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Lipid and fatty acid composition of canine lipoproteins

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Abstract

Lipid classes and their fatty acids were studied in the major lipoprotein fractions from canine, in comparison with human, plasma. In dogs, high-density-lipoprotein (HDL), the main carrier of plasma phospholipid (PL), cholesterol ester (CE) and free cholesterol, was the most abundant lipoprotein, followed by low and very-low density lipoproteins (LDL and VLDL). Notably, LDL and VLDL contributed similarly to the total dog plasma triacylglycerol (TG). The PL composition was similar in all three lipoproteins, dominated by phosphatidylcholine (PC). Even though the content and composition of lipids within and among lipoproteins differed markedly between dog and man, the total amount of circulating lipid was similar. All canine lipoproteins were relatively richer than those from humans in long-chain (C20-C22) n-6 and n-3 polyunsaturated fatty acids (PUFA) but had comparable proportions of total saturated and monoenoic fatty acids, with 18:2n-6 being the main PUFA in both mammals. The fatty acid profile of canine and human lipoproteins differed because they had distinct proportions of their major lipids. There were more n-3 and n-6 long-chain PUFA in canine than in human plasma, because dogs had more HDL, their HDL had more PC and CE, and both these lipids were richer in such PUFA. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Dog lipoproteins; HDL; LDL; VLDL; Phospholipids; Cholesterol esters; Triacylglycerols; Polyunsaturated fatty acids

Abbreviations: CE, cholesterol esters; FAME, fatty acid methyl esters; FC, free cholesterol; GC, gas chromatography; HDL, high density lipoproteins; LCAT, lecithin: cholesterol acyltransferase; LDL, low density lipoproteins; MFA, monoenoic fatty acids; PC, phosphatidylcholine; PL, phospholipid; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TC, total cholesterol; TLC, thin-layer chromatography; TG, triacylglycerols; VLDL, very low density lipoproteins.

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1. Introduction

Vertebrates differ in their relative amounts of plasma lipoproteins. In the vast majority of mammals, including the dog, HDL is the predominant fraction (Chapman, 1986; Lehmann et al., 1993; Bauer, 1996). Those mammalian species where high density lipoproteins (HDL) account for 50% or more of the total particles of density less than 1.21 g/ml in plasma are considered 'HDL mammals' (Chapman, 1986). Physicochemical properties of canine lipoproteins and the enzymes involved in their metabolism are of interest because, in contrast to humans and other so termed 'LDL mammals', the dog is substantially resistant to the development of atherosclerosis and hypercholesterolemia (Watson and Barrie, 1993). Mahley and Weisgraber (1974) characterized dog lipoproteins including description of microscopic appearance, major apoprotein constituents and lipid classes. The fatty acid composition of the total plasma lipid was also reported in dogs in comparative studies with other mammals (Pekiner and Pennock, 1995). However, quantitative and qualitative information about the lipid constituents of dog lipoprotein fractions is still fragmentary. The aim of this work was to present a description of the lipid and fatty acid composition of the major lipoprotein classes in the dog. For that purpose, plasma lipoproteins were separated by applying the method developed by Vieira et al. (1996) for the rapid isolation of human LDL. The good separation between the three main lipoprotein fractions, and the short time of processing, made this method attractive and suitable for the study of lipoprotein lipids and their fatty acids. The present results compare, on the same methodological bases, the lipoprotein lipid constituents of dog and man. The latter is mostly used as a control, since the composition of human lipoproteins is widely known. Dog and man are shown to have a phosphatidylcholine-rich and a cholesterol ester-rich fasting plasma, respectively. Canine plasma is shown to be relatively richer in long-chain n-6 and n-3 PUFA than human plasma because of its distinctive lipoprotein profile, dominated by HDL.

2. Materials and methods

2.1. Blood samples and isolation of lipoproteins

Four male Beagle dogs, eight years old, apparently healthy, were housed and fed under standard laboratory conditions. The major fatty acids of the chow diet used were 16:0, 16:1, 18:0, 18:1, 18:2n-6 and 18:3n-3 (19.1. 3.4. 6.4. 33.6. 29.2 and 3.2 %, respectively). The sums of n-6 and n-3 fatty acids were 29.7 and 5.1%, respectively (n-6/n-3 ratio: 5.9). After an overnight fast, at 09.00 h, blood samples were obtained by venipuncture of the cephalica antebrachial vein using heparinized material. Plasma was separated immediately by centrifugation at 3000 rev/min for 15 min at 15°C, and used for lipid and lipoprotein analysis. Canine lipoproteins were obtained by the procedure described for the isolation of human plasma LDL by Vieira et al. (1996). Blood samples from two healthy human normolipidemic male donors, aged 35 and 40, were studied for comparison. Both subjects were on an ordinary Mediterranean diet. The samples were also taken at 09.00 h, after a fasting period of 12 h and processed together with those from dogs. Briefly, solid KBr was added to 2.5 ml of plasma to adjust the density to 1.21 g/ml. A discontinuous gradient was made by overlying the plasma with 6 ml phosphate-buffered saline containing 110 mM NaCl, 20 mM phosphate pH 7.4 and 1 mM EDTA. The samples were then centrifuged at 40 000 rev./min for 3 h at 15°C, using a TFT 65.13 fixed-angle rotor with brakes off. After centrifugation, 1 ml of the top fraction containing VLDL was collected by aspiration. The next 3 ml were discarded, and the next 1 ml containing LDL was carefully collected by suction. After discarding the next 1.5 ml, the last 2 ml remaining at the bottom of the tube and containing HDL were aspirated. The purity of canine and human LDL preparations was monitored by SDS-PAGE (Vieira et al., 1996), which showed the presence of a single protein band with a molecular mass close to that expected for the major LDL apoprotein, apoB100 (~ 500 kDa in both). The proper separation between LDL and the other two fractions was monitored in preparations run in parallel to which the lipophilic dye Sudan Black had been added. Lipoprotein fractions were analyzed without further purification.

