supernatant was assayed as described above. PLTP activity was expressed as the rate of radiolabeled phosphatidylcholine transferred from radiolabeled liposomes to exogenous human HDL₃ after deduction of non-incubated controls.

2.5. Plasma lipid assays

All chemical assays were performed on a Cobas-Fara Centrifugal Analyzer (Roche). Total and unesterified cholesterol were measured by enzymatic methods using Boehringer reagents. Phospholipids and triglycerides were measured by enzymatic assays using BioMérieux or Roche Diagnostics reagents, respectively. Protein concentration was measured with the bicinchoninic acid reagent (Pierce).

2.6. Statistical evaluation

Correlations between plasma parameters were analyzed by Spearman rank correlation analysis. The significance of the difference between data means was determined by using the Mann–Whitney test.

Table 1
Plasma lipid concentrations in 14 vertebrate species

Species		Total cholesterol (g l ⁻¹)	Unesterified cholesterol (g l ⁻¹)	Esterified cholesterol (g l ⁻¹)	Triglycerides (g l ⁻¹)	Phospholipids (g l ⁻¹)
Cat	<i>Felix catus</i>	1.68–1.42	0.54–0.46	1.14–0.96	0.30–0.54	1.62–2.40
Chicken	<i>Gallus domest-</i> <i>cus</i>	0.88–0.96	0.43–0.53	0.45–0.43	1.22–1.96	1.44–1.56
Cow	<i>Bos taurus</i>	0.67–0.67	0.17–0.17	0.50–0.50	0.24–0.22	0.78–0.77
Dog	<i>Canis familiaris</i>	2.41–1.86	0.80–0.63	1.61–1.23	0.24–0.39	3.68–1.69
Duck	<i>Anas boschas</i>	1.94–2.82	0.87–1.16	1.07–1.66	3.18–3.09	3.94–5.12
Goat	<i>Capra hircus</i>	0.82–0.80	0.24–0.24	0.58–0.56	0.18–0.21	1.18–1.20
Horse	<i>Equus caballus</i>	0.73–0.72	0.23–0.22	0.50–0.50	0.62–0.62	1.21–1.19
Man	<i>Homo sapiens</i>	1.86 ± 0.27	0.67 ± 0.11	1.19 ± 0.16	0.63 ± 0.20	1.96 ± 0.33
Mouse	<i>Mus musculus</i>	0.94–0.80	0.37–0.34	0.57–0.46	1.04–1.05	1.36–1.60
Pig	<i>Sus domesticus</i>	0.72–0.67	0.17–0.16	0.55–0.51	0.22–0.20	0.51–0.79
Rabbit	<i>Oryctolagus sp.</i>	0.57–0.66	0.18–0.26	0.39–0.40	0.94–1.24	0.90–1.16
Rat	<i>Rattus rattus</i>	0.62–0.58	0.22–0.15	0.40–0.43	2.79–1.93	1.22–1.27
Sheep	<i>Ovis aries</i>	0.56–0.54	0.15–0.15	0.41–0.39	0.18–0.18	0.73–0.71
Trout	<i>Oncorhynchus</i> <i>mykiss</i>	1.73–1.64	0.84–0.77	0.89–0.87	0.85–1.01	4.37–4.04

For each animal species, the two lipid concentration values correspond to two different animals, with the exception of mouse for which lipid concentrations were assayed in two plasma pools from six distinct animals each. Man's values are mean ± S.D. of four plasma samples from normolipidemic subjects.

3. Results

3.1. Plasma lipid concentrations among various animal species

Lipid concentrations were measured in total plasma of 14 vertebrate species (Table 1). Relatively low plasma cholesterol levels were found in chicken, cow, goat, horse, mouse, pig, rabbit, rat, and sheep, while they tended to be in the upper range in cat, dog, duck, man, and trout. Triglyceride and phospholipid concentrations were widely distributed among the studied species, with high triglyceride levels in chicken, duck, and rat, and high phospholipid levels in dog, duck, and trout.

