Metabolic Profile and Adipokine Levels in Overweight and Obese Dogs

Paula Nassar De Marchi[1]

Luiz Henrique de Araújo Machado[1]

Luciane Holsback[2]

Jéssica Calesso Ragazzi[2]

Rafael Fagnani[3]

Ademir Zacarias Júnior[2]

Fabíola Soares Zahn[1]

Mauro José Lahm Cardoso[4]*

[1]College of Veterinary Medicine and Animal Science, UNESP, Botucatu, Brazil;

[2]College of Veterinary Medicine ,UENP, Bandeirantes, Brazil;

[3]College of Production Engeneering, UNOPAR, Londrina, Brazil;

[4]Agricultural Sciences Center, UEL, Londrina, Brazil.

*Corresponding author

The Araucária Foundation (Project number 931/2013) financed the kits for adipokines evaluation.
CNPq and CAPES provided study grants for some of the authors.

KEY WORDS: Adipokines, Systolic Hypertension, Obesity, Insulin Resistance.

ABSTRACT

Obesity in dogs is increasingly present in the veterinary practice. In humans, it's known that there is a significant correlation between obesity and the development of hypertension and insulin resistance, a clinical picture called metabolic syndrome. In dogs, however, there is only anecdotal about it. The objectives of the study were1 to determine serum levels of adiponectin, leptin, triglycerides, cholesterol, insulin, glucose, HOMA-B, HOMA-IR, and systolic blood pressure in dogs with different body conditions (BC) without endocrine disease. Seventy six healthy dogs submitted to evaluation of BC and morphometry. The dogs were separated into four groups: optimal BC (ECC 3,4 or 5 of 9 and GC% of 13 to 27-G1), overweight (ECC 6 and 7 of 9 and GC% of 14 to 38- G2), obese (ECC 8 and 9 of 9 and GC% greater than or equal to 34-G3). G3 presented higher serum levels of total protein, triglycerides, glucose, insulin, and HOMA-IR. Adipokines didn't correlate to any other parameter, but the occurrence of hyperinsulinemia was higher in G3. The results have shown that obese dogs presented IR and alterations in fat metabolism.

INTRODUCTION

Obesity is a disease that is increasingly present in veterinary routine, representing more than 50% of attended dogs.[1] Certain risk factors contribute to the increased incidence in recent years, such as spaying, longevity,

quality and quantity of food, and lifestyle of the owners.[1]

Obesity reduces longevity and predisposes to the development of several diseases such as musculoskeletal, cardiorespiratory and reproductive disorders.[2] It is known that adipose tissue acts on the supply of energy, protection of the organs, and is responsible for the synthesis and secretion of various hormones and inflammatory substances, called adipokines.[3]

In humans, the term "metabolic syndrome" (MS) is used to expose a metabolic condition in which a person has risk factors (among them the visceral obesity) that predispose to cardiovascular disease and Type 2 Diabetes mellitus (T2DM).[4] Moreover, hyperlipidemia and changes in the levels of adipokines caused by obesity appear to be the keys to the development of IR and T2DM, although the mechanisms involved are poorly understood.[5]

In dogs, the use of the term "MS" is controversial, as it has not been confirmed whether the obese dogs are in fact more likely to develop cardiovascular diseases and metabolic diseases.[6,7] For this reason, Tvarijonaviciute et al.[8] suggest the use of the term "obesity-related metabolic dysfunction" (ORMD) and the characterization of the syndrome based on different parameters and cut-off values. Among them is the need that the dog presents body condition score higher or equal to 7 (out of 9), associated to at least two of the following parameters: triglycerides higher than 200mg/dL, total cholesterol higher than 300mg/dL, systolic blood pressure (SBP) higher than 160mmHg, and fasting blood glucose higher than 100mg/dL or confirmed diagnosis of diabetes mellitus.

It is believed that obese dogs have a predisposition to develop clinical manifestations secondary to obesity. Thus, the present study aimed to investigate the occurrence of changes in the metabolic and lipid profiles in overweight dogs.

