Feline Obesity Causes Hematological And Biochemical Changes And Oxidative Stress

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Research Article

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Abstract

Obesity, an extremely important factor in feline clinical practice, is estimated to affect up to one third of the feline population. Moreover, it can trigger chronic inflammation, which could predispose to oxidative stress by increasing reactive oxygen species, thereby generating potentially irreversible cellular damage. This study analyzed hematological, biochemical and oxidative stress profiles at various degrees of feline obesity. Forty-five cats were selected and divided into three groups: control (n=17), overweight (n=13) and obese (n=15), after clinical and laboratory evaluation and body condition score. Biochemical and oxidative stress analyses were performed using a photocolorimeter and hematological analyses were performed in a veterinary cell counter. The hematological profile of obese cats showed increased mean corpuscular volume (MCV) and red cell distribution width (RDW), while their biochemical profile revealed increased HDL cholesterol and triglycerides and decreased activity of gamma-glutamyl transferase (GGT). As for oxidative stress, obese cats showed higher total antioxidant capacity (TAC), by the inhibition of 2,2’-Azino-Bis-3-Ethylbenzthiazoline-6-Sulfonic Acid (ABTS), inhibition of ABTS associated with horseradish peroxidase (ABTS+HRP), cupric ion reducing antioxidant capacity (CUPRAC) and ferric reducing antioxidant power (FRAP) methods, while overweight cats had a higher TAC-ABTS+HRP and TAC-FRAP. We conclude that the conditions of natural obesity and overweight in the feline species alter its hematological, biochemical and oxidative stress parameters.

1. Introduction

Sedentary lifestyle and inadequate nutrition are undesirable results of the domestication of felines, which account for 25 to 35% of obesity among cats (Butterwick 2000; Hess et al. 2003; Zoran 2009; German 2010). Obesity leads to a predisposition for numerous secondary diseases, such as diabetes mellitus (Hess et al. 2003; Laflamme 2006; German 2010), changes in the lipid profile (Hess et al. 2003), cardiorespiratory, dermatological and oral abnormalities (Laflamme 2006; German 2010), lower urinary tract and orthopedic diseases (Laflamme 2006; German 2010; Radakovich et al. 2017), in addition to hepatic lipidosis (German 2010) and neoplasms (Laflamme 2006; German 2010).

Adipose tissue is an active endocrine organ, releasing several important hormones that play a role in the development of obesity-associated changes, such as metabolic syndrome and insulin resistance (Kil and Swanson 2010). Adiponectin is a protein hormone secreted exclusively by adipose tissue, whose expression decreases as adipose tissue increases, leading to a reduction in (Hess et al. 2003; German 2010) serum concentration in cats (Hoenig et al. 2007; Ishioka et al. 2009) and in humans (Spranger et al. 2003). Leptin, which acts by increasing energy expenditure, reducing food intake and modulating glucose (Hess et al. 2003; German 2010) and lipid metabolism (Kil and Swanson 2010), tends to increase with weight gain and excess adipose tissue, and can be used as a marker of obesity in dogs (Ishioka et al. 2002).

Considering the human and canine species, obesity causes a chronic inflammatory condition, responsible for the increase in platelets, neutrophils, lymphocytes and monocytes, in addition to elevated levels of
pro-inflammatory cytokines (Nemet et al. 2005; Zaldivar et al. 2006; Radakovich et al. 2017). The pro-inflammatory state can trigger the excessive production of reactive oxygen species (ROS) (Dandona et al. 2001, 2004; Tanner et al. 2007) and although ROS are necessary in physiological processes such as cell signaling and defense against microorganisms, excessive production leads to oxidative damage as a result of oxidative stress (Pacher et al. 2007). In obese cats, the association of intense protein and lipid oxidation with an increase in inflammatory cytokines such as interleukins 1 and 6, C-reactive protein and tumor necrosis factor-alpha, constitutes a typical picture of chronic inflammatory disease (Coppack 2001).