3.2. Plasma cholesteryl ester transfer activity

As described in Section 2, cholesteryl ester transfer activity among various vertebrate species was evaluated by using two distinct assays, i.e. a lipoprotein-dependent assay, and a lipoprotein-independent assay. Table 2 shows plasma cholesteryl ester transfer rates measured in all studied species by using the two methods. Substantial cholesteryl ester transfer activity was detected only in five species (chicken, duck, man, rabbit, and trout), whereas cholesteryl ester transfer rates did not exceed a few percent in cat, cow, dog, goat, horse, mouse, pig, rat, and sheep by using either of the two assays (Table 2). Only duck, rabbit, and trout displayed higher cholesteryl ester transfer activity than man, and consistent observations could be made by using the lipoprotein-dependent and the lipoprotein-independent

assays. Among all studied species ($n = 14$), Spearman rank correlation analysis revealed a significant correlation between cholesteryl ester transfer rates obtained with the two methods ($r = 0.81$; $P < 0.005$).

3.3. Plasma phospholipid transfer activity

Plasma phospholipid transfer activity was measured as the rate of radiolabeled phosphatidylcholine transferred from [¹⁴C]PC-liposomes towards either endogenous plasma HDL (lipoprotein-dependent assay) or an excess of exogenous human HDL₃ (lipoprotein-independent assay). The experimental procedure involving artificial phospholipid liposomes was previously proven to specifically reflect the activity of plasma PLTP, independently of the phospholipid exchange activity of CETP [6,28].

Unlike cholesteryl ester transfer activity, substantial phospholipid transfer rates could be measured in all studied species. Some discordances in phospholipid transfer activity could be noted by using either the lipoprotein-dependent assay or the lipoprotein-independent assay (Table 3). Indeed, the highest phospholipid transfer rates were displayed by duck and trout when evaluated with the lipoprotein-dependent assay, but by mouse and pig when measured with the lipoprotein-independent assay (Table 3). Nevertheless, a significant correlation between the two phospholipid transfer rate series was observed ($r = 0.65$; $P < 0.05$).

No significant correlations between CETP and PLTP activities were observed among studied species when using either lipoprotein-dependent or lipoprotein-independent assays.

Table 2
Cholesteryl ester transfer activity in plasma from 14 vertebrate species

Species	Lipoprotein-dependent cholesteryl ester transfer rate		Lipoprotein-independent cholesteryl ester transfer rate	
	%	Relative to man	%	Relative to man
Cat	ND	ND	0.5 ± 0.8	0.02 ± 0.03
Chicken	10.7 ± 3.5	0.87 ± 0.28	7.6 ± 2.9	0.30 ± 0.11
Cow	ND	ND	0.4 ± 0.8	0.02 ± 0.03
Dog	0.1 ± 0.2	0.01 ± 0.01	0.4 ± 0.5	0.01 ± 0.02
Duck	27.5 ± 7.4	2.23 ± 0.60	34.5 ± 9.6	1.36 ± 0.38
Goat	0.1 ± 0.1	0.01 ± 0.01	0.7 ± 0.5	0.03 ± 0.02
Horse	ND	ND	1.0 ± 0.4	0.04 ± 0.02
Man	12.3 ± 2.7	1.00 ± 0.22	25.5 ± 7.4	1.00 ± 0.29
Mouse	ND	ND	1.7 ± 0.7	0.07 ± 0.03
Pig	0.1 ± 0.2	0.01 ± 0.01	2.1 ± 1.0	0.08 ± 0.04
Rabbit	22.9 ± 1.9	1.87 ± 0.16	48.4 ± 1.6	1.90 ± 0.06
Rat	0.1 ± 0.1	0.01 ± 0.01	3.0 ± 0.6	0.12 ± 0.02
Sheep	0.1 ± 0.1	0.01 ± 0.01	0.7 ± 0.5	0.03 ± 0.02
Trout	15.3 ± 4.6	1.24 ± 0.37	44.9 ± 5.9	1.76 ± 0.23

Cholesteryl ester transfer activity was determined as the rate of radiolabeled cholesteryl esters transferred from [³H]CE-HDL₃ towards either the endogenous plasma apoB-containing lipoproteins (lipoprotein-dependent assay) or an excess of exogenous LDL (lipoprotein-independent assay) (see Section 2). For each animal species, triplicate determinations were performed in plasma samples from two distinct animals, with the exception of mouse for which CETP activity measurements were performed in triplicate in pooled plasmas from six distinct animals. In the case of man, triplicate determinations were performed in plasma samples from four normolipidemic subjects. ND, not detectable.