OBJECTIVES

This study aimed to define the metabolic and lipid profiles and SBP in dogs with adequate body condition (BC), overweight or obesity without endocrine diseases. And, thus, determine serum levels of glucose, insulin, triglycerides, cholesterol, leptin, adiponectin, and SBP in 76 dogs with different BC.

Also, this study aimed to evaluate the occurrence of IR in overweight and obese dogs (by measurement of blood glucose, serum insulin and HOMA indexes) and the relation to the concentrations of adipokines and insulin.

METHODS

Location and Experimental Groups

The samples for this multicenter study were obtained at University FMVZ UNESP, Botucatu-SP, at UENP campus Luiz Meneghel and at UEL, Londrina. The Universities are located in Brazil.

The animals in this study were separated into four groups:

- Optimal body condition (G1, n=23)
- Overweight (G2, n=27) and obese (G3, n=26). The G1 was composed of dogs with ECC 3,4 or 5 of 9 and GC% of 13 to 27
- G2 was composed of dogs with ECC 6 and 7 of 9 and GC% of 14 to 38
- G3 was composed of dogs with ECC 8 and 9 of 9 and GC% greater than or equal to 34. Author, please confirm that these groupings are correct

In relation to the sex there were six females castrated in G1, six in G2 and 11 in G3; and seven uncastrated females in G1,7 in G2 and 5 in G3. There were five castrated males on the G1, five in G2 and G3; five uncastrated males in G1, 7 in G2 and 4 in G3.

Evaluation of Body Condition, Morphometric Measurements and Exclusion Criteria

BC was measured according to the 1-9 Body Condition Score System (BCS).9 Morphometry[10] was evaluated in addition to the BCS. Those animals in which the results of morphometry and BCS were conflicting were excluded from the experiment. Only three

trained examiners did the BCS assessment, strictly following the description of scale 1-9 proposed by Laflamme.[9]

The exclusion criteria were: endocrine, liver, and kidney diseases, and those that were being treated with glucocorticoid, anticonvulsants, hypo or hyperglycemic, and hypotensive drugs. The diseases were ruled out by clinical examination, hematologic evaluation, urinalysis, and imaging.

Hyperadrenocorticism was ruled out after suppression test with low-dose dexamethasone[11] and hypothyroidism after the thyroid function test.

Specimen Collection and Laboratory Tests

Samples were collected after 12 hour fasting, in the morning, by jugular venipuncture. Blood samples were immediately stored in sterile tubes containing EDTA and clot activator gel, being centrifuged before 1 hour after collection. Blood serum samples were fractionated into five vials and frozen at -70°C in freezer until the moment of testing.

Samples for glucose measurement were collected in a flask containing sodium fluoride, and sent for laboratory analysis immediately after collection.

Complete blood count, urinalysis, glucose measurement and serum biochemistry exams were processed at the Veterinary Clinical Laboratory of FMVZ UNESP.

Serum concentrations of adiponectin and leptin (Canine Adiponectin/Leptin ELISA, Millipore®) were measured using commercial kits of enzyme-linked immunosorbent assay (ELISA), according to the manufacturers' recommendations. Inter-assay sensitivity was 6.34% and 7.38% and intra assay sensitivity was 4.19% and 5.41% respectively for adiponectin and leptin.

The measurement of serum insulin was analyzed by the IMMULITE 1000 analyzer using a commercial kit (Millipore®), with intra-assay and inter-assay sensitivity being 3.23% and 3.65%, respectively. Samples were measured in duplicate.

The measurement of serum levels of total thyroxine, free thyroxine and cortisol were performed by the technique of radioimmunoassay (WIZARD, 1470 Automatic Gamma Counter, Perkin Elmer®), according to the recommendations of the kit's manufacturer (MP Biomedicals®).

HOMA Index Calculation and Noninvasive Measurement of Systolic Blood Pressure (SBP)

The calculation of the homeostasis assessment (HOMA) was performed as proposed by Mattews,12 based on the following equations: HOMA-IR = [Fasting insulin level (μU/mL) X Fasting glucose level (mmol/L)] / 22.5, and HOMA-%B= [20 X Fasting insulin level (μU/mL)] / [fasting glucose level (mmol/L) – 3.5].