Thus, oxidative stress occurs when there is an imbalance between antioxidants and oxidants in the body, a condition associated with several pathological conditions in felines, such as diabetes mellitus (Webb and Falkowski 2009), chronic kidney failure (Keegan and Webb 2010), infection with feline immunodeficiency virus (FIV) (Webb et al. 2008), and a carcinogenic potential (Shacter et al. 1989; Biezus et al. 2017). There is a paucity of studies that evaluate oxidative stress in feline obesity. A single study that induced obesity in cats over a short period of 12 weeks and maintained it for an additional 8-week period revealed increased oxidative stress through increased oxidation of proteins, lipids and DNA (Tanner et al. 2007). However, as obesity was induced, it is difficult to compare these results with actual clinical conditions found in chronically obese cats. Furthermore, the total antioxidant and oxidant capacities were not evaluated. In this context, our study aimed to evaluate the hematological, biochemical and oxidative stress profiles at various levels of feline obesity.

2. Materials And Methods

2.1. Animal selection

The experiment was conducted according to the ethical principles of the Animal Research Ethics Committee of the University Center of the Integrated Faculties of Ourinhos (Protocol no. 007/2019). The participation of each feline was authorized by its owner, who signed a free and informed consent form.

After clinical and laboratory evaluation, 45 cats were selected and allocated to three groups, according to the body condition score (BCS) proposed by Laflamme (1997) and the characteristics listed in Table 1:

− Control group: 17 adult cats (mean age 4.62 ± 1.70 years), with BCS 5 (mean weight 3.61 ± 0.67 kg), clinically healthy, with no changes in the clinical examination and laboratory evaluation (complete blood count – CBC, albumin, alanine aminotransferase – ALT, creatinine, total cholesterol, gamma-glutamyl transferase – GGT, glucose, urea, total protein and triglycerides).

− Overweight group: 13 adult cats (mean age 5.04 ± 2.93 years), with BCS 6–7 (mean weight 4.5 ± 0.78 kg) for at least one year.

− Obese group: 15 adult cats (mean age 6.07 ± 2.88 years), presenting BCS 8–9 (mean weight 5.89 ± 0.94 kg) for at least one year.
The only cats included in this study were those fed exclusively with commercial cat food containing similar compositions, and which accepted physical restraint to draw blood samples without struggling. Animals treated in the preceding month with any type of medication, particularly drugs that lead to obesity or that have antioxidant and/or anti-inflammatory action, were not included in this study.

2.2. Collection of blood samples and laboratory analysis

The cats were fasted for 12 hours, after which 5 mL of blood was drawn by jugular venipuncture into tubes with K$_2$EDTA (BD Vacutainer®, Becton-Dickson, New Jersey, USA) for complete blood count (CBC), tubes with sodium fluoride (Injex Vacuo, Injex Indústrias Cirúrgicas, São Paulo, Brazil) for the biochemical determination of glucose, and tubes with clot activator (BD Vacutainer®, Becton-Dickson, New Jersey, USA) to obtain serum for other analyses. The fluoridated blood was immediately centrifuged (3,000 rpm for 10 min), while the tube used to obtain serum was centrifuged 20 min after collection. All serum processing took place in the absence of light and samples were stored away from light at -20°C until analysis, for a maximum period of 20 days.

CBC was performed as previously described(Costa et al. 2020; Oliveira et al. 2020). Briefly, red blood cells (RBC), white blood cells (WBC) and platelet (PLT) concentrations, mean corpuscular volume (MCV), red cell distribution width (RDW), mean platelet volume (MPV) and hemoglobin were examined in a veterinary automated cell counter (ABX Micros ESV 60, Paris, France) previously calibrated and checked with commercial controls (ABX Minotrol 16, Paris, France). Hematocrit (HCT) was determined by the Strumia microcapillary method (11,400 rpm for 5 minutes), the differential leukocyte count was performed using a blood smear stained with commercial hematological dye (Instant-Prov, Newprov, Pinhais, PR, Brazil), and the icterus index test was performed as recommended by Jain (1986). Total plasma protein (TPP) was determined in a portable clinical refractometer (ATAGO, Mod. Master-SUR-NM, Tokyo, Japan).