2.2. Extraction and analysis of lipoprotein lipids and fatty acids

Lipids were extracted from each lipoprotein fraction with chloroform:methanol (1:2, v/v) (Bligh and Dyer, 1959). Aliquots of the extracts were taken for the determination of total lipid

phosphorus, of the fatty acid composition of the total lipid, and for the separation of lipids by thin layer chromatography (TLC). Phospholipids (PL) were separated into classes using chloroform/methanol/acetic acid/0.15 M NaCl (50:25:8:2.5, by vol.) as the solvent (Brown and Subbaiah, 1994) and the bands were located under ultraviolet light after spraying the plates with dichlorofluorescein in methanol. The PL classes other than phosphatidylcholine (PC) were scraped from the plates to tubes for phosphorus analysis. PC was eluted quantitatively from the plates with chloroform/methanol/acetic acid/water (50:39:1:10, by vol.) (Arvidson, 1968) and aliquots were taken for phosphorus and for fatty acid analysis. The neutral lipids at the top of the TLC plates were eluted, as was PC, dried under N_2 and subjected to TLC using n-hexane/diethyl ether (80:20, v/v) to resolve free cholesterol (FC), triacylglycerols (TG) and cholesterol esters (CE). The bands were located, scraped into tubes, and eluted as above (Arvidson, 1968). FC and TG were quantified by a standard colorimetric method used in clinical settings (Boehringer Mannheim Gmbh, Mannheim, Germany).

CE and TG were quantified by gas chromatography (GC) of their fatty acid methyl esters (FAME), using methyl heneicosanoate (21:0) as an internal standard. Lipid methanolysis was performed using 14% BF_3 in methanol, placing the samples overnight at 45°C under N_2 in Teflon®-sealed tubes. The resulting FAME were recovered into hexane and purified on TLC plates that had been previously washed with acetonitrile/diethyl ether (75:25, v/v), by using hexane/diethyl ether (95:5, v/v). FAME areas were located as above and recovered by partition in water/methanol/hexane (1:1:2, v/v/v), with vigorous agitation followed by centrifugation. Fatty acid analysis was performed using a Varian 3700 gas chromatograph equipped with two (2 m × 2 mm) glass columns packed with 15% SP 2330 on Chromosorb WAW 100/120 (Supelco Inc., Bellefonte, PA). The column oven temperature was programmed from 155°C to 230°C at a rate of 5°C/min. Injector and detector temperatures were 220 and 230°C, respectively, and N_2 (30 ml/min) was the carrier gas. Fatty acid peaks were detected with a flame ionization detector, operated in the dual-differential mode, and quantified by electronic integration.

3. Results

3.1. Lipid classes of canine and human plasma and plasma lipoprotein classes

The lipid composition of plasma and of plasma HDL, LDL and VLDL in canine and human species is shown in Table 1. The main lipid classes of plasma, namely PL, CE, FC and TG, were in quite different proportions in both mammals. The most conspicuous contrasts were in the concentration of PL (higher in dogs), and in that of cholesterol and TG (higher in humans), leading to total PL/total cholesterol (TC) and PL/TG ratios considerably higher in dogs. The sum of

Table 1
Lipid content and composition of canine and human plasma and plasma lipoprotein classes[a]

Lipid class	Canine				Human			
	Plasma	HDL	LDL	VLDL	Plasma	HDL	LDL	VLDL
Content (μmol/dl)								
Phospholipids	301.7 ± 35.9	276.8 ± 27.2	24.4 ± 3.7	6.5 ± 1.1	186.1	56.9	112.5	17.4
Cholesterol esters	171.0 ± 8.2	157.4 ± 10.2	15.3 ± 0.2	3.8 ± 0.1	233.6	25.9	193.6	4.6
Free cholesterol	77.6 ± 5.0	56.2 ± 3.6	12.8 ± 1.8	3.4 ± 0.6	112.1	10.2	90.2	8.7
Triacylglycerols	24.5 ± 5.0	2.4 ± 0.2	11.0 ± 2.0	11.7 ± 3.0	57.3	8.3	15.2	42.6
Composition (mol%)								
Phospholipids	52.4 ± 2.6	56.5 ± 1.7	38.4 ± 1.5	25.6 ± 3.2	31.6	56.1	27.2	23.7
Cholesterol esters	29.9 ± 1.8	31.6 ± 1.4	24.1 ± 3.3	15.0 ± 0.8	39.7	25.5	47.0	6.2
Free cholesterol	13.5 ± 0.6	11.5 ± 0.4	20.2 ± 0.4	13.4 ± 1.4	19.0	10.2	21.0	11.9
Triacylglycerols	4.3 ± 0.6	0.5 ± 0.04	17.3 ± 0.5	46.1 ± 2.0	9.7	8.2	3.7	58.2

[a] Data are the mean values ± S.D. from four dogs and the mean of two in the case of men. PL were determined by phosphorous analysis, and the neutral lipids after their separation by TLC as described in Section 2. CE and TG were quantified after analysis of their fatty acids by GC.

these four major lipid constituents gave similar values in dog and human plasma (a total of 420 and 413 mg/dl, respectively).