SBP was measured by the noninvasive method, using Doppler flowmeter (Doppler Vascular DV 610, MedMega®), as described by Henik et al.[13]

The confirmation of hypertension was based on average SBP levels above 160mmHg, following the ORMD classification standards[8,14].

Analysis of the results

The experimental design has considered the effects of BCS on the variables:

- Percentage of body fat (%BF)
- SBP
- Triglycerides
- Cholesterol
- Glucose
- Insulin
- HOMA-IR, HOMA-B
- Adiponectin, and
- Leptin.

The variables didn't show normality and homoscedasticity by the Kolmogorov-Smirnov test and Liliefors test ($p < 0.05$). Thus, the differences between dogs grouped according to BCS (G1, G2 and G3) were analyzed by Kruskal-Wallis test with $p < 0.05$.

All the variables were related to each other through the Spearman correlation and $p < 0.05$. All analyzes were done in Statistica 10.0 software.

RESULTS

The results are shown in Figures 1, 2, and 3 as box plots. Dogs classified as obese (G3) had higher triglycerides, blood glucose, insulin and HOMA-IR. Medians without letters or followed by equal letters don't differ in the Kruskal Wallis test ($p<0,05$).

Note 1: trig = triglycerides; SBP= systolic blood pressure. Medians without letters don't differ in the Kruskal Wallis test ($p<0,05$). Medians without letters or followed by equal letters don't differ in the Kruskal Wallis test ($p<0,05$). Note 2: HOMA-IR = model for assessment of the homeostasis of insulin resistance; HOMA-B= model for assessment of the homeostasis of B cell function

Figure 1. Graphical representation of triglycerides, cholesterol and SBP in dogs with different body conditions (G1, G2 and G3).

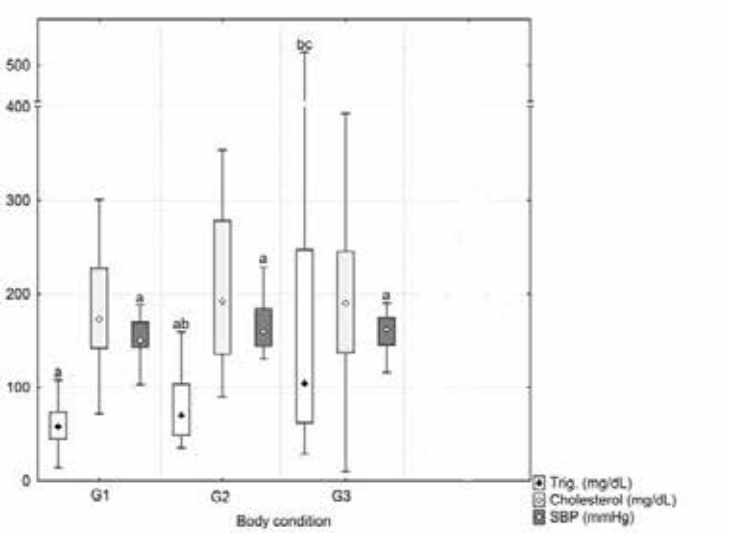
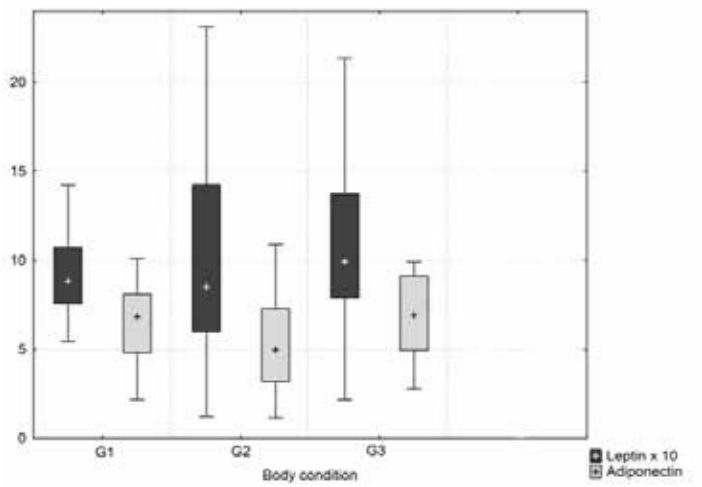


Table 1 shows the values obtained for leptin and adiponectin in the different groups studied. It is noted that there is no variation of adiponectin and leptin values according to body condition. Besides that, adipokines showed no significant correlation with any of the studied variables.