Biochemical analyses were performed in a semi-automated photocolorimeter (BIO 2000, BioPlus, Barueri, SP, Brazil) in duplicate, using a set of commercial reagents (Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil) according to the manufacturer's instructions, after calibration with calibrator (Calibra H, Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil) and verification with commercial control levels I (Qualitrol 1H, Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil) and II (Qualitrol 2H, Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil). HDL cholesterol levels were determined after precipitation with phosphotungstic acid and magnesium chloride, with subsequent determination of cholesterol. Uric acid, total cholesterol and triglyceride levels were determined by the enzymatic Trinder method, glucose by the glucose oxidase Trinder method, and ALT and AST by the ultraviolet (UV) kinetic method. Albumin was analyzed by the colorimetric method using bromocresol green, total calcium by the colorimetric cresolphthalein method, creatinine by the alkaline picrate colorimetric method, and ALP by Bowers and McComb's modified kinetic procedure. Phosphorus was evaluated by UV determination according to Daly and Ertingshausen's modified method, GGT by the Szasz modified method, amylase by the substrate 2-chloro-p-nitrophenyl-alpha-D-maltotrioside, lipase by colorimetric enzymatic method, total protein by biuret colorimetric method, urea by UV enzymatic method and fructosamine by nitroblue tetrazolium (NBT) reduction. The globulin content was quantified by subtracting the albumin from total proteins.
Oxidative stress was determined as previously described (Almeida et al. 2021; Bonatto et al. 2021), by measuring total antioxidant capacity (TAC) using four different methods, total oxidant capacity (TOC), lipid peroxidation and the antioxidants uric acid and albumin. TAC was determined in a semi-automated photocolorimeter (BIO 2000, BioPlus, Barueri, SP, Brazil) by inhibiting the reduction of the ABTS cation alone (TAC-ABTS) (Erel 2004) or in association with peroxidase (TAC-ABTS + HRP) (Rubio et al. 2016a), using the cupric ion reducing antioxidant capacity assay (TAC-CUPRAC) (Rubio et al. 2016b) and the ferric reducing antioxidant power assay (TAC-FRAP) (Benzie and Strain 1996). TOC was determined by the colorimetric method of xylenol orange (Erel 2005), while lipid peroxidation was determined using thiobarbituric acid reactive substances (TBARS) (Hunter et al. 1985). All the reagents were from Sigma-Aldrich Chemical Co.

2.3. Statistical analysis

The data were tested for normality using the Shapiro-Wilk test and for homoscedasticity using the Bartlett test. Differences between groups were verified by ANOVA and Tukey’s post-hoc test or Kruskal-Wallis and Dunn’s post-hoc test. All the statistical analyses were performed using a computer program (GraphPad Prism, v.6.00 for Windows, GraphPad Software, La Jolla, CA, USA, www.graphpad.com) and differences were considered significant when p < 0.05.

3. Results

3.1. Hematological and biochemical parameters

With regard to hematological parameters, obese cats showed higher RDW than control cats and higher MCV than cats in the overweight group, but no differences were found in the other hematological variables (Table 1).

As for biochemical parameters, obese cats had higher levels of HDL cholesterol and triglycerides than control cats and lower GGT activity than overweight cats, but showed no significant differences in other biochemical parameters (Table 1).