3.4. Comparison of lipid transfer activities in plasmas from species 'resistant' or 'susceptible' to atherosclerosis

As previously proposed [31], two distinct groups could be constituted among studied species on the basis of their atherosclerosis susceptibility. Thus, cat, dog, mouse, and rat formed the 'resistant' group, while chicken, pig, rabbit, and man formed the 'susceptible' group. As shown in Fig. 1A,B, mean cholesteryl ester transfer activity, as measured with either the lipoprotein-dependent assay or the lipoprotein-independent assay, was significantly higher in the 'susceptible' group than in the 'resistant' group. Conversely, mean plasma phospholipid transfer activity was lower in the 'susceptible' group than in the 'resistant' group (Fig. 1C,D). In the latter case, differences reached the statistical significance level only with the lipoprotein-dependent assay (Fig. 1C).

4. Discussion

Previous studies indicated that plasma cholesteryl ester transfer and phospholipid transfer activities, determined in endogenous lipoprotein-independent assays, can vary from one species to another [6,13,20,41]. Since the lipid transfer process requires the direct interaction of lipid transfer proteins with lipoprotein substrates, both the amount and composition of plasma lipoproteins are susceptible to greatly influence the lipid

transfer reaction [25]. Thus, plasma lipid transfer activity values result from a combination of the amount of lipid transfer proteins, of their ability to interact with lipoproteins, as well as of the amount and composition of endogenous lipoprotein substrates. Since the amount and composition of plasma lipoproteins are known to vary widely among various vertebrate species [4], specificities in the plasma lipoprotein profile may account, at least in part, for differences in lipid transfer activities from one species to another. Therefore, two distinct experimental approaches were set up in the present study, reflecting either the mass concentration of lipid transfer protein (lipoprotein-independent assay), or the activity of the lipid transfer protein as modulated by endogenous lipoproteins (lipoprotein-dependent assay). In accordance with previous findings [6,13,41,48], striking variations in plasma cholesteryl ester transfer activity were observed among studied species. Indeed, by using a lipoprotein-independent assay, substantial levels of cholesteryl ester transfer activity were measured in plasma from chicken, man, rabbit, and trout, while CETP activity was virtually absent from plasma of cow, dog, horse, mouse, pig, and rat. Overall, observed variations confirmed those reported by others [6,13,41,48]. In addition, new species were studied, and cat, goat, and sheep were shown to be deficient in plasma cholesteryl ester transfer activity, while duck was shown to constitute a new member of the high activity group. In spite of some differences in species ranking determined with either lipoprotein-dependent or lipoprotein-independent assays, a good correspon-

Table 3
Phospholipid transfer activity in plasma from 14 vertebrate species

Species	Lipoprotein-dependent phospholipid transfer rate		Lipoprotein-independent phospholipid transfer rate	
	%	Relative to man	%	Relative to man
Cat	21.4 ± 4.3	1.46 ± 0.29	18.8 ± 3.3	0.93 ± 0.17
Chicken	4.8 ± 0.9	0.32 ± 0.06	8.4 ± 0.6	0.42 ± 0.03
Cow	11.6 ± 1.4	0.79 ± 0.10	10.9 ± 1.0	0.54 ± 0.05
Dog	23.7 ± 0.9	1.62 ± 0.06	21.4 ± 1.5	1.07 ± 0.07
Duck	19.4 ± 0.4	1.32 ± 0.03	15.3 ± 1.6	0.76 ± 0.08
Goat	14.5 ± 0.8	0.99 ± 0.05	12.6 ± 2.4	0.63 ± 0.12
Horse	4.8 ± 0.4	0.33 ± 0.03	9.5 ± 1.0	0.46 ± 0.05
Man	14.6 ± 2.7	1.00 ± 0.19	20.1 ± 3.3	1.00 ± 0.16
Mouse	15.7 ± 4.3	1.07 ± 0.29	29.9 ± 1.9	1.49 ± 0.09
Pig	10.4 ± 1.5	0.71 ± 0.10	24.6 ± 1.6	1.24 ± 0.07
Rabbit	9.1 ± 0.7	0.62 ± 0.05	10.2 ± 1.5	0.51 ± 0.08
Rat	12.4 ± 1.6	0.85 ± 0.11	17.7 ± 2.3	0.88 ± 0.11
Sheep	8.6 ± 0.4	0.59 ± 0.03	11.2 ± 0.7	0.56 ± 0.03
Trout	30.9 ± 6.0	2.11 ± 0.41	15.4 ± 2.0	0.77 ± 0.10