Figure 2. *Graphical representation of adiponectin and leptin values in dogs with different body conditions (G1, G2 and G3). Medians without letters don't differ in the Kruskal Wallis test ($p<0,05$).*

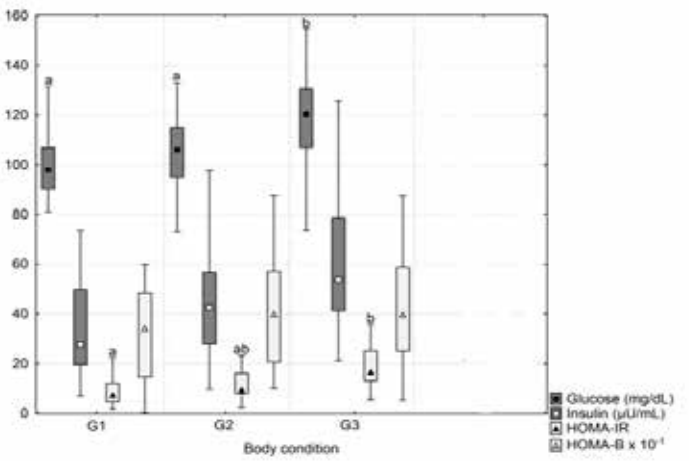


DISCUSSION

Melhman et al.[15] found that SBP values were higher in obese dogs, although they all were within the normal range. This was also observed in this study, suggesting that both the amount and the period of adiposity can influence SBP values. It is possible that we didn't detect statistical difference in SBP values between G1, G2, and G3 by the fact that this was a study involving only animals with spontaneous obesity. It is inferred that the

rate of weight gain and the type of diet can both influence the occurrence of hypertension in dogs. Pérez-Sánchez et al.[14] On the other hand, do not consider obesity as a risk factor for the development of hypertension, believing that there is always association with other diseases. In the current study, however, other comorbidities were ruled out.

Figure 3. *Graphical representation of glucose, insulin, HOMA-IR e HOMA-B values in dogs with different body conditions (G1, G2 and G3).*



In this, higher levels of triglycerides in overweight and obese dogs are similar to those described by others.[5,16,17] It is inferred, therefore, that may be a risk factor for the development of hyperlipidemia. The mechanisms involved aren't clear, but it is believed that genetic factors, fat distribution, diet imbalance, and physical inactivity are associated.

In this study, higher levels of glucose, insulin and HOMA-IR in G3, suggests that obese dogs do develop IR. Similar findings of increased HOMA-IR in obese dogs was described by Respondek et al.,18 demonstrating the occurrence of IR. As observed in the present study, others have shown that obese dogs tend higher concentrations of glucose.[8,16,19,20,21]

It is noteworthy that most of the studies that found no hyperglycemia in obese dogs worked with portable glucometer and obesity induced by high-energy diets. It is considered that the use of glucometer must be associated with laboratory serum glucose analysis. Spontaneously obese dogs manifest more changes in glucose levels than experimentally-induced obese dogs, possibly because of the adiposity time, the level of physical inactivity and the type of diet.

It is known that there is variation in the measurement of adipokines depending on the brand of the kits and techniques. Also, it's believed that sample and tubes storage time cause alterations, and there are differences in the sensitivity of the tests for the various existing forms of adiponectin. In this study, specific and previously reported commercial kits were used[5,19] and the samples

Table 1. *Average, standard deviation and median of adiponectin and leptin in G1, G2 and G3.*

		G1	G2	G3
Leptin (ng/dL)	Average	1,13	1,61	1,15
	Standard D.	0,76	3,33	0,56
	Medium	0,88	0,85	0,99
Adiponectin (µg/mL)	Average	6,87	5,53	6,99
	Standard D.	2,76	2,62	2,90
	Medium	6,84	4,98	6,91

Medians without letters did not differ in the Kruskal Wallis test (p <0.05).

were stored for a shorter time than that recommended by the manufacturer. There were no differences in leptin and adiponectin concentrations between groups and adipokines were not correlated with any variable.