Table 1: Gonadal status, sex, age (mean and standard deviation), weight (mean and standard deviation), body condition score (BCS), hematological and biochemical parameters (mean and standard deviation) in cats with body score condition 5 (Control, n=17), 6/7 (Overweight, n=13) and 7/8 (Obese, n=15) according to Laflamme (1997).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Overweight</th>
<th>Obese</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonadal state (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutered</td>
<td>88%</td>
<td>100%</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Unneutered</td>
<td>12%</td>
<td>0%</td>
<td>0%</td>
<td>-</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>53%</td>
<td>62%</td>
<td>40%</td>
<td>-</td>
</tr>
<tr>
<td>Male</td>
<td>47%</td>
<td>38%</td>
<td>60%</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>4.62 ± 1.70</td>
<td>5.04 ± 2.93</td>
<td>6.07 ± 2.88</td>
<td>-</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>3.61 kg ± 0.67</td>
<td>4.5 kg ± 0.78</td>
<td>5.89 kg ± 0.94</td>
<td>-</td>
</tr>
<tr>
<td>BCS</td>
<td>5 (100%)</td>
<td>6 (69.2%)</td>
<td>8 (93.3%)</td>
<td>-</td>
</tr>
<tr>
<td>Hematological profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT (%)</td>
<td>43.12 ± 3.42</td>
<td>42.08 ± 3.22</td>
<td>43.20 ± 3.52</td>
<td>24 – 45</td>
</tr>
<tr>
<td>RBC (x10^{12}/L)</td>
<td>9.74 ± 0.93</td>
<td>9.77 ± 1.05</td>
<td>9.43 ± 0.79</td>
<td>5.0 – 10.0</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.99 ± 1.00</td>
<td>13.65 ± 0.85</td>
<td>13.96 ± 1.28</td>
<td>8.0 – 15.0</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>44.39 ± 2.48 b</td>
<td>43.29 ± 3.52 b</td>
<td>45.94 ± 3.80 a</td>
<td>39 – 55</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>32.48 ± 1.91</td>
<td>32.53 ± 1.83</td>
<td>32.32 ± 1.47</td>
<td>31 – 35</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>18.17 ± 0.59 b</td>
<td>18.87 ± 0.58 a</td>
<td>18.70 ± 0.60 a</td>
<td>17 – 21</td>
</tr>
<tr>
<td>WBC (x10^9/L)</td>
<td>7.96 ± 2.5</td>
<td>5.78 ± 2.0</td>
<td>7.10 ± 2.9</td>
<td>5.5 – 19.5</td>
</tr>
<tr>
<td>Band neutrophils (x10^9/L)</td>
<td>0.18 ± 0.04</td>
<td>0.02 ± 0.05</td>
<td>0.006 ± 0.01</td>
<td>0 – 0.3</td>
</tr>
<tr>
<td>Segmented neutrophils (x10^9/L)</td>
<td>4.62 ± 2.37</td>
<td>3.02 ± 1.29</td>
<td>4.27 ± 2.60</td>
<td>2.5 – 12.5</td>
</tr>
<tr>
<td>Lymphocytes (x10^9/L)</td>
<td>2.59 ± 1.57</td>
<td>2.01 ± 1.23</td>
<td>2.05 ± 0.85</td>
<td>1.5 – 7.0</td>
</tr>
<tr>
<td>Monocytes (x10^9/L)</td>
<td>0.29 ± 0.18</td>
<td>0.19 ± 0.13</td>
<td>0.26 ± 0.22</td>
<td>0 – 0.85</td>
</tr>
<tr>
<td>Eosinophils (x10^9/L)</td>
<td>0.49 ± 0.30</td>
<td>0.52 ± 0.29</td>
<td>0.49 ± 0.46</td>
<td>0 – 1.5</td>
</tr>
<tr>
<td>Basophils (x10^9/L)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>Rare</td>
</tr>
<tr>
<td>TPP (g/dL)</td>
<td>7.62 ± 0.58</td>
<td>7.72 ± 0.53</td>
<td>7.90 ± 0.57</td>
<td>6 – 8</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
