The variability in the canine lipid profile values and its possible relationship with the measurement method used

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ABSTRACT: The lipid profile in clinical biochemistry is a very important tool for research and diagnosis of several pathologies related to an abnormal lipid metabolism. Lipid profile for samples of 127 healthy mixed bred adult canines (61 males and 66 females) divided in three age groups (1 to 3 years; 3,1 to 7 years; and over 7, 1 years) were obtained using the Friedewald method in order to establish reference values for some blood lipids. A statistically significant difference was not found comparing the obtained values for males and females. However, there are clear differences in the values obtained in this study compared to others, and it was also noted that in the consulted literature the above-mentioned method, which calculates the VLDL cholesterol and LDL cholesterol levels with a formula, presents different results when compared with the direct method.

Key words: dogs, metabolism, blood lipids, cholesterol

La variabilidad en los valores del perfil lipídico canino y su posible relación con el método de determinación utilizado

RESUMEN: El perfil lipídico en bioquímica clínica es una herramienta muy importante para la investigación y diagnóstico de diferentes patologías relacionadas con un metabolismo lipídico. El perfil lipídico de las muestras de 127 caninos adultos sanos (61 machos y 66 hembras) divididos en tres grupos de edad (1 a 3 años; 3,1 a 7 años y mayores de 7,1 años) fue determinado utilizando el método de Friedewald, buscando establecer valores de referencia para algunos lípidos sanguíneos. No se encontró diferencia estadísticamente significativa entre machos y hembras. Sin embargo, se observan claras diferencias en los valores obtenidos en el presente estudio al compararlo con otros; además, en la literatura consultada se encuentran diferencias entre los valores obtenidos por el método usado por nosotros, el cual calcula los valores de colesterol VLDL y colesterol LDL mediante fórmula, y otros como el método directo.

Palabras clave: perros, metabolismo, lípidos sanguíneos, colesterol

Abbreviations: VLDL, Very Low-Density Lipoprotein; IDL, Intermediate-Density Lipoprotein; LDL, Low-Density Lipoprotein; HDL, High-Density Lipoprotein; CHD, Coronary Heart Disease; CETP, Cholesteryl Ester Transfer Protein, TG, Triglycerides.

Introduction

The Enzymatic assay (oxidase-peroxidase) is the most common method for determination of total cholesterol, HDL cholesterol and triglycerides levels in serum or plasma. The Friedewald method is a routinely analysis which provides the option of calculating the VLDL cholesterol dividing the triglyceride values by five and the LDL cholesterol levels using the following formula: LDL-cholesterol= Total cholesterol-HDL cholesterol- VLDL cholesterol.

The main reports of lipid profile in canines only present values of total cholesterol, HDL cholesterol and LDL cholesterol probably due to the lack of studies validating the Friedewald method for animals. The present study present the complete lipid profile for three groups of age in canines using the Friedewald method showing the similarity and the difference for some parameters reported for others and its possible causes.

Subjects and Methods

Samples of 127 healthy mixed bred adult canines (61 males and 66 females) divided in three groups of age (1 to 3 years old; 3,1 to 7 years old , and more than 7,1 years old) were analyzed using the Friedewald method (Friedewald et al., 1972) in order to establish reference values for some blood lipids. They were admitted to our university teaching veterinary hospital for clinical and laboratory routine analysis, and selected by rigorous clinical exam; obese or bad nourished animals were not admitted to the study, neither animals suspected of any disease as hypo or hyperthyroidism or under any pharmacological treatment which can alter lipid profile, and all animals were consuming a standard commercial diet.

Blood samples from the overnight fasting subjects were collected by venopuncture. Serum of samples was separated immediately. Total cholesterol, triglycerides, and high-density lipoprotein cholesterol were measured using a Cobas Bio 8326, Hoffman-La Roche (enzymatic methods). The very low density cholesterol and low density cholesterol were calculated by formula. Comparison between groups was made using *t* student test.

Results and Discussion

In the present study, statistically significant difference was not found related to sex, and average values (95% confidence interval) were obtained for the group of studied canines (Table 1). A significant difference ($p < 0.05$) was found for total cholesterol and LDL-cholesterol for the aging group (>7 years old) which shown higher levels compared to others. The HDL-cholesterol was significantly higher ($p < 0.05$) for the group of 3,1 to 7 years old.

Table 1. Lipid profile in dogs divided into three groups of age.