German et al. [22] used only dogs with owners and the same kit used in this study for the evaluation of total adiponectin serum levels. Similarly, Verkest et al.[6] did not observe changes in adiponectin levels in dogs due to overweight. These data suggest that dogs show no variation in adiponectin concentrations.

Other authors, however, reported the occurrence of either decreased adipokines levels in canine obesity or of increased levels in obese dogs after weight loss.[5,8,16,19] It is noticeable, however, that most of these studies were conducted with experimentally induced obese dogs fed high energy diets and measurements were performed using human kits.

In humans, it is believed that the development of obesity-related disorders is directly associated to decrease serum levels adiponectin.[8] In the studied population, there were no changes in the levels of adipokines, and probably this is a protective factor in the development of MS and T2DM, as reported in other species.

This strengthens the hypothesis, wherein the obese dogs, despite developing IR, are resistant to the development of DM similar to type-2 in humans because of compensatory mechanisms that prevent changes in adiponectin and even leptin levels, as occurred in the population studied here.

Several researchers have found the occurrence of hyperleptinaemia in obese dogs.[5,16,17] As mentioned previously, the interpretation of these findings must be careful. In the present study, there were no changes in leptin levels. German et al.[22] using the same kits as this study found values below the detection threshold. As observed here, Müller et al.[23] found no correlation between adiposity or leptin concentrations and insulin levels or IR.

The constancy in the levels of adipokines observed in different groups can be justified by the fact that this was a clinical study, ie, all included animals had owners and none had experimentally induced obesity, which means that it is not possible to determine the diet and to estimate how long each individual dog was in overweight or obese.

Therefore, a key to detect changes in levels of adipokines and even the development of metabolic disorders in dogs with increased BF may be the determination of the diet and the duration of overweight or obesity, as well as performing longitudinal studies. Furthermore, it is important to emphasize the distinction between quickly induced obesity and those cases in which the dogs weigh is slowly increased over the years, since the resulting disorders may be different.

Dogs seem to keep higher adiponectin levels than humans and may act as a protective factor in dogs, preventing the development of T2DM.

CONCLUSION

The results obtained allowed concluding that obese dogs have higher levels of triglycerides, glucose, insulin, HOMA-IR, and higher SBP when compared to dogs with optimal BC and overweight.

However, adipokines do not seem to correlate with any of the other studied variables and do not vary according to BC. This constancy in the level of adipokines probably is a protective factor in the development of MS and T2DM in dogs.

Conflict of Interests

The authors declare that they have no conflicts of interests.

Approval of the Ethics Committee and Informed Consent

The present study was submitted to the Ethics Committee on Animal Use of FMVZ, UNESP and was approved under the protocol number 107/2014-CEUA.

All owners signed a free and informed

consent authorizing the collection of materials and use of data in publications.

Health and Safety

During the accomplishment of this experimental work all the obligatory procedures of health and safety of the laboratory were fulfilled.

