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CHOLESTEROL, GLUCOSE AND TRIGLYCERIDES ROLE IN THE PREVALENCE OF HYPERLIPIDEMIA IN DOGS AT HIGHER ELEVATIONS

Rol del colesterol, la glucosa y los triglicéridos en la prevalencia de hiperlipidemia canina a gran altitud

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ABSTRACT

Hyperlipidemia is a metabolic disorder that is characterized by a fat concentration increase in blood serum and it is known to be common in dogs. However, there is no information about how this disorder behaves in different environmental conditions related to metabolic changes, such as elevation, which may reduce metabolic efficiency. It was determined the clinic variables involved in hyperlipidemia prevalence in dogs dwelling at >3000 m elevation. Blood samples were taken from 80 dogs in La Paz, Bolivia; a city located at 3600 m.a.s.l. Cholesterol (total, HDL, and LDL), triglycerides and glucose concentrations were determined. Data was analyzed using an Akaike Information Criterion (AIC) model selection approach. Hyperlipidemia prevalence was estimated in 12.5%, which is lower than expected for the sea level. Hyperlipidemia was present in five-year or older dogs, irrespective of gender, breed, and diet. Competing models obtained showed that, besides the main role of cholesterol, a significant effect of cholesterol + glucose, as well as cholesterol + glucose + triglycerides determined hyperlipidemia prevalence in the surveyed animals. Despite being important, cholesterol is not the only clinical variable involved, there are significant interactions with glucose and triglycerides concentrations related to lower metabolic efficiencies, as it happens at higher elevations, which should be taken in account when conducting clinic examinations.

Key words: Hyperlipidemia, lipid metabolism, altitude metabolism, metabolic disorder.

RESUMEN

La hiperlipidemia es un desorden metabólico común en el perro, caracterizado por un incremento de la concentración de grasas en el suero sanguíneo, sin embargo, no se tiene información acerca de cómo se comporta este desorden bajo diferentes condiciones ambientales, capaces de ocasionar cambios metabólicos como lo es la altitud, factor capaz de reducir la eficiencia metabólica. Se determinaron las variables clínicas relacionadas con la prevalencia de hiperlipidemia en perros a más de 3000 m de altitud. Para ello se tomaron muestras de sangre a 80 canes de la ciudad de La Paz, Bolivia, ubicada a una elevación media de 3600 m. Se determinaron las concentraciones de colesterol (total, HDL y LDL), glucosa y triglicéridos, los datos fueron analizados mediante una aproximación de selección de modelos mediante el Criterio de Información de Akaike (AIC). Se estimó una prevalencia de hiperlipidemia del 12,5%, por debajo de lo esperado a nivel del mar, estando presente en perros de cinco años de edad o más, independientemente del sexo del animal, la raza o su dieta. Los modelos en competición obtenidos mostraron que, a pesar del rol protagónico del colesterol, las combinaciones de colesterol + glucosa así como colesterol + glucosa + triglicéridos determinaron la prevalencia de hiperlipidemia en los animales estudiados. A pesar de ser una variable importante, el colesterol no fue la única variable clínica involucrada en la presencia de hiperlipidemia, sino que existen interacciones significativas con la glucosa y los triglicéridos relativas a eficiencias metabólicas menores, como sucede a grandes alturas. Dichas variables también deberían tomarse en cuenta al realizar exámenes clínicos en este tipo de condiciones.

Palabras clave: Hiperlipidemia, metabolismo lipídico, metabolismo en altura, desorden metabólico.

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INTRODUCTION

Hyperlipidemia is a metabolic disorder that represents the increasing of cholesterol, triglycerides, or both in blood serum [1, 3]. This disorder may result from a genetic lipoprotein metabolism deficiency, or as a consequence of an underlying systemic disease [17, 18]. Another possible cause of this disorder is related to animal feeding habits, because diets rich in high fats and cholesterol increase the likelihood of developing hyperlipidemia. This disorder may be caused by hypercholesterolemia, hypertriglyceridemia, or both [14].