Age (years)	n	Col-Total (mg/dl)	Col-HDL (mg/dl)	TAG (mg/dl)	Col-VLDL (mg/dl)	Col-LDL (mg/dl)
1 to 3	46	205.3 ± 71.2	69.4 ± 8.6	60.4 ± 7.2	12.1 ± 1.5	124.4 ± 16.3
3.1 to 7	46	210.7 ± 18.9	81.5 ± 10.7	59.8 ± 8.5	12 ± 1.7	117.2 ± 18.6
More than 7.1	35	224.0 ± 111.0	65.6 ± 35.0	67.5 ± 55.1	13.7 ± 11.2	145.7 ± 103.1

Some reports of lipid profile in dogs have been presented previously (Table 7). The difference between the studies probably depends of some factors like breed, age, and diet of the dogs, or the techniques used for determinations. It is necessary to emphasize about the lifestyle of the animals in their respective environments, however the reported values have sometimes a very high difference. Even when the different studies of our group are compared, it can be observed that some times there is no a logical correlation between them (Tables 1 and 3), the same situation is presented when compared to the results obtained by others (Tables 4 and 5), while some parameters are found elevated or diminished in some groups, for others studies the same parameters are found completely different for the same groups.

Table 2. Lipid profile in dogs divided in normal and obese animals (Osorio & Giraldo 1999).

Condition	n	Col-Total (mg/dl)	Col-HDL (mg/dl)	TAG (mg/dl)	Col-VLDL (mg/dl)	Col-LDL (mg/dl)
Normal	46	200.3 ± 71.2	76.5 ± 40.0	61.6 ± 26.8	12.3 ± 5.4	111.5± 68.2
Obese	46	224.0 ± 111.0	65.6 ± 35.0	67.5 ± 55.1	13.7 ± 11.2	145.7 ± 103.1

Table 3. Lipid profile in dogs divided into two groups of age (Osorio, 2006).

Age (years)	n	Col-Total (mg/dl)	Col-HDL (mg/dl)	TAG (mg/dl)	Col-VLDL (mg/dl)	Col-LDL (mg/dl)
less than 7	35	201.3 ± 11	71.9±6.3	58.1±4.7	11.62	117,8
more than 7	35	163.6± 22.5	47±19.4	59±18.3	11.83	104,8

Table 4. Lipid profile in obese Labrador dogs divided by gender (Duarte et al., 2005).

Age (years-gender)	n	Col-Total (mg/dl)	Col-HDL (mg/dl)	TAG (mg/dl)	Col-VLDL (mg/dl)	Col-LDL (mg/dl)
4 to 5.99 Male		176.13	45.96	161.26	-	76.7
4 to 5.99 Female		125.3	66.63	43.49	-	99.56
More than 6 Male		205.9	47.96	55.4	-	89.13
More than 6 Female		277.17	59.06	87.46	-	188.69

Table 5. Lipid profile in obese poodle dogs divided in males and females (Duarte et al., 2005).

Age (years)	n	Col-Total (mg/dl)	Col-HDL (mg/dl)	TAG (mg/dl)	Col-VLDL (mg/dl)	Col-LDL (mg/dl)
4 to 5.99 Male		186.8	47	99.56	-	56.6
4 to 5.99 Female		245.3	62.8	71.96	-	73.76
More than 6 Male		-	69.23	69.56	-	63.9
More than 6 Female		-	50.73	116.3	-	242.6

Some authors have found difference in the total cholesterol level depending of the diet or sex (Coles, 1989), age (Groslambert et al., 1985) or breed (Downs et al., 1993), while others did not find it which means that there is no agree between the different groups. However, it is necessary to analyze the methods used which can be an important factor to influence in the lipid measurement.

In humans, the association between increased concentrations of low-density lipoprotein cholesterol (LDL-C) and increased rate of premature coronary heart disease (CHD) has been clearly demonstrated, and currently, most clinical laboratories use the Friedewald equation to calculate the LDL-C levels, since the reference method, β-quantification by ultracentrifugation, is not suitable for routine use (NCEP 2001).