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<td>------------------------</td>
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<td>--------------------</td>
</tr>
<tr>
<td>Icterus index (U)</td>
<td>2 ± 0</td>
<td>2 ± 0</td>
<td>2 ± 0</td>
<td>0 – 5</td>
</tr>
<tr>
<td>PLT (10⁹/L)</td>
<td>348.0 ± 112.0</td>
<td>413.8 ± 197.2</td>
<td>319.5 ± 123.1</td>
<td>300 – 800</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>12.97 ± 1.26</td>
<td>13.12 ± 1.91</td>
<td>12.66 ± 1.25</td>
<td>12 – 17</td>
</tr>
<tr>
<td><strong>Biochemical profile</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.71 ± 0.21</td>
<td>2.86 ± 0.35</td>
<td>2.88 ± 0.35</td>
<td>2.1 – 3.3^1</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>30.5 ± 12.5</td>
<td>43.5 ± 20.4</td>
<td>37.1 ± 12.5</td>
<td>25 – 93^1</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>62.7 ± 39.9</td>
<td>62.4 ± 39.3</td>
<td>49.5 ± 33.9</td>
<td>6 – 83^1</td>
</tr>
<tr>
<td>Amylase (IU/L)</td>
<td>2472 ± 805</td>
<td>2062 ± 474</td>
<td>2340 ± 851</td>
<td></td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>52.4 ± 22.7</td>
<td>49.0 ± 19.3</td>
<td>44.0 ± 8.6</td>
<td>26 – 43^1</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.6 ± 1.05</td>
<td>9.4 ± 0.94</td>
<td>9.8 ± 0.83</td>
<td>6.2 – 10.2^1</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.37 ± 0.36</td>
<td>1.34 ± 0.26</td>
<td>1.47 ± 0.39</td>
<td>0.8 – 1.8^1</td>
</tr>
<tr>
<td>Fructosamine (µmol/L)</td>
<td>220.3 ± 40.5</td>
<td>238.6 ± 39.9</td>
<td>212.1 ± 39.4</td>
<td>146 – 271^2</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>0.51 ± 0.53 ab</td>
<td>0.86 ± 0.45 b</td>
<td>0.40 ± 0.51 a</td>
<td>1.3 – 5.1^1</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>4.10 ± 0.75</td>
<td>4.09 ± 0.55</td>
<td>4.21 ± 0.74</td>
<td>2.6 – 5.1^1</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>90.3 ± 18.0</td>
<td>95.6 ± 22.9</td>
<td>92.0 ± 24.5</td>
<td>73 – 134^1</td>
</tr>
<tr>
<td>HDL Cholesterol (mg/dL)</td>
<td>58.8 ± 15.7 b</td>
<td>63.3 ± 18.2 ab</td>
<td>74.0 ± 14.8 a</td>
<td></td>
</tr>
<tr>
<td>Lipase (IU/L)</td>
<td>13.9 ± 4.9</td>
<td>13.8 ± 5.5</td>
<td>14.7 ± 3.9</td>
<td>0 – 83^1</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>5.5 ± 1.46</td>
<td>4.9 ± 1.01</td>
<td>4.5 ± 0.84</td>
<td>4.5 – 8.1^1</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>119.9 ± 31.3</td>
<td>138.8 ± 41.8</td>
<td>118.2 ± 28.9</td>
<td>95 – 130^1</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>6.81 ± 0.77</td>
<td>6.94 ± 0.71</td>
<td>7.09 ± 0.83</td>
<td>5.4 – 7.8^1</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>48.5 ± 17.3 b</td>
<td>63.0 ± 31.5 ab</td>
<td>74.5 ± 27.0 a</td>
<td>10 – 114^1</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>56.7 ± 11.7</td>
<td>55.2 ± 8.6</td>
<td>57.0 ± 9.9</td>
<td>42 – 64^1</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>0.38 ± 0.13</td>
<td>0.47 ± 0.18</td>
<td>0.41 ± 0.17</td>
<td>0 – 1.0^1</td>
</tr>
</tbody>
</table>