Considering the lipoprotein lipid content (Table 1 upper panel), HDL transported as much as 84% of the total circulating lipid, being the predominant lipoprotein in dogs, and the main carrier of plasma PL, CE and FC, in that order. In human plasma, it was LDL that carried the bulk (65%) of the total circulating lipid, though in a different quantitative order (CE > PL > FC). TG accounted for a significant proportion of the lipids in VLDL, although VLDL was more abundant in human than in dog plasma. It is noteworthy that, while in human plasma most of the TG was in VLDL, in canine plasma VLDL and LDL contributed similarly to total plasma TG. Calculated horizontally from the data in the upper panel of Table 1, it follows that HDL provided as much as 90% of the total PL, 90% of the total CE, and 78% of the total FC in dog plasma. By the same token, LDL supplied nearly 60% of the total PL, 86% of the CE and 83% of the FC in human plasma. A usual way for the quantitative expression of lipoprotein concentrations is by referring to their total cholesterol content. By adding up the content of free and esterified cholesterol, HDL in dogs and LDL in humans were similar in that they contributed approximately 85% to the total cholesterol in plasma. The most important distinctive feature of dog circulating lipid was that it was substantially richer in PL, mostly contributed by HDL.

The lower panel of Table 1 shows the lipid composition of plasma and lipoprotein fractions, calculated from the concentration data. In both mammals, HDL were the richest in PL and the poorest in TG, and VLDL were the richest in TG and the poorest in PL. The most marked interspecies differences in lipoprotein composition were found in LDL, especially in the PL/TC molar ratio (0.8 in dogs and 0.4 in men) and in the percentage of TG (in dogs, five-fold that in men).

Table 2 shows the phospholipid composition of canine and human plasma and plasma lipoproteins. The major PL components were similar in all three lipoprotein fractions in mammals, phosphatidylcholine (PC) being the prevalent class, sphingomyelin and lysoPC being relatively minor. Also in both mammals, the relative contribution of PC to the total lipid of lipoprotein was the highest in HDL, and the lowest in VLDL (lower panel). The LDL fraction was richer in PC and poorer in total neutral lipids in the dog than in man.

3.2. Lipid fatty acids of canine and human plasma and lipoproteins

General features shared by the total lipid fatty acid profiles of canine and human plasma and lipoproteins were that n-6 polyunsaturated fatty acids (PUFA) were major constituents, mainly 18:2n-6 and 20:4n-6, as well as C16 and C18 saturated (SFA) and monoenoic fatty acids (MFA;

Table 2
Phospholipid proportions in canine and human plasma and plasma lipoprotein classes[a]

Phospholipid class	Canine				Human			
	Plasma	HDL	LDL	VLDL	Plasma	HDL	LDL	VLDL
% of the total phospholipid								
Phosphatidylcholine (PC)	77.3 ± 2.2	81.3 ± 1.4	79.6 ± 2.0	70.8 ± 0.8	71.3	67.4	76.2	75.6
LysoPC	7.8 ± 0.1	7.3 ± 0.8	6.0 ± 0.8	7.8 ± 0.6	10.0	14.6	8.0	7.6
Phosphatidylinositol	3.2 ± 0.8	2.0 ± 0.2	5.1 ± 0.7	4.0 ± 0.3	4.4	3.8	3.8	6.4
Phosphatidylethanolamine	2.6 ± 0.5	1.6 ± 0.2	2.9 ± 0.3	7.7 ± 0.7	3.0	2.8	3.5	2.9
Sphingomyelin	8.7 ± 0.1	7.8 ± 0.7	6.4 ± 0.4	9.7 ± 0.2	11.3	11.4	8.5	7.5
% of the total lipid								
Phosphatidylcholine (PC)	40.6	45.9	30.6	18.1	22.5	37.8	20.8	17.9
LysoPC	4.1	4.1	2.3	2.0	3.2	8.2	2.3	1.8
Phosphatidylinositol	1.7	1.1	2.0	1.0	1.4	2.1	1.0	1.5
Phosphatidylethanolamine	1.4	0.9	1.1	2.0	1.0	1.6	0.9	0.7
Sphingomyelin	4.6	4.4	2.5	2.5	3.6	6.4	2.2	1.8
Neutral lipids	47.6	43.6	61.5	74.4	68.5	43.9	72.8	76.3

[a] Data are the mean values ± S.D. from four independent samples of dogs and the mean of two in the case of men.