However, the use of the equation has been repeatedly questioned, particularly since it is based on the assumption that the majority of triglycerides reside in the VLDL fraction and that the relationship between triglycerides and cholesterol in this fraction is constant. But, the equation is considerably inaccurate even at TG concentrations of 200–400 mg/dl (Warnick et al., 1990). Homogeneous LDL-C assays have been introduced in recent years for which additional validation is still needed (Nauck ete al., 2002). At the same time, alternative ways of LDL-C calculation have been proposed taking also into consideration total serum apolipoprotein B (apoB) concentration. Thus, the equation LDL-C = 0,41TC – 0,14TG + 0.66apoB – 10,43 has been proposed for the estimation of LDL-C (Planella et al., 1997),and the equations LDL-C = 0,94TC - 0,94HDL-C - 0,19TG and LDL-apoB = apoB - 0.09TC + 0.09HDL-C - 0.08TG have been proposed for the estimation of LDL-C and of LDL contained apoB (LDL-apoB), and therefore for the calculation of the ratio LDL-C/LDL-apoB (Hattori et al., 1998). This ratio has been associated with the size of the LDL particles.

But there is not just the methods used to analyze the lipid profile, the responsible for the possible variations. There are two factor which can influence the result, first of all the Friedewald method for humans, does not hold in certain conditions associated with hypertriglyceridemia, in type III hyperlipidemia, and certain secondary dyslipidemias, possibly including uremia. In fact, in renal failure, accumulation of partly metabolized triglyceride-rich particles (predominantly VLDL and IDL remnants) is observed, causing hypertriglyceridemia and low HDL-C concentrations (Baigent et al., 2000). Furthermore, even although LDL-C levels

are typically similar to those in the general population (or lower), this pattern often conceals a highly abnormal lipid subfraction profile with a predominance of atherogenic small dense LDL-particles (Cattran et al., 1976).

Secondly, there is a special situation related to dogs as dogs present a HDL pattern for lipid metabolism and on the other hand, in dogs there is not appreciable CETP activity in circulation (McTigue et al., 2006; Watson et al., 1995), and then the exchange of TG for cholesteryl esters between HDL and VLDL or LDL can not be considered. Also dogs secrete apo-B48 containing VLDL (Bauer, 1996), which leads to low concentrations of apo-B containing lipoproteins in dogs as a result of more efficient hepatic clearance, because of the rapid recognition and hepatic receptor binding of these particles (Table 8). In general terms, dogs present less level of apo- B containing lipoproteins, and lower levels of cholesteryl esters in VLDL and LDL. These two facts decrease the risk for CHD in dogs compared to humans by diminishing the offer of saturated cholesterol to peripheral tissues, such as coronary arteries.

Table 6. Lipid profile in dogs divided intofive groups of age (Coppo et al., 2003).

age (years)	n	Col-Total (mg/dl)	Col-HDL (mg/dl)	TAG (mg/dl)	Col-VLDL (mg/dl)	Col-LDL (mg/dl)
Less than 1	6	201±55	116±35	-	-	43±24
1 to 2	13	174±32	111±34	-	-	41±21
3 to 4	7	188±46	119±28	-	-	27±19
5 to 6	12	184±31	122±31	-	-	31±16
7 to 12	11	193±41	119±30	-	-	40±17

Table 7. Different values for lipid profile in canines.

Total-cholesterol	HDL-cholesterol	LDL-Cholesterol
60-150 (Angel, 1997)	40-100 (Angel et al.,)	10 (Rodríguez et al., 2002)
70-160 (Coppo, 2001)	61-88 (Forstner, 1985)	20-60 (Coppo, 2001)
121,5 (Rajan et al., 1973)	80-120 (Coppo, 2001)	
149 (Gleeson et al., 1990)	156 (Rodríguez et al., 2002)	
190,5 (Bergeman et al., 1971)		
110-300 (Forstner, 1985)		
130-210 (Florio et al., 1971)		
120-255 (Sodikoff 1996)		
135-270 (Kaneko 1989)		
193 (Johnson1994)		
229,7 (Carvalho et al., 1958)		
211 (Coles, 1989)		
243 (Rodriguez et al., 2002)		

Table 8. Canine lipoproteins.

Lipoprotein	Size (nm)	Hydrated density (g/ml)	Mobility (electrophoresis)	Major Apo-proteins
Chylomicrons	75-1200	< 0.960	origin	B_{48}
VLDL	30-80	0.093-1006	Pre-ß	B_{100}, B_{48}, E, C
LDL	18-25	1.019-1.087	ß	B_{100}, B_{48}
HDL_1	10-35	1.025-1.100	α_1	E,A,C
HDL_2	9-12	1.063-1.100	α_1	E,A,C
HDL_3	5-9	1.100-1.185	α_1	A,C