REFERENCES

1. Corbee RJ. Obesity in show dogs. *J Anim Physiol an* N. 2013; 97:904-10.
2. German AJ. The growing problem of obesity in dogs and cats. *J Nutr*. 2006; 136:1940S-46S.
3. Zoran DL. Obesity in dogs and cats: a metabolic and endocrine disorder. *Vet Clin N Am-Small.* 2010; 40:221-39.
4. Toth PP, Henriksson KM, Palmer MK. Metabolic syndrome and low-density lipoprotein cholesterol (LDL-C) goal attainment in the national health and nutrition examination survey (NHANES). In: Anais do NATIONAL LIPID ASSOCIATION SCIENTIFIC SESSIONS. New Orleans: Painel. 2016; 2003-12
5. Park H, Lee S, Oh J, SEO K, Song K. Leptin, adiponectin and serotonin levels in lean and obese dogs. *BMC Vet Res.* 2014; 10(113):1-8.
6. Verkest KR, Rand JS, Fleeman LM, Morton JM, Richards AA, Rose FJ, et al. Distinct adiponectin profiles might contribute to differences in susceptibility to type 2 diabetes in dogs and humans. *Domest Anim Endocrin*. 2011a; 4:67-73.
7. Verkest KR, Fleeman LM, Morton JM, Ishioka K, Rand JS. Compensation for obesity-induced insulin resistance in dogs: assessment of the effects of leptin, adiponectin, and glucagon-like peptide-1 using path analysis. *Domest Anim Endocrin.* 2011b; 41:24-34.
8. Tvarijonaviciute A, Cerón JJ, Holden SL, Cuthbertson DJ, Biourge V, Morris PJ, et al. Obesity-related metabolic dysfunction in dogs: a comparison with human metabolic syndrome. *BMC Vet Res.* 2012; 8(147):1-8.
9. Laflamme DP. Development and validation of a body condition score system for dogs. *Canine Pract.* 1997; 22:10-15.
10. Burkholder WJ, Toll PW. Obesity. In: Hand MS, Tatcher CD, Remillard RL, Roudebusch. Small animal clinical nutrition. 4th ed. Topeka: Mark Morris Institute; 2000; 401-30.
11. Behrend EN, Kooistra RN, Nelson CE, Reusch CE, Scott-Moncrieff JC. Diagnosis of spontaneous canine hyperadrenocorticism: 2012 ACVIM consensus statement (small animal). *J Vet Intern Med.* 2013; 27:1-13.
12. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and B-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985; 28:412-19.
13. Henik RA, Dolson MK, Wenholz LJ. How to obtain a blood pressure measurement. *Clin tech small an* P. 2005; 20:144-50.
14. Pérez-Sánches AP, Del-Angel-Caraza J, Quijano-Hernández IA, Barbosa-Meireles MA. Obesity-hypertension and its relation to other diseases in dogs. *Vet Res Commun*. 2015; 39:45-51.
15. Melhman E, Bright JM, Jeckel K, Porsche C, Veeramachaneni DN, Frye M. Echocardiographic evidence of left ventricular hypertrophy in obese dogs. *J Vet Intern Med.* 2013; 27:62-68.
16. Piendetosi D, DI Loria A, Guccione J, De Rosa A, Fabbri S, Cortese L, et al. Serum biochemistry profile, inflammatory cytokines, adipokines and cardiovascular findings in obese dogs. *Vet J.* 2016; 216:72-78.
17. Söder J, Wernersson S, Hagman R, Karlsson I, Malmlöf K, Höglund K. Metabolic and hormonal response to a feed-challenge test in lean and overweight dogs. *J Vet Intern Med.* 2016; 30:574-92.
18. Respondek F, Swanson KS, Belsito KR, Vester BM, Wagner A, Istasse L, et al. Short-chains fructooligossacharides influence insulin sensitivity and gene expression of fat tissue in obese dogs. *J Nutr*. 2008; 138:1712-18.
19. Adolphe JL, Silver TI, Childs H, Drew MD, Weber LP. Short-term obesity results in detrimental metabolic and cardiovascular changes that may not be reversed with weight loss in an obese dog model. *Brit J Nutr.* 2014; 112:647-56.
20. Cardoso MJ, Fagnani R, Cavalcante CZ, Zanutto MS, Zacarias-Júnior A, Melussi M, et al. Blood pressure, serum glucose, cholesterol and triglycerides in dogs with different body scores. *Vet Med Int*. 2016; 1-7.
21. Verkest KR, Rand JS, Fleeman LM, Morton JM. Spontaneously obese dogs exhibit greater postprandial glucose, triglyceride, and insulin concentrations than lean dogs. *Domest Anim Endocrin*. 2012; 42:103-12.
22. German AJ, Hervera M, Hunter L, Holden SL, Morris PJ, Biourge V, et al. Improvement in insulin resistance and reduction in plasma inflammatory adipokines after weight loss in obese dogs. *Domest Anim Endocrin*. 2009; 37:214-26.
23. Müller L, Kollár E, Balogh L, Pösténvi Z, Márián T, Garai I, et al. Body fat distribution and metabolic consequences – examination opportunities in dogs. *Acta Vet Hung*. 2014; 62(2):169-79.