Table 3
Fatty acid composition of the total lipid of canine and human plasma and lipoproteins[a]

	Canine				Human			
	Plasma	HDL	LDL	VLDL	Plasma	HDL	LDL	VLDL
14:0	0.2 ± 0.1	0.3 ± 0.1	1.0 ± 0.3	0.8 ± 0.2	0.3	0.5	0.3	0.2
15:0	0.1 ± 0.1	0.1 ± 0.02	0.9 ± 0.1	0.6 ± 0.1	0.1	0.2	0.3	0.3
16:0	12.0 ± 0.7	13.5 ± 0.3	21.8 ± 0.9	21.6 ± 1.0	23.3	33.7	19.7	25.7
17:0	0.7 ± 0.04	0.7 ± 0.02	1.3 ± 0.2	1.1 ± 0.1	0.6	0.8	0.4	0.4
18:0	21.3 ± 0.6	22.6 ± 0.6	23.8 ± 1.4	13.4 ± 2.8	7.8	13.0	7.7	6.3
14:1	0.1 ± 0.1	0.1 ± 0.1	1.1 ± 0.3	0.5 ± 0.1	0.1	0.1	0.1	0.1
15:1	0.1 ± 0.02	0.1 ± 0.03	0.2 ± 0.04	0.3 ± 0.3	0.1	0.1	0.1	0.1
16:1	2.8 ± 0.4	2.8 ± 0.5	4.9 ± 0.3	7.0 ± 1.7	2.2	1.6	1.9	2.9
17:1	0.5 ± 0.2	0.5 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	0.2	0.2	0.2	0.3
18:1	14.1 ± 1.0	13.1 ± 0.5	10.8 ± 1.1	22.1 ± 2.0	20.2	12.8	16.0	29.5
20:1	0.1 ± 0.1	0.2 ± 0.01	0.2 ± 0.1	0.4 ± 0.2	0.4	0.5	0.2	0.4
20-3n-9	0.1 ± 0.04	0.1 ± 0.02	0.2 ± 0.1	0.2 ± 0.1	0.5	0.3	0.3	0.3
18:2n-6	23.5 ± 1.5	22.5 ± 0.9	15.5 ± 1.7	18.5 ± 1.4	30.5	26.1	36.9	25.4
18:3n-6	0.3 ± 0.1	0.2 ± 0.03	0.5 ± 0.2	0.3 ± 0.2	0.8	0.7	0.8	0.5
20:3n-6	1.0 ± 0.2	1.1 ± 0.3	0.4 ± 0.04	0.3 ± 0.1	1.3	1.1	2.3	1.2
20:4n-6	14.4 ± 1.6	14.3 ± 1.6	9.8 ± 1.6	5.1 ± 0.1	6.6	6.1	8.5	3.1
22:4n-6	0.1 ± 0.1	0.1 ± 0.04	0.3 ± 0.1	0.3 ± 0.3	0.7	0.2	0.4	0.1
22:5n-6	0.7 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	0.9 ± 0.6	1.5	0.1	0.9	0.04
18:3n-3	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	1.1 ± 0.3	0.6	0.4	0.4	0.6
20:5n-3	2.4 ± 0.4	2.0 ± 0.3	2.2 ± 0.9	3.1 ± 0.2	0.9	0.5	0.7	1.0
22:5n-3	2.3 ± 0.5	2.1 ± 0.6	1.2 ± 0.2	1.0 ± 0.1	0.4	0.2	0.3	0.3
22:6n-3	2.7 ± 0.7	2.6 ± 0.5	2.0 ± 0.7	1.1 ± 0.3	1.0	0.8	1.5	1.2

[a] The fatty acid composition is expressed as weight%. The figures represent mean values ± S.D. from three independent samples in the case of dogs, and mean of two in the case of men.

Table 3). Lipoproteins from dogs were all richer in long-chain n-3 PUFA (20:5n-3, 22:5n-3 and 22:6n-3) than those from humans. In canines, the proportion of 20:4n-6 was the highest in HDL, intermediate in LDL, and the lowest in VLDL, while the % of 20:5n-3 followed an opposite order. Regarding saturated fatty acids, the 16:0/18:0 ratio differed markedly, the proportion of 18:0 being higher in dogs, and that of 16:0 higher in man. The opposite was true for the main MFA, 16:1 and 18:1, the former being higher in dogs and the latter in man, either in plasma or in lipoprotein classes.

The fatty acid composition of the three major lipid constituents of canine and human plasma and plasma lipoprotein classes is depicted in Table 4. In each of the lipoprotein fractions of both mammals, the fatty acid profile of PC, CE and TG differed substantially. However, each of these lipid classes had quite a similar fatty acid profile whether isolated from HDL, LDL or VLDL, especially in men, and with only two exceptions in dogs. This similarity was most evident for the fatty acid composition of PC, virtually the same in the three lipoprotein fractions from canine, and also from human plasma. The only compositional differences noted within a lipid class were in two relatively minor lipid constituents, the CE of canine VLDL and in the TG of canine HDL. Interspecies differences were noted in the ratios between some of the major fatty acids. The major SFA of canine and lipoprotein PC was 18:0 while in human PC was 16:0. In turn, the major PUFA of dog lipoprotein PC was 20:4n-6, followed by 18:2n-6, whereas in humans the same two PUFA predominated, although in opposite ratios. In canine, but more so in human lipoproteins, the most abundant acyl group of TG was 18:1n-9.