Hypercholesterolemia is manifested by the increase in blood cholesterol concentrations and may have diverse consequences such as corneal rings, xanthomas, xanthelasmas, and atherosclerosis [21]. However, hypertriglyceridemia, which is a high concentration of triglycerides in blood, is manifested through abdominal pain, vomiting, diarrhea, convulsions, hepatomegaly, and abnormal lipid deposits in some tissues [1, 2].

Dog *(canis familiaris)* breeds most prone to manifest hyperlipidemia are Miniature Schnauzers and Brittany Spaniels, due to an autosomic recessive hereditary defect in the lipid metabolism [2, 19, 20]. Dogs from these breeds, clinically healthy, usually show persistent basal hyperlipidemia levels during the routine clinic tests. There is no gender predisposition to hyperlipidemia, and this disorder is usually found in four-year or older dogs [2]. Nevertheless, there are no previous reports regarding how hyperlipidemia disorder behaves when metabolic efficiencies become reduced, as happens in dogs bred at high altitude (>2000m above the sea level). Knowing and understanding the role of other clinical variables than the cholesterol in the occurrence and prevalence of hyperlipidemia at higher elevations, where the metabolism is slower, could provide new insights about other not-obvious factors, as related to any possible accompanying systemic disease that might play an important role besides the cholesterol concentrations at non-optimum metabolic conditions.

The scope of this research was: 1, to determine the prevalence of hyperlipidemia in dogs dwelling at >3000 m.a.s.l. elevation, and 2, to determine the clinic variables involved in hyperlipidemia prevalence at those conditions.

MATERIALS AND METHODS

Study site

This study was conducted in La Paz, northwestern Bolivia (16°30'S lat, 68°09'W long), this city has an average elevation of 3600 m. The city of La Paz correspond to the Altiplano tableland, a zone characterized by a cold weather (mean temperature 8°C), a rainy season in summer (January to March), and vegetation is characterized by native grassed and schlerophyll shrubs, with some introduced tree species, such as pines and eucalyptus [12].

Sampling criteria and protocols

Sample size was determined using Epi Info 2002 software [6] with a 99.5% confidence level for an expected hyperlipidemia prevalence of 14%, following the literature [10]. According to the last zoonosis Municipal reports, dog population is assumed to be 410,508; therefore, agreeing to the study protocols sample size was determined to be 80 individuals. Study individuals were selected randomly, excluding homeless dogs and considering all breeds (including cross-bred dogs). Age distribution of the sampled animals followed a normal distribution (Ryan-Joiner test, RJ= 0.99, P > 0.10) [15].

Samples were collected from October 2006 to February 2007. During blood sampling, the following zoometric data were collected: age, gender, breed, weight, and type of feeding (homemade, commercial pellets, or both). For each sampled dog, diabetes was discarded through a laboratory test. Blood samples were taken early in the morning, studied individuals were kept under control the night before sampling to assure that the animals has not eaten anything before the blood extraction, all the sampled animals were in fasting at least for 8 hours [4, 13]. Samples were taken at De la Peña Veterinary Clinic. It was collected 5mL of blood in vaccutainer tubes without anticoagulant, which were spun to separate the serum. Samples were kept refrigerated (at -20°C; Macsa refrigerator, model JDC-2K4-AL, Mexico) in eppendorf tubes until the serological tests were completed. Sampling procedures followed higher ethical and welfare standards, as stated by the Veterinary Professional Association of La Paz, Bolivia.

Serological tests

Collected samples were analyzed to determine cholesterol, triglycerides, and glucose concentrations. In hyperlipidemic dogs, additionally, high-density (HDL) and low-density lipoprotein (LDL) concentrations were determined. All serological tests were performed using commercial LABTEST kits (cholesterol kits # 13, 76 and 86, triglycerides kit # 87, glucose kit # 84; MG, Brazil), with visible spectrophotometry (Spectronic equipment, model 20D, United Kingdom) quantification at 520nm [8]. Quality controls using solutions with known concentrations were performed for each assay in order to ensure data reproducibility and repeatability, calibration curves adjusted with values of $R^2 > 0.9$. Normal values were taken from Elliot et al. [7], and Bonagura and Kirk [4]. All laboratory routines were conducted at the clinical laboratory of San Gabriel Hospital.

