Metabolic profile and adipokine levels in overweight and obese dogs

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1. Introduction
Obesity is a disease that is increasingly present in veterinary routine, representing more than 50% of attended dogs. Certain risk factors have contributed to the increased incidence in recent years, such as spaying, longevity, the quality and quantity of food, and the 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, the 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 explain 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 the 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 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 cutoff values. Among them is the need that the dog present a body condition score higher than or equal to 7 (out of 9), associated with at least 2 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 the 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.
This study aimed to define the metabolic and lipid profiles and SBP in dogs with adequate BCs, overweight or obesity without endocrine diseases and thus to determine the serum levels of glucose, insulin, triglycerides, cholesterol, leptin, adiponectin, and SBP in 76 dogs with different BCs.

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 its association with the concentrations of adipokines and insulin.

2. Material and Methods

2.1. Location and experimental groups

The samples for this multicenter study were obtained at the College of Veterinary Medicine and Animal Science, Paulista State University Júlio de Mesquita Filho, Botucatu-SP, Brazil, at the State University of Northern Parana, Bandeirantes-PR, Brazil, and at the State University of Londrina, Londrina-PR, Brazil.

All the animals were healthy and were selected randomly for the present study. The age of the animals ranged from 2 to 16 years. The evaluated breeds were diverse and consisted of Golden retrievers, Labradors, poodles, Yorkshires, mixed breeds, malts, blue heelers, Rottweilers, and Lhasa Apso.

The animals in this study were separated into 3 groups: optimal body condition [Group (G1), n = 23], overweight [Group (G2), n = 27], and obese [Group (G3), n = 26]. G1 was composed of dogs with BCS 3, 4, or 5 and BF% of 13 to 27, G2 was composed of dogs with BCS 6 and 7 of 9 and BF% of 14 to 38, and G3 was composed of dogs with BCS 8 and 9 of 9 and BF% greater than or equal to 34.

In relation to sex, there were 6 females spayed in G1, 6 in G2, and 11 in G3, and 7 uncastrated females in G1, 7 in G2, and 5 in G3. Furthermore, there were 5 castrated males in G1, 5 in G2 and G3, and 5 uncastrated males in G1, 7 in G2, and 4 in G3.

2.2. Evaluation of body condition, morphometric measurements, and exclusion criteria

BCS 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 3 trained examiners did the BCS assessment, strictly following the description of scale 1–9 proposed by Laflamme [9].

The exclusion criteria included endocrine, liver, and kidney diseases and those which were being treated with glucocorticoid, anticonvulsants, and 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.

2.3. Specimen collection and laboratory tests

The samples were collected after 12 h of fasting, in the morning, by jugular venipuncture. The blood samples were immediately stored in sterile tubes containing EDTA and clot activator gel, being centrifuged within 1 h after collection. The blood serum samples were fractionated into 5 vials and frozen at −70°C in freezer until the moment of testing.

The 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 Paulista State University Júlio de Mesquita Filho.

The serum concentrations of adiponectin and leptin (Canine Adiponectin/Leptin ELISA, Millipore®, Billerica, MA, USA) were measured using commercial kits of enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s recommendations. Interassay sensitivity was 6.34% and 7.38% and intraassay 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®, Billerica, MA, USA), with intraassay and interassay sensitivity being 3.23% and 3.65%, respectively. The samples were measured in duplicate.

The measurement of serum levels of total thyroxine, free thyroxine, and cortisol were performed by radioimmunoassay technique (WIZARD, 1470 Automatic Gamma Counter, Perkin Elmer Inc., Waltham, MA, USA) according to the recommendations of the kit’s manufacturer (MP Biomedicals, California, CA, USA).

2.4. HOMA index calculation and noninvasive measurement of systolic blood pressure (SBP)

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

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

The confirmation of hypertension was based on the average SBP levels above 160mmHg following the ORMD classification standards [8,14].
2.5. Analysis of the results
The experimental design has considered the effects of BCS on the variables of the percentage of body fat (%BF), SBP, triglycerides, cholesterol, glucose, insulin, HOMA-IR, HOMA-B, adiponectin, and leptin.

Although the groups differ in quantity, the number of animals per group allowed statistical analyses to be performed.

The variables did not show normality and homoscedasticity by the Kolmogorov-Smirnov test and Liliefors test (P < 0.05). Thus, the differences among dogs grouped according to BCS (G1, G2, and G3) were analyzed by the Kruskal-Wallis test with P < 0.05.

All the variables were related to each other through the Spearman correlation and P < 0.05. All the analyses were carried out in the Statistica 10.0 software.

3. Results
The results are presented in Figures 1, 2, and 3 as box plots. The dogs classified as obese (G3) had higher levels of triglycerides, blood glucose, insulin, and HOMA-IR.

The table 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.

4. Discussion
Mehlman et al. [15] found that the 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 did not detect a statistical difference in the SBP values between G1, G2, and G3 due to 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 an association with other diseases. In the current study, however, other comorbidities were ruled out.