The main factor determining the differences in fatty acid profile of the total lipid of the three lipoprotein classes under study (Table 3) was the disparate proportions of each of the main lipid

Table 4
Fatty acid composition of the major lipid constituents of canine and human plasma and plasma lipoprotein classes[a]

	Canine											
	Phosphatidylcholine				Cholesterol esters				Triacylglycerols			
	P	H	L	V	P	H	L	V	P	H	L	V
14:0	0.3 ± 0.1	0.2	0.3 ± 0.1	0.5 ± 0.3	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.3	1.0 ± 0.7	1.0 ± 0.6	1.8 ± 0.8	0.6 ± 0.4	0.6 ± 0.1
15:0	0.1 ± 0.1	0.03	0.1 ± 0.1	0.2 ± 0.1	0.1	0.1	0.3 ± 0.2	0.4 ± 0.1	0.6 ± 0.4	0.5 ± 0.1	0.6 ± 0.6	0.4 ± 0.3
16:0	13.4 ± 2.8	11.5 ± 1.6	12.6 ± 1.7	15.0 ± 1.0	8.6 ± 1.3	7.0 ± 0.7	7.8 ± 0.9	19.0 ± 1.8	19.2 ± 1.9	23.7 ± 0.2	12.7 ± 2.0	19.1 ± 2.7
17:0	0.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.3	0.2 ± 0.1	0.4 ± 0.1	1.5 ± 0.2	1.1 ± 0.3	1.2 ± 0.7	0.9 ± 0.3	0.8 ± 0.2
18:0	28.8 ± 2.2	27.3 ± 1.6	30.8 ± 2.1	28.1 ± 2.9	1.9 ± 0.5	1.1 ± 0.2	2.3 ± 0.7	21.8 ± 5.2	6.3 ± 0.5	10.4 ± 1.5	4.1 ± 0.2	4.9 ± 2.2
14:1	0.03	0.01	0.1	0.3 ± 0.2	0.1	0.1	0.4 ± 0.3	0.3 ± 0.2	0.2 ± 0.1	0.9 ± 0.1	0.9 ± 1.0	0.2 ± 0.1
15:1	0.3 ± 0.4	0.1	0.2 ± 0.1	0.5 ± 0.3	0.2	0.1 ±	0.1 ± 0.1	0.5 ± 0.3	0.8 ± 0.2	1.4 ± 0.1	0.5 ± 0.3	0.4 ± 0.2
16:1	1.8 ± 0.6	1.7 ± 0.4	1.8 ± 0.3	3.8 ± 1.4	3.3 ± 1.1	3.2 ± 0.4	4.5 ± 1.6	7.3 ± 0.9	6.5 ± 0.9	6.4 ± 1.4	10.1 ± 1.2	7.5 ± 2.3
17:1	0.6 ± 0.3	0.7 ± 0.5	0.4 ± 0.2	0.6 ± 0.3	0.2	0.2 ± 0.0	0.3 ± 0.1	0.9 ± 0.3	0.6 ± 0.1	0.4 ± 0.2	0.5 ± 0.3	0.5 ± 0.2
18:1	11.9 ± 2.8	9.6 ± 1.5	10.0 ± 1.0	11.2 ± 1.3	15.6 ± 4.3	14.5 ± 1.4	16.5 ± 2.4	19.4 ± 2.6	31.2 ± 5.7	16.3 ± 1.1	28.5 ± 3.5	25.8 ± 1.4
20:1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.3	0.4 ± 0.2	1.0 ± 0.3	0.04 ± 0.1	0.5 ± 0.2	0.4 ± 0.2
20:3n-9	0.3 ± 0.1	0.3 ± 0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
18:2 n-6	15.9 ± 1.9	12.1 ± 2.2	12.7 ± 2.6	11.8 ± 1.4	52.0 ± 2.2	53.1 ± 1.8	49.9 ± 4.1	16.1 ± 4.3	18.3 ± 1.6	17.2 ± 6.5	19.9 ± 1.8	24.0 ± 1.5
18:3 n-6	0.2 ± 0.1	6.0 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	1.0 ± 0.1	0.4 ± 0.5	0.9 ± 0.4	1.0 ± 0.4	0.8 ± 0.1	0.7 ± 0.1	1.2 ± 0.5	0.7 ± 0.3
20:3 n-6	1.7 ± 0.6	1.8 ± 0.3	1.7 ± 0.4	1.3 ± 0.4	0.5 ± 0.3	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.3	0.7 ± 0.1	0.9 ± 1.3	0.4 ± 0.3	0.7 ± 0.3
20:4 n-6	17.7 ± 2.6	18.2 ± 3.1	19.8 ± 2.4	18.3 ± 2.6	13.4 ± 4.5	14.8 ± 1.9	11.9 ± 1.9	4.3 ± 1.1	4.7 ± 1.4	9.4 ± 2.8	6.4 ± 1.6	4.3 ± 0.6
22:4 n-6	0.5 ± 0.3	0.4 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.1 ± 0.1	0.1	0.1	0.9 ± 0.6	0.3 ± 0.1	1.5 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
22:5 n-6	0.1 ± 0.1	0.1	0.1	0.2 ± 0.2	0.1 ± 0.1	0.1	0.2 ± 0.1	0.5 ± 0.2	0.8 ± 0.5	1.4 ± 0.7	0.5 ± 0.3	0.5 ± 0.3
18:3 n-3	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.9 ± 0.2	0.6 ± 0.3	0.5 ± 0.2	0.8 ± 0.5	0.8 ± 0.1	1.1 ± 0.1	1.6 ± 0.3
20:5 n-3	0.5 ± 0.5	1.8 ± 0.9	1.5 ± 0.4	1.4 ± 0.4	1.0 ± 0.7	2.2 ± 0.6	1.8 ± 0.1	3.2 ± 1.6	2.5 ± 1.4	3.7 ± 1.3	6.4 ± 2.2	3.3 ± 1.0
22:5 n-3	2.3 ± 1.1	3.3 ± 0.4	2.9 ± 0.5	2.0 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.1	0.1 ± 0.1	1.5 ± 0.6	0.5 ± 0.4	2.4 ± 0.8	2.0 ± 1.0
22:6 n-3	2.6 ± 1.0	4.0 ± 0.4	3.5 ± 0.5	2.8 ± 0.5	0.2 ± 0.2	0.3 ± 0.2	0.4 ± 0.1	0.3 ± 0.2	1.1 ± 0.7	0.6 ± 0.4	1.6 ± 0.3	1.7 ± 0.8