Different letters on the same line indicate a statistically significant difference (p<0.05).
Reference intervals for the feline species: Hematology: Rizzi et al. (2010); Biochemistry 1Kaneko et al. (2008) and 2Thoresen and Bredal (1995).

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BSC, body condition score; GGT, gamma glutamiltransferase; HCT, hematocrit; HDL, high density lipoprotein; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; PLT, platelet count; RBC, red blood cell count; RDW, red blood cell distribution width; TPP, total plasma protein; WBC, white blood cell count.

3.2. Oxidative stress parameters

Obese cats showed higher TAC by the ABTS (Figure 1C), ABTS+HRP (Figure 1D), CUPRAC (Figure 1E) and FRAP (Figure 1F) methods than the control group. Overweight cats presented only higher TAC-ABTS+HRP (Figure 1D) and TAC-FRAP (Figure 1F) than the control group and presented similar levels of TAC-ABTS (Figure 1C) and TAC-CUPRAC (Figure 1E). No differences were observed among the groups with respect to the antioxidants albumin (Figure 1A) and uric acid (Figure 1B), TOC (Figure 1G) and to lipid peroxidation (Figure 1H).

4. Discussion

Studies evaluating laboratory changes in different stages of feline obesity are scanty. We observed increased TAC by four different methods, with no alteration in TOC and lipid peroxidation in obese cats. Moreover, changes in CBC such as increased MCV and RDW and in biochemical parameters such as increased triglycerides and HDL cholesterol levels, as well as decreased GGT activity, were also observed in different stages of obesity. In this regard, the current study sheds greater light on the pathophysiological mechanisms of feline obesity.

The average age of obese cats in this study was 6 years. Several authors have linked obesity to increasing age (Burkholder and Toll 1998; Colliard et al. 2009; Courcier et al. 2010). Laflamme (2012), Oh (2011) and Diez and Nguyen (2008) state that cats between 5 and 10 years old are more prone to obesity, and that this risk increases greatly from the age of 10 years onwards. Thus, we show that middle-aged cats start out overweight and become obese.

All the animals of the obese group were neutered and this group was predominantly composed of males. Previous studies have demonstrated that neutered male cats are more prone to obesity (Diez and Nguyen 2008; Cave et al. 2012; Rowe et al. 2015). Male cats are approximately 13 times more likely to develop obesity than females, and if neutered, that chance increases to 15 times (Robertson 1999; Russell et al. 2000; Lund et al. 2005). In dogs, obesity is usually more common among neutered females, as males have a higher resting metabolic rate than females (Burkholder and Toll 1998; German 2006; Diez and Nguyen 2008; Kil and Swanson 2010; Courcier et al. 2010).
Obese cats showed increased MCV and RDW, showing not only an increase in the size of red blood cells but also greater variation in cell size. The lifespan of erythrocytes in feline species is shorter than in other species (Christian 2010; Tasker 2012; Korman et al. 2013; Lalor et al. 2014); hence, we hypothesize that the removal of senescent erythrocytes in obese cats is even faster. This means there is an increase in the replacement of young red blood cells, leading to an increase in MCV and RDW. However, further studies are needed to expand our understanding of the results reported here, since these changes are discrete and are insufficient to exceed the reference range for the species.

As for the WBC count, no changes were observed in the population of blood leukocytes. Previous studies have shown that obesity leads to a pro-inflammatory state with increased inflammatory cytokines in humans (Nemet et al. 2005; Zaldivar et al. 2006), dogs (Radakovich et al. 2017) and cats (Tanner et al. 2007), and changes in WBC, such as increased neutrophils, monocytes and lymphocytes in obese children (Nemet et al. 2005; Zaldivar et al. 2006) and obese dogs due to increased levels of neutrophils and monocytes (Radakovich et al. 2017).