Phospholipid transfer activity was determined as the rate of radiolabeled phosphatidylcholine transferred from [¹⁴C]PC-liposomes towards either the endogenous plasma HDL fraction (lipoprotein-dependent assay) or an excess of exogenous HDL₃ (lipoprotein-independent assay) (see Section 2). For each animal species, triplicate determinations were performed in plasma samples from two distinct animals, with the exception of mouse for which PLTP activity measurements were performed in triplicate in pooled plasmas from six distinct animals. In the case of man, triplicate determinations were performed in plasma samples from four normolipidemic subjects.

dence between the two series of homologous lipid transfer values was noted. In contrast with cholesteryl ester transfer activity that was absent from plasma of several species, substantial phospholipid transfer activity was detected in plasma from all studied species by using either of the two assays. In fact, cholesteryl ester and phospholipid transfer activities did not correlate significantly with either assay suggesting further that, although structurally related, CETP and PLTP expressions are independently regulated *in vivo*. The remarkable preservation of facilitated plasma phospholipid transfer among vertebrates might indicate an essential role of PLTP *in vivo*. Overall, phospholipid transfer rates determined with the lipoprotein-independent assay were in good agreement with previously reported data [6,20,41]. In particular, mouse has the highest phospholipid transfer activity, rabbit belongs to the low activity group, and dog and pig display activity similar to that of man. In addition to previous studies, phospholipid transfer activities among eight new species, i.e. cat, chicken, cow, duck, goat, horse, sheep, and trout, are given in the present report. Although a significant correlation between phospholipid transfer rates measured with the lipoprotein-dependent and the lipoprotein-independent assays was observed among the 14 studied species, there were some discrepancies between the two methods. For instance, the endogenous lipoprotein-dependent phospholipid transfer activity was markedly higher in trout plasma relative to man plasma, while the opposite conclusion could be drawn when the lipoprotein-independent assay was used instead.

It is noteworthy that several vertebrate species, for which plasma lipid transfer activities were determined, were previously used as animal models in understanding the atherosclerotic process [1]. In fact, comparative atherosclerosis allowed to define two distinct groups with low or high atherosclerosis susceptibility [31]. Thus, as previously proposed [31], cat, dog, mouse and rat belong to the 'resistant' group, while chicken, pig, rabbit and man belong to the 'susceptible' group. In the present study, mean CETP activity, as measured by using either lipoprotein-dependent or lipoprotein-independent assays, was shown to be markedly and significantly lower in the 'resistant' group than in the 'susceptible' group. Interestingly, a similar tendency can be worked out from data of Ha and Barter [13]. However, in the latter study, 'resistant' and 'susceptible' groups were not constituted, and CETP activity data were not analyzed in the light of atherosclerosis susceptibility of individual species. The association of high plasma cholesteryl ester transfer activity with elevated atherosclerosis susceptibility, as observed in the present study, is sustained by other experimental data obtained in cholesterol-fed monkeys [40], in some dyslipidemic populations [2,44], and in CETP-transgenic mice [34]. Nevertheless, more recent observations obtained in probucol-treated patients [10,32], in CETP-deficient patients [16,17,49], and in CETP-CIII transgenic mice [15], rather support an antiatherogenic effect of CETP. In fact, it has been proposed that the effect of CETP might vary according to the metabolic context [43], and on a biochemical point of view CETP might exert either a positive effect by generating pre β HDL or a negative