Table 4 (*Continued*)

	Human											
	Phosphatidylcholine				Cholesterol esters				Triacylglycerols			
	P	H	L	V	P	H	L	V	P	H	L	V
14:0	0.3	0.5	0.2	0.4	0.5	0.4	0.4	0.3	0.9	0.3	0.3	0.05
15:0	0.2	0.4	0.3	0.4	0.2	0.2	0.3	0.6	0.3	0.1	0.2	0.3
16:0	29.5	28.2	27.7	29.3	11.9	11.3	11.2	12.3	24.8	27.3	22.2	25.2
17:0	0.4	0.6	0.4	0.6	0.3	0.4	0.3	0.5	0.6	0.8	0.9	0.4
18:0	15.2	14.5	15.0	15.3	1.2	1.1	0.9	3.9	4.0	3.9	4.1	3.3
14:1	0.02	0.1	0.2	0	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.1
15:1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1
16:1	1.2	1.2	1.0	1.4	2.5	2.9	2.6	3.6	3.2	2.1	3.0	3.4
17:1	0.1	0.2	0.1	0.2	0.2	0.3	0.2	0.2	0.3	0.4	0.5	0.3
18:1	9.5	9.3	9.1	10.0	18.9	18.2	19.4	17.0	36.7	36.1	37.2	36.8
20:1	0.1	0.1	0.2	0.1	0.3	0.3	0.2	0.4	0.4	0.4	0.4	0.5
20:3n-9	0.1	0.4	0.4	0.1	0.1	0.3	0.1	0.3	0.2	0.5	1.0	0.4
18:2n-6	24.2	24.3	23.4	24.2	52.5	52.6	53.4	47.5	23.2	24.4	21.5	24.6
18:3n-6	0.2	0.1	0.2	0.3	1.2	1.4	1.4	0.8	0.7	0.6	0.7	0.6
20:3n-6	4.0	3.9	4.0	3.8	1.0	1.0	0.8	1.0	0.5	0.4	0.9	0.3
20:4n-6	10.8	11.8	10.8	10.2	7.0	7.7	7.0	7.4	1.8	1.0	3.0	0.4
22:4n-6	0.3	0.2	0.6	0.2	0.1	0.2	0.1	0.1	0.2	0.3	0.5	0.1
22:5n-6	0.2	0.1	1.7	0.1	0.03	0.01	0.02	0.1	0.1	0.1	0.4	0.01
18:3n-3	0.1	0.1	0.5	0.2	0.5	0.5	0.2	0.9	0.7	0.4	1.2	0.8
20:5n-3	0.3	0.5	0.6	0.1	0.6	0.2	0.9	2.5	0.4	0.3	0.4	1.3
22:5n-3	0.4	0.4	0.6	0.3	0.2	0.1	0.03	0.1	0.2	0.1	0.3	0.3
22:6n-3	2.7	3.0	2.7	2.5	0.7	0.4	0.4	0.3	0.7	0.3	1.2	0.7

[a] Data are presented as in Table 3. S.D. smaller than 0.05% are not depicted. P, plasma; H, HDL; L, LDL; V, VLDL.

classes (Table 1) having a distinctive fatty acid composition (Table 4). For example, within dog lipoproteins, VLDL were the richest in 18:1 (Table 3) because 18:1 was a major component of TG (Table 4), in turn the major lipid class of VLDL (Table 1). This was also the case for most of the interspecies differences. Thus, human LDL were the richest in 18:2n-6 because of their high CE content, a lipid in which this fatty acid prevailed; dog LDL had less 18:2n-6 because they had less CE, and more PL, than their human counterparts. Also, canine plasma lipid was considerably richer than human plasma in long-chain PUFA (Table 3) because dog plasma was much richer in HDL-PC (Table 2), and PC had more of such PUFA in dogs than in humans (Table 4).

The distribution of each of the 3 major lipid classes and their main groups of fatty acids among the three major lipoprotein fractions under study is summarized in Fig. 1. PC prevailed in canine HDL but in humans abounded in LDL followed by HDL. The PC of HDL alone was the main source of the circulating n-3 PUFA in dogs, larger than that obtained by adding up the n-3 PUFA of the PC carried by LDL and HDL in humans. The CE were mostly concentrated in the HDL of dogs but in the LDL of humans. The CE from both sources were very rich in n-6 PUFA, although there was somewhat more n-3 PUFA in dogs. Finally, as seen by the profiles of TG from VLDL and LDL, this lipid also carried a significantly larger amount of n-3 PUFA in canine than in human plasma.