As for lipid metabolism, obese cats showed higher levels of triglycerides, as described in the literature of different species (Vasan 2003; Alberti et al. 2006; Hoenig 2006; Mori et al. 2012). Lipid alterations are relatively common in veterinary medicine, especially in obese animals, often as a result of excessive intake of high calorie diets containing large amounts of carbohydrates and lipids (Barrie et al. 1993; Chikamune et al. 1995; Bailhache et al. 2003; Johnson 2005; Jeusette et al. 2005; Hoenig 2006). However, in the present study, all the owners reported feeding their cats solely with commercial cat food, which suggests that this change in lipid metabolism is not due to increased lipid intake. In addition, total cholesterol levels did not change as a function of obesity levels, corroborating the findings of previous studies that found no change in cholesterol levels in feline obesity (de Freitas et al. 2017; Aguiar et al. 2018). Higher HDL cholesterol levels in obese cats have been previously demonstrated (de Freitas et al. 2017). Unlike humans, cats have predominantly circulating HDL lipoprotein (Bauer 1996). Hoenig et al. (2003) emphasizes that obese cats have high levels of HDL cholesterol, which suggests the presence of cholesteryl ester transfer protein deficiency.

Obese cats showed reduced GGT activity, although it still remained within the reference range for the species. Increased GGT activity has been reported in obese humans, which is directly related to metabolic syndrome and its comorbidities (Saely et al. 2008). However, the reasons that led to the reduction in the activity of this enzyme in feline obesity are still unknown, and further studies are needed to clarify this issue.

Few earlier studies have evaluated oxidative stress in feline obesity. All the methods employed in this study indicated that obese cats had higher TAC, although TOC and lipid peroxidation remained unchanged. On the other hand, overweight cats showed an increase in TAC only by the ABTS+HRP and FRAP methods. Thus, oxidative stress seems to be related to animal weight, since TAC was higher in obese and overweight cats, depending on the method of analysis. Other authors have observed protein oxidation and lipid peroxidation in cats that had obesity induced and maintained for an 8-weeks period,
as well as an increase in inflammatory cytokines, indicating an inflammatory condition induced by obesity (Tanner et al. 2007). In our study, the inclusion criterion was that the animals had to have been obese for at least one year, which means that they had already experienced obesity for a long period, making the process more chronic. Therefore, it was assumed that the animals were already adapted to obesity and that the TAC increased in order to fight oxidative damage during this condition.

The TAC evaluation methods showed differences, with overweight cats showing an increase in TAC only by the ABTS+HRP and FRAP methods and obese cats an increase in TAC by the four evaluated methods. A comparison of overweight and obese cats showed a difference only by the CUPRAC method, with obese cats showing higher CUPRAC than overweight animals. The differences observed in the TAC can most likely be attributed to the biochemical assays used in each method. The FRAP method primarily assesses uric acid, bilirubin, vitamin C and polyphenols (Benzie and Strain 1996). The CUPRAC method predominantly assesses non-enzymatic antioxidants from the thiol group (Rubio et al. 2016b), while the ABTS method assesses protein-based antioxidants such as glutathione and albumin (Erel 2004). The difference found between the methods may be related to antioxidant compounds not evaluated in the present study, since albumin and uric acid did not differ between groups. It is known that about 60% of TAC in human plasma is composed of uric acid (Benzie and Strain 1996) and the increase in this analyte has already been reported in human obesity (Abdul-Majeed 2009), in rodents (Tsushima et al. 2013) and in obese dogs, although in the latter species this increase was not enough to prevent systemic oxidative stress (Bosco et al. 2018). Thus, chronic feline obesity induces increased TAC by substrates other than uric acid and albumin.