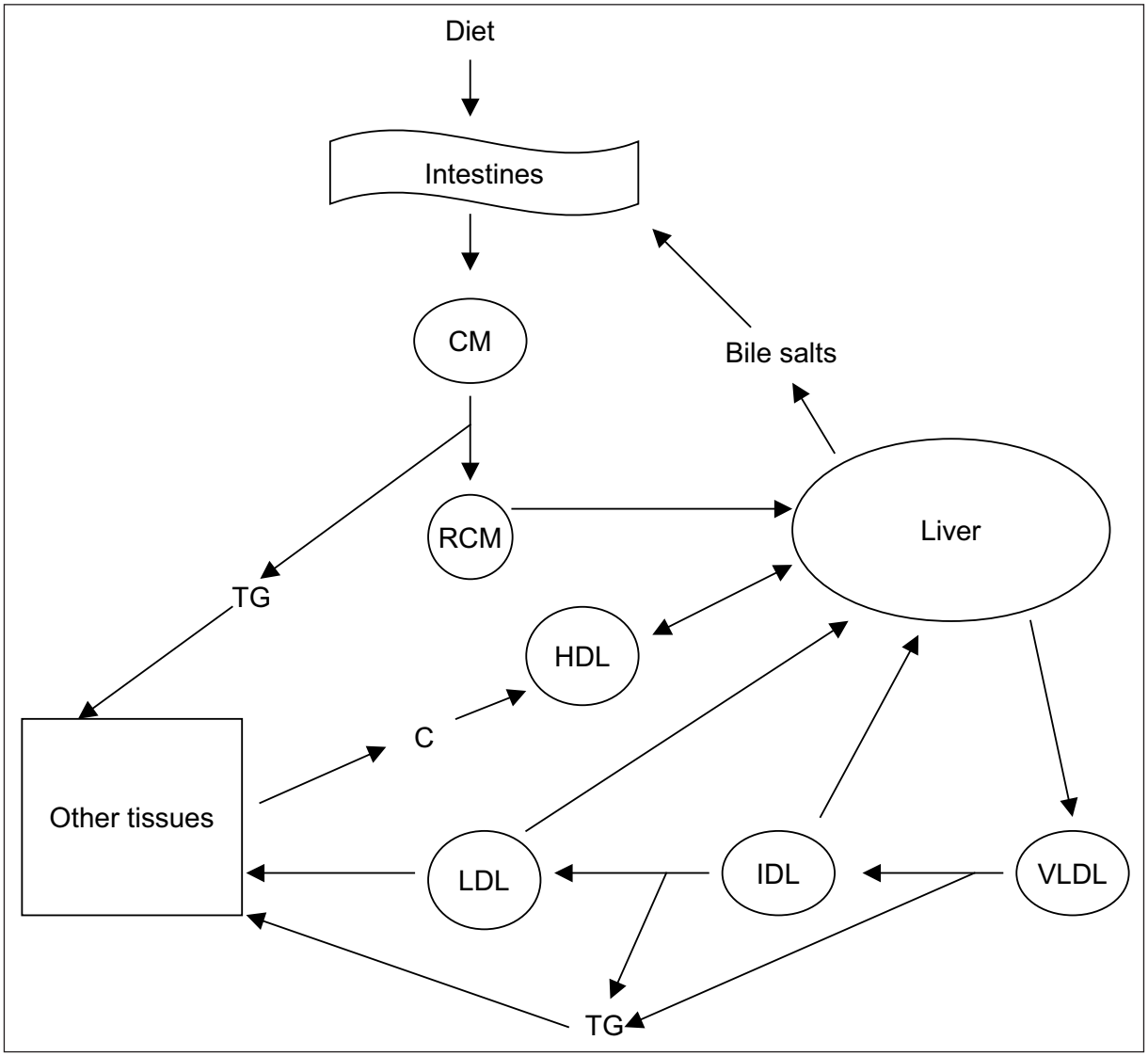

Figure 1. Metabolism and transport of plasma lipoproteins. C, cholesterol; TG, triglycerides; CM, chylomicrons; CR, chylomicron remnants; HDL, high-density lipoprotein; IDL, intermediate-density lipoproteins; LDL, Low-density lipoproteins; VLDL, very low-density lipoprotein.

Conclusion

It seems that the method used to measure blood lipids in dogs can influence the values obtained, however the methods proposed for these determinations in humans have been postulated considering the fact that humans have a LDL pattern for lipid metabolism, conversely to dogs where the pattern is HDL. That is why it is necessary to perform research to validate the different methods used in humans to be used in animals.

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