Data analyses

Data was analyzed first with a discriminant analysis, in order to determine which variables to include in the logistic regression analysis, based on the variable loadings. Proportions between healthy and hyperlipidemic animals related to the categorical variables were assessed using a two-tailed Fisher exact probability test [15]. It was performed a logistic regres-

sion analysis, using gender as categorical variable, and age, glucose, triglycerides and cholesterol concentrations as continuous variables. All predictor variables combinations were tested separately as additive models, because interactions were not significant. Competing models were evaluated using an Akaike Information Criterion (AIC) [5]. As the sample size / number of parameters (n/K) ratio were <40 in some cases, the second-order AIC values (AIC_c) was estimated, following Burnham and Anderson [5]. The most parsimonious model subset was defined as $\Delta AIC \leq 2$. Continuous variables were tested for multicollinearity; in all cases, the Variation Inflation Factor was <5 indicating no multicollinearity. Finally, it was conducted linear regressions between body mass (log transformed) and cholesterol (log transformed), glucose (rank transformed), and triglycerides (rank transformed) in order to evaluate relationships between those variables. All procedures were conducted in STATISTICA 7 [16]. Results are shown as mean value ± one standard error, in all cases.

TABLE I

ESTIMATED MODEL SUBSET FROM LOGISTIC REGRESSION ANALYSIS. MAXIMUM LOG-LIKELIHOOD VALUE IS SHOWN IN BOLD, K = NUMBER OF PARAMETERS IN THE MODEL.

Model	Log-likelihood	K	AIC_c	ΔAIC_c	w_i
Cholesterol	-14.93	1	31.85	0.00	0.13
Glucose + Cholesterol	-14.03	2	32.05	0.20	0.11
Triglycerides + Cholesterol	-14.48	2	32.96	1.11	0.07
Glucose + Triglycerides + Cholesterol	-13.63	3	33.29	1.44	0.06
Glucose + Cholesterol + Sex	-13.80	3	33.60	1.75	0.05
Cholesterol + Sex	-14.78	2	33.55	1.70	0.05

RESULTS AND DISCUSSION

Estimated canine hyperlipidemia prevalence was 12.5%, from which 70% of the dogs were older than five years. The 60% of the hyperlipidemic dogs were females, however sex proportions on hyperlipidemic dogs do not differed significantly among females and males (Fisher exact test P = 0.51). Hyperlipidemia was found in five breeds (Schnauzer, German Shepherd, Cocker Spaniel, Bichon Frise, and cross-bred dogs), with occurrence frequencies ranging from 10 to 30%. Sixty percent of the hyperlipidemic dogs were fed with mixed food (pellets + home-made) and a 30% were fed with homemade food. According to the discriminant analysis performed, dogs' breed and type of feeding had no significant influence on the hyperlipidemia prevalence (Fisher exact test P = 0.41 and 0.89, respectively). Estimated clinical values were: cholesterol 178 ± 9 mg /dL (n = 80), triglycerides 109 ± 7 mg/dL (n = 80), and glucose 77 ± 4 mg/dL.

Cholesterol concentration provided the most parsimonious explanation of the hyperlipidemia prevalence at high elevation, but cholesterol + glucose, triglycerides + cholesterol, and glucose + triglycerides + cholesterol interactions also provided significant explanations for the hyperlipidemia phenomenon in the assessed population. Additionally, sex and age may also interact with the glucose and/or cholesterol concentrations to explain a 14% of the data variability (TABLE I). LDL values in hyperlipidemic dogs ranged from 29 to 118 mg/dL (56 ± 8 mg/dL (n = 10); 90% of the animals above the reference levels); whereas HDL values ranged from 5 to 38 mg/dL (15 ± 3 mg/dL (n = 10); 80% of the animals were below the reference levels; FIG. 1). Linear regression between body mass and the serological variables measured do not presented any significant relationship for cholesterol (R^2 = 0.004, P = 0.56), glucose (R^2 = 0.01, P = 0.36), or triglycerides (R^2 = 0.039, P = 0.08).