In this study, higher levels of triglycerides in overweight and obese dogs are similar to those described by others [5,16,17]. It is inferred, therefore, that this may be a risk factor for the development of hyperlipidemia. The mechanisms involved are not 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 suggest that obese dogs do develop IR. Similar findings of increased HOMA-IR in obese dogs were described by Respondek et al. [18], demonstrating the occurrence of IR. As observed in the present study,
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). G1: Group 1 (Optimal BC); G2: Group 2 (Overweight); G3: Group 3 (Obese).

Figure 3. Graphical representation of glucose, insulin, HOMA-IR e HOMA-B values in dogs with different body conditions (G1, G2 and G3). Medians without letters or followed by equal letters don't differ in the Kruskal Wallis test (P < 0.05). G1: Group 1 (Optimal BC); G2: Group 2 (Overweight); G3: Group 3 (Obese); HOMA-IR: model for assessment of the homeostasis of insulin resistance; HOMA-B: model for assessment of the homeostasis of B cell function.
It is noticeable, however, that most of these studies were conducted with experimentally induced obese dogs fed high energy diets and the measurements were performed using human kits.

In humans, it is believed that the development of obesity-related disorders is directly associated with decreases in the serum levels of adiponectin [8]. Previous studies in cats have shown adiponectin behavior similar to humans [23], although more recent studies have not observed significant changes in adiponectin levels in obese cats and after weight loss [24]. 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 that obese dogs, despite developing IR, are resistant to the development of DM similar to type-2 in humans because of the compensatory mechanisms that prevent changes in adiponectin and even leptin levels, as occurred in the population studied here. There are different isoforms of adiponectin from different molecular weights [25,26]. Brunson et al. [27] detected a similarity between the canine adiponectin molecule and that of humans and rodents, and the expression of the adiponectin gene was identified in the canine visceral adipose tissue, which supports the hypothesis that adipokines play an important role in canine metabolism. Muñoz-Prieto et al. [28] showed the use of selective protease digestion to detect canine serum as a procedure to detect different isomers of adiponectin. This technique was not used in the present study, and therefore, some isoforms may not have been detected.

It is emphasized that metabolic studies are necessary to investigate the differences in dog metabolism.

Several researchers have found the occurrence of hyperleptinemia in obese dogs [5,16,17]. As mentioned before, these findings must be carefully interpreted because there is a range of kits and methodologies used, as well as the storage time and the type of sample used (serum or plasma). In addition, the studies are not identical, as there seems to be a difference between subcutaneous and visceral adipose tissue on adipokine levels [29], which was not evaluated in these studies. In the present study, there were no changes in leptin levels. German et al. [22] found values below the detection threshold using the same kits of this study. As observed here, Müller et al. [30] 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, all the 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 overweight or obese.

### Table

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
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<tbody>
<tr>
<td><strong>Leptin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average (ng/dL)</td>
<td>1.13</td>
<td>1.61</td>
<td>1.15</td>
</tr>
<tr>
<td>Standard D.</td>
<td>0.76</td>
<td>3.33</td>
<td>0.56</td>
</tr>
<tr>
<td>Medium</td>
<td>0.88</td>
<td>0.85</td>
<td>0.99</td>
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<tr>
<td><strong>Adiponectin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average (μg/mL)</td>
<td>6.87</td>
<td>5.53</td>
<td>6.99</td>
</tr>
<tr>
<td>Standard D.</td>
<td>2.76</td>
<td>2.62</td>
<td>2.90</td>
</tr>
<tr>
<td>Medium</td>
<td>6.84</td>
<td>4.98</td>
<td>6.91</td>
</tr>
</tbody>
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Medians without letters did not differ in the Kruskal Wallis test (P < 0.05).

G1: Group 1 (Optimal BC); G2: Group 2 (Overweight); G3: Group 3 (Obese).
Recent studies have shown that different diets can influence the levels of circulating adiponectin and leptin [31], which can also justify differences between the levels of adipokines detected in different studies cited, since this parameter has not been evaluated.

Therefore, a key to detect the changes in the 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 dog’s weight slowly increases over the years, since the resulting disorders may be different.

In humans, low levels of adiponectin are related to disorders such as atherosclerosis, T2DM, and insulin resistance [26]. Dogs seem to keep higher adiponectin levels than humans, which may act as a protective factor in dogs, preventing the development of T2DM. When comparing the levels of adiponectin with cats, there also seem to be differences, since in cats, which develop DM similar to T2 in humans, there are differences between the levels of adiponectin according to body condition[23]. However, recent studies have not confirmed that this is significant [24].

5. 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.

Approval of the Ethics Committee and Informed Consent
The present study was submitted to the Ethics Committee on Animal Use of Paulista State University Júlio de Mesquita Filho, Botucatu-SP and was approved under the protocol number 107/2014-CEUA.

All the owners signed a free and informed consent authorizing the collection of materials and the use of data in publications.

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References