In the obesity levels evaluated in this study, no evidence was found of change in TOC and lipid peroxidation. This may be explained, at least partially, by the increase in TAC, which could contribute to the inactivation of oxidizing compounds in feline obesity, preventing lipid peroxidation. A previous study demonstrated that obese dogs underwent oxidative stress as a result of increased TOC and lipid peroxidation, while TAC remained unchanged (Bosco et al. 2018). In studies on human obesity by Cătoi et al. (2013) and Pirgon et al. (2013), an increase detected in the oxidative stress index was attributed to increased TOC and reduced TAC. In rodents, a decrease in TAC was also observed among obese animals (Beltowski et al. 2000; Furukawa et al. 2004). In addition to obesity, oxidative stress has been described in pathologies such as chronic kidney disease (CKD) in dogs (Silva et al. 2013), in cats (Keegan and Webb 2010) and in humans (Rysz et al. 2004), and in feline infectious peritonitis (Pedersen 2014). In CKD in felines, an increase was detected in reduced glutathione: oxidized glutathione (GSH:GSSG) ratios. This suggests the activation of antioxidants to fight ROS, despite the significantly lower TAC of sick animals, precisely because they are unable to maintain a balance between oxidizing substances and antioxidants (Keegan and Webb 2010). Increased lipid peroxidation has already been described in human obesity (Ozata et al. 2002; Konukoglu et al. 2006) and in rodents (Beltowski et al. 2000; Furukawa et al. 2004). In canine obesity, the increase in TBARS was not detected in animals on a short-term fattening diet (van de Velde et al. 2012). Thus, lipid peroxidation and TOC do not seem to be suitable markers for the evaluation of oxidative stress in natural feline obesity.
It is essential for further research to evaluate the role of oxidative stress and inflammation in feline obesity, given the paucity of studies with this species in the obese condition and the fact that most of the data found are extrapolated from other species. However, since felines have nutritional, metabolic, physiological and pathological characteristics that render many of these extrapolations meaningless, further investigations into this subject are needed to understand its clinical implications in feline obesity.

5. Conclusions

Natural obesity and overweight in felines alter their hematological, biochemical and oxidative stress parameters. Furthermore, the best way to assess oxidative stress in feline obesity is by determining the TAC, preferably using different methods.

Declarations

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Statement of Animal Ethics

The experiment was conducted according to the ethical principles of the Animal Research Ethics Committee of the University Center of the Integrated Faculties of Ourinhos (Protocol no. 007/2019). The participation of each feline was authorized by its owner, who signed a free and informed consent form.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

The study conception and design were performed by Beatriz Perez Floriano, Marcel Gambin Marques and Breno Fernando Martins de Almeida. Animal selection, material preparation and sample collection were
performed by Tainara de Oliveira Martins, Rebecca Cápera Ramos, Geovana Possidonio, Vinicius Aquiles Zamboni, Marcel Gambin Marques and Breno Fernando Martins de Almeida. Laboratory analysis were performed by Tainara de Oliveira Martins, Rebecca Cápera Ramos, Maria Rachel Melo Bosculo, Paula Lima Oliveira, Leticia Ramos Costa, Vinicius Aquiles Zamboni and Breno Fernando Martins de Almeida. The first draft of the manuscript was written by Tainara de Oliveira Martins and Rebecca Cápera Ramos and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The experiment was conducted according to the ethical principles of the Animal Research Ethics Committee of the University Center of the Integrated Faculties of Ourinhos (Protocol no. 007/2019).

Consent to participate

The participation of each feline was authorized by its owner, who signed a free and informed consent form.

References


Figures
Figure 1

Oxidative stress markers albumin (A), uric acid (B), total antioxidant capacity (TAC) determined by ABTS cation inhibition method alone (ABTS, C) or in association with peroxidase (ABTS+HRP, D), cupric reducing antioxidant capacity assay (CUPRAC, E) and by ferric reducing antioxidant power assay (FRAP, F), total oxidant capacity (TOC, G) and lipid peroxidation determined by thiobarbituric acid reactive substances (TBARS, H) in cats with body score condition 5 (Control, n=17), 6/7 (Overweight, n=13) and 8/9 (Obese, n=15) according to Laflamme (1997).