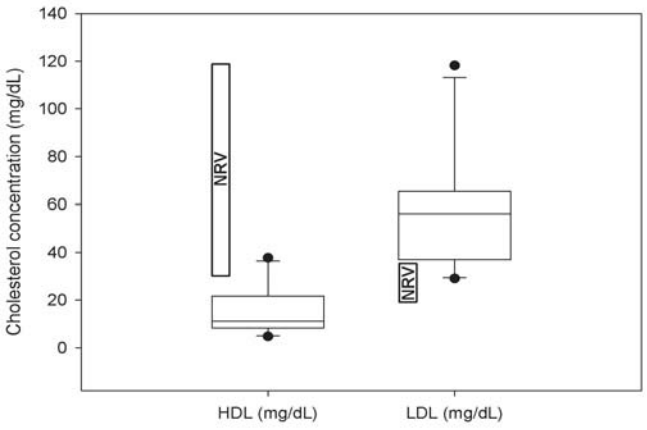

FIGURE 1. HDL AND LDL CONCENTRATIONS DETERMINED IN HYPERLIPIDEMIC DOGS (MEAN, 25 AND 75% PERCENTILES ARE SHOWN). NRV = NORMAL REFERENCE VALUES.

Hyperlipidemia prevalence estimated in this study was about 2% less than the expected according to previous sea-level reports [10, 17]. The obtained results were consistent to the literature [2] about the non-discriminating gender prevalence. Only 10% of the hyperlipidemic dogs were fed with commercial food (pellets) and the other 90% were fed with homemade food or a combination of both, suggesting that homemade food, usually rich in fats, may predispose animals to suffer hyperlipidemia, due to the increase in their blood lipid contents, and the slower lipid metabolism at high elevation conditions. It was discarded the possibility of postprandial hyperlipidemia because all the sampled animals were in fasting for 8 or more hours. Interestingly this study found a hyperlipidemia prevalence of 20% in Schnauzer dogs, which are known to be more prone to suffer this condition [19, 20], whereas other

breeds such as German Shepherd and Cocker Spaniel showed similar (20%) prevalence values.

In the model subset estimated, cholesterol concentration provided the most parsimonious explanation, but cholesterol interactions with glucose (sampled dogs were not diabetic), in a first instance, and then with triglycerides, also provided significant explanations, suggesting that cholesterol is not the only responsible variable for hyperlipidemia presence and it may also interact with glucose and triglycerides metabolism. It is well know that metabolism at elevations > 2000 m became slower, food digestion is also slow and the digestive system performs poorly compared to the sea level [9, 11]. Consequently there are more chances to fat and carbohydrate accumulation in blood serum, especially in dogs older than five years.

There was no body mass effect over the cholesterol, glucose, or triglycerides concentrations, consequently the hyperlipidemia prevalence estimated responded to metabolic traits and not to a simple body mass increasing artifact. An interesting finding is the relationship between HDL and LDL cholesterol. Most hyperlipidemic dogs presented higher LDL ("good" cholesterol) concentrations but lower HDL ("bad" cholesterol) concentrations, being a favorable scenario for ill animals and their treatment.

CONCLUSIONS AND RECOMMENDATIONS

The obtained results provide new evidence about metabolic interactions at reduced metabolic efficiencies, such as happens at high elevation conditions. They may be a valuable tool for small animal veterinaries in order to provide better diagnosis and treatments, based on a better understanding of the problem, and considering other involved clinical variables (i.e., triglycerides and glucose) as well as the cholesterol. Despite this study was focused on the higher elevation effects, it could be extrapolated to other situations in which the metabolism is lowered by environmental and/or clinical factors (e.g., other metabolic disorders). It is recommended conducting regular checks in dogs older than five year, irrespective of the gender or breed, and also performing triglyceride and glucose analyses in dogs with cholesterol concentrations above the normal values.

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