4. Discussion

The results presented here show that canine plasma is rich in HDL compared to human plasma rich in LDL and VLDL. The dog has a PC-rich fasting plasma, poorer in total cholesterol and TG and richer in long-chain (C20-C22) n-6 and n-3 PUFA than that from humans. According to our results, dog HDL and LDL contribute with nearly 87 and 11% of the total cholesterol of plasma, respectively, quite opposite to the case in humans, where HDL and LDL contribute with nearly 11 and 86% of the TC, respectively. This quantitative contrast, along with the differences in the total PL content of these two main lipoprotein fractions, point to possible differences in the way lipoproteins and lipids are metabolized and transported in canine and human plasma.

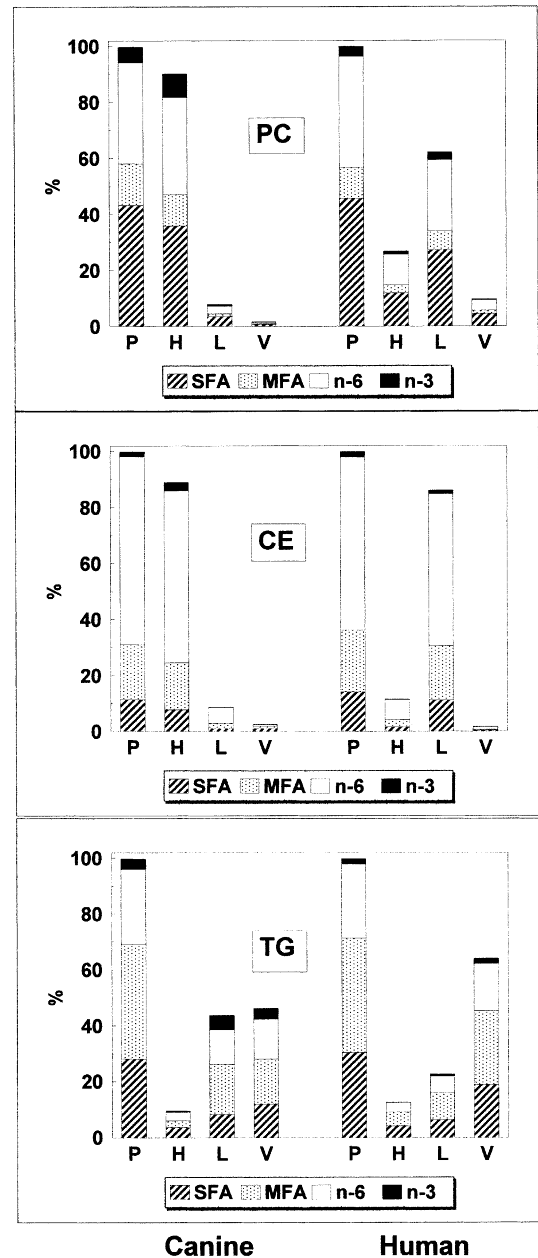

Fig. 1. Percent distribution of the major groups of fatty acids contributed by phosphatidylcholine (PC), cholesterol ester (CE), and triacylglycerol (TG) in canine and human plasma lipoprotein classes. The % distribution of each lipid class among HDL, LDL and VLDL (H, L and V, respectively), was calculated from the data in mg/dl, with plasma (P) accounting for the 100% bar. Within each bar, the fatty acids of each lipid class, grouped as the sum of SFA, MFA, n-6 PUFA and n-3 PUFA are included, as calculated from the data in Table 4.

It is widely accepted that liver-derived VLDL

and the product of its peripheral metabolism, LDL, are involved in the delivery of cholesterol from the liver to extrahepatic tissues (forward cholesterol transport). The return of cholesterol from tissues to the liver (reverse cholesterol transport) is attributed mainly to HDL particles, which are rich in PL and carry cholesterol from tissues, mostly in the form of CE, back to the liver. The formation of such CE is mediated by lecithin:cholesterol acyltransferase (LCAT), a liver-derived plasma enzyme that transfers a fatty acid (mostly 18:2n-6) from PC to cholesterol in lipoprotein particles for esterification. It is also known that an active exchange of CE and PL exists between circulating lipoprotein classes, mediated by the CE transfer protein and the PL transfer protein, respectively (Fielding and Fielding, 1991). The large amount of PL transported in the canine HDL fraction and the high PL-free cholesterol ratio in HDL are obviously advantageous to the free cholesterol capturing function of HDL, operated by LCAT to seclude cholesterol in the form of cholesterol ester in the particle core.

In vitro studies showed that of all lipoproteins, HDL is the preferred substrate for the esterification of free cholesterol by LCAT (Subbaiah and Liu, 1996), and that the preferred phospholipid substrates of LCAT are 16:0-18:2n-6 PC and 18:0-18:2n-6 PC in most mammals, except the dog, where it is 18:0-20:4n-6 PC (Yamamoto et al., 1980). In dogs, as in other HDL-mammals, the exact role played by HDL is still unknown. However, it is possible that part of the natural resistance of these animals to develop atherosclerotic lesions may be due to the presence of these PL-rich particles, with high proportions of 18:0 and 20:4 in PC. Given the high concentration of HDL (Tables 1 and 2), and given the fatty acid composition of the lipoprotein constituents (Tables 3 and 4), it is apparent that most of the fatty acids of dog plasma are furnished by the PC of HDL. Because of this, these particles also transport more long-chain (C20-C22) PUFA of the n-3 series in dogs than in humans, a condition that may also be related to the reduced susceptibility of the former to atherogenesis.