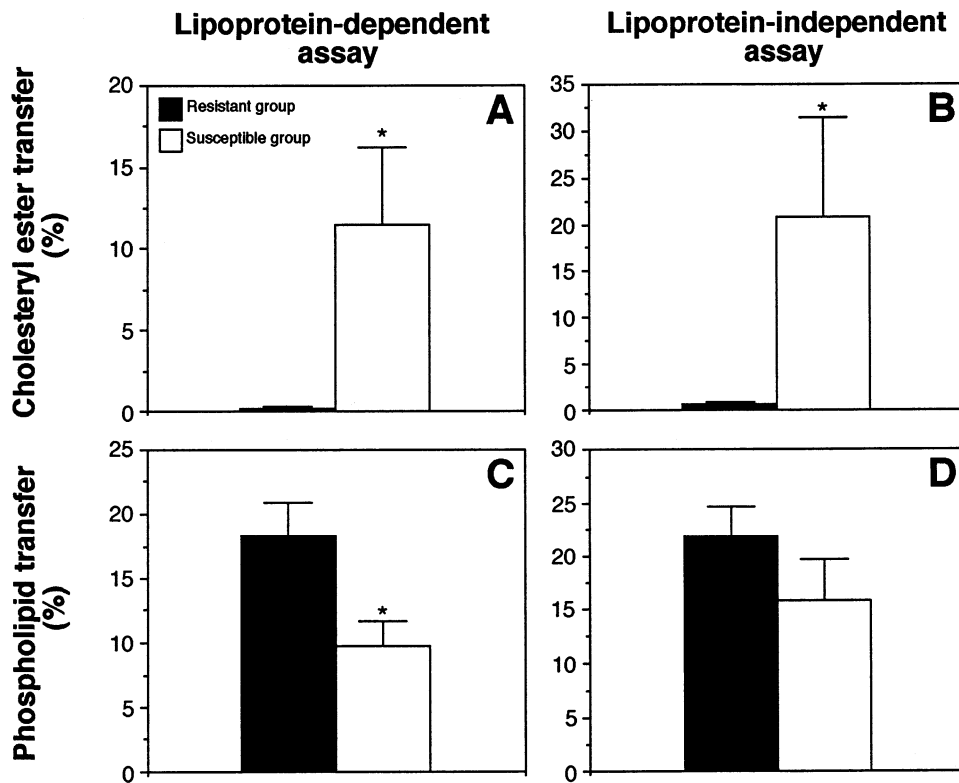


Fig. 1. Cholesteryl ester transfer and phospholipid transfer rates in plasma from vertebrate species 'resistant' or 'susceptible' to atherosclerosis. Chicken, pig, rabbit, and man formed the 'susceptible' group, while cat, dog, mouse, and rat formed the 'resistant' group. CETP and PLTP activities, expressed as the percentage of radioactivity transferred, were measured by using assays that were dependent (lipoprotein-dependent assay) or not (lipoprotein-independent assay) on endogenous plasma lipoprotein substrates (Section 2). Vertical bars are mean \pm S.E.M. of transfer values from four species. * $P < 0.05$.

effect by carrying cholesteryl esters from α HDL towards proatherogenic apoB-containing lipoproteins. In contrast to CETP activity, we observed that mean plasma PLTP activity was significantly higher in the 'resistant' group than in the 'susceptible' group when measured with the lipoprotein-dependent assay, and a similar observation, but not statistically significant, could be made with the lipoprotein-independent assay. Although not conclusive at this stage, the interspecies comparisons of plasma phospholipid transfer activity support recent studies which suggested that PLTP might exert a dual beneficial effect by favoring simultaneously the formation of small, pre β HDL, that constitute the initial acceptors of cellular cholesterol, and large, cholesteryl ester-rich α HDL, that have been longer associated with a low risk for coronary artery disease.

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