A comparative study in the plasma from 14 vertebrates showed that PL transfer protein activity levels were remarkably preserved in evolution, and that of all animals studied, this activity was among the highest in dogs (Guyard-Dangremont et al., 1998). This may explain our results showing a virtually equal PL composition (Table 2), and a similar fatty acid composition of PC (Table 4), in each lipoprotein fraction. By contrast, it was clear from the same study (Guyard-Dangremont et al., 1998) that a few species including man displayed substantially high, while others, among them the dog, had no detectable CE transfer protein (CETP). This may also explain our results that the fatty acids of VLDL-CE differed from those of the other two, HDL-CE and LDL-CE in dogs, while the fatty acids of EC from all three lipoproteins were similar in humans. Thus, the dog VLDL-CE, formed after the activity of acyl-CoA:cholesterol acyltransferase, poor in 18:2 and 20:4, came with these particles from the liver, while the CE within HDL and LDL, rich in 18:2 and 20:4, would be mainly formed in the circulation by the high activity of LCAT. In the absence of CETP, this picture may remain as such in the dog, whereas in man it does not, because of the superimposed activity of CETP, exchanging molecular species of CE among lipoproteins. This view is supported by the finding that, while in normal humans LDL and HDL are similar in CE fatty acid composition, in CETP-deficient humans they are not, and, if purified CETP is added to CETP-lacking plasma, an equilibration of CE between lipoproteins results (Bisgaier et al., 1991).

In dogs, VLDL was a relatively small lipoprotein fraction rich in TG and HDL a large lipoprotein fraction poor in TG. The proportion of TG was relatively small in LDL, but since LDL were in larger concentrations than VLDL, both contributed similarly to the total TG in dog plasma. The concentrations of TG were smaller in each of the canine than in the corresponding human lipoproteins. Whereas in human plasma the TG from the three lipoprotein fractions were similar in fatty acid composition, in dogs HDL-TG differed from the other two. This can also be explained by the negligible CE transfer protein activity in dogs, but not in men (Guyard-Dangremont et al., 1998), since this protein is known to mediate the movement of CE from HDL into VLDL in a reciprocal exchange for TG (Moulin, 1996).

The TG of VLDL and LDL (Fig. 1) had monoenoic and saturated fatty acids as major constituents and were the main source of n-3 PUFA metabolically available to tissues. The fact that the TG of dogs (Table 4) were richer in 20:5

n-3 and other n-3 PUFA than human TG may point to differential properties of lipoprotein lipase, the endothelial enzyme involved in the hydrolysis of TG from TG-rich particles. This enzyme has been shown to display selectivity in vitro towards TG with fatty acids of the n-6 series, especially 18:2n-6, in comparison with TG with n-3 PUFA (Botham et al., 1997). The much smaller amounts of TG, and the relatively higher n-3 to n-6 PUFA ratio in this lipid class, are consistent with the possibility of a more active lipoprotein lipase in dog than in man.

The present results allow the speculation that, from the lipid point of view, the relative scarcity of PL could be as an important factor as the relative excess of cholesterol in determining the unfavorable susceptibility to atherogenesis of LDL mammals like humans, as opposed to HDL mammals like dogs. By dietary or pharmacological manipulations or by lifestyle changes, the fatty acid, lipid, and eventually the lipoprotein profile of plasma is usually modified in humans and animals as well (Watson and Barrie, 1993; Harris, 1996; Downs et al., 1997). The ultimate goal of these changes is to decrease potentially atherogenic components like total cholesterol or TG. It would be worth devising experimental ways of increasing, in LDL mammals, the relative proportions of PL circulating in LDL or HDL. The latter is considered an important factor in lowering the risk of developing atherogenesis (Stein and Stein, 1999).

The low CETP activity of dogs (Guyard-Dangremont et al., 1998) could be responsible for the fact that they retain much more of the total CE in HDL than in LDL, thus having, from the human point of view, a favorable HDL-cholesterol/LDL-cholesterol ratio. Trying to control the level or inhibit the activity of this protein, quite active in LDL mammals (Guyard-Dangremont et al., 1998), could be one of the tools worth investigating, since low CT transfer protein activity is potentially antiatherogenic. In fact, various CE transfer-protein gene mutations that result in increased HDL levels have been reported in humans, although the real impact of CETP on atherosclerosis is still controversial (Moulin, 1996).

The study of HDL-mammals like the dog is valuable for gaining insight into the metabolism of the lipid constituents of HDL. While the metabolic fate of the major lipid components of VLDL (TG) and LDL (CE) is well established, this is not the case for those of HDL (PL and CE), other than that they are freely exchangeable with those of other circulating lipoproteins (Fielding and Fielding, 1991; Cheung et al., 1996) and that they are cleared from plasma when HDL enter the liver. This process involves two uptake pathways, one selective (through the scavenger receptor BI) and other depending on endocytosis (Silver et al., 2000; Tall et al., 2000). Given its virtual lack of CE transfer protein, and since it has plenty of PL/CE-rich HDL particles in readily available form, the dog may be a good model to study this basic problem.

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