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ORIGINAL ARTICLE

Feline obesity causes hematological and biochemical changes and oxidative stress – a pilot study

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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. Obese cats showed increased mean corpuscular volume (MCV), red cell distribution width (RDW), HDL cholesterol and triglycerides and decreased activity of gamma-glutamyl transferase (GGT) than control cats, although within the reference ranges for the species. 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 than control cats. We conclude that the conditions of natural obesity and overweight in the feline species alter its hematological, biochemical and oxidative stress parameters.

Keywords Complete blood count (CBC) · Reactive oxygen species · Antioxidants · Cats

Introduction

Sedentary lifestyle and inadequate nutrition are undesirable results of the domestication of felines, which commonly leads to obesity, a condition observed in 25 to 35% of cats (Butterwick 2000; Zoran 2009; German 2010). Obesity leads to a predisposition for numerous secondary diseases, such as diabetes mellitus (Biourge et al. 1997; Osto et al. 2013), cardiorespiratory (Chandler 2016), dermatological (Lund et al. 2005) and oral abnormalities (German 2010), lower urinary tract and orthopedic diseases (Öhlund et al. 2018) in addition to hepatic lipidosis (Blanchard et al. 2004) and neoplasms (Chandler et al. 2017).

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). 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; Radakovich et al. 2017; Zaldivar et al. 2006). 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

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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 cats, 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 (Biezus et al. 2017; Shacter et al. 1989). 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.

Materials and methods

Animal selection

The study 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 cat was authorized by its owner, who signed a free and informed consent form.

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

– Control group: 17 adult cats (mean age 4.62 ± 1.70 years), 9 females and 8 males, 15 neutered and 2 unneutered, 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), 8 females and 5 males, all neutered, 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), 6 females and 9 males, all neutered, presenting BCS 8–9 (mean weight 5.89 ± 0.94 kg) for at least one year.

BCS was proposed previously (Laflamme 1997): cats on control group with BCS 5 presented well-proportioned, ribs could be felt with slight fat covering, waist could be seen behind ribs, but was not pronounced and abdominal fat pad was minimal; cats on overweight group presented BCS 6 and 7, ribs were felt with slight excess of fat covering or were not easily felt through moderate fat covering, waist was not easily seen, abdomen may be slight rounding and moderate abdominal fat pad could be seen; cats on obese group presented BCS 8 and 9, ribs could not be felt due to excessive fat covering, waist was absent, abdomen presented obvious rounding with prominent fat pad and fat deposits were also present over lower back area. All estimates were disclosed by the same evaluator. All animals were photographed for a second blind opinion. In case of divergence, a third blind researcher was consulted and the most prevalent classification was considered.

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.

Collection of blood samples and laboratory analysis

The cats were fasted for 12 h, after which 5 mL of blood was drawn by jugular venipuncture into tubes with K_2EDTA (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 min), 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 1 H, Labtest Diagnóstica SA, Lagoa Santa, MG, Brazil) and II (Qualitrol 2 H, 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.

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" target="_blank">www.graphpad.com) and differences were considered significant when $p < 0.05$.

Results

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).

Hematological and biochemical changes still remain within the species reference ranges (Table 1).

Oxidative stress parameters

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

Discussion

Studies evaluating laboratory changes in different stages of feline obesity are scant. We observed increased TAC by four different methods, with no alteration in TOC and lipid

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)

Parameter	Control	Overweight	Obese	Reference interval
Gonadal state (%)				
Neutered	88%	100%	100%	-
Unneutered	12%	0%	0%	-
Sex (%)				
Female	53%	62%	40%	-
Male	47%	38%	60%	-
Age (years)	4.62 ± 1.70	5.04 ± 2.93	6.07 ± 2.88	-
Weight (kg)	3.61 kg ± 0.67	4.5 kg ± 0.78	5.89 kg ± 0.94	-
BCS	5 (100%)	6 (69.2%) 7 (30.8%)	8 (93.3%) 9 (6.7%)	-
Hematological profile				
HCT (%)	43.12 ± 3.42	42.08 ± 3.22	43.20 ± 3.52	24–45
RBC ($x10^{12}$/L)	9.74 ± 0.93	9.77 ± 1.05	9.43 ± 0.79	5.0–10.0
Hemoglobin (g/dL)	13.99 ± 1.00	13.65 ± 0.85	13.96 ± 1.28	8.0–15.0
MCV (fL)	44.39 ± 2.48 ab	43.29 ± 3.52 b	45.94 ± 3.80 a	39–55
MCHC (%)	32.48 ± 1.91	32.53 ± 1.83	32.32 ± 1.47	31–35
RDW (%)	18.17 ± 0.59 b	18.87 ± 0.58 a	18.70 ± 0.60 a	17–21
WBC ($x10^9$/L)	7.96 ± 2.5	5.78 ± 2.0	7.10 ± 2.9	5.5–19.5
Band neutrophils ($x10^9$/L)	0.18 ± 0.04	0.02 ± 0.05	0.006 ± 0.01	0–0.3
Segmented neutrophils ($x10^9$/L)	4.62 ± 2.37	3.02 ± 1.29	4.27 ± 2.60	2.5–12.5
Lymphocytes ($x10^9$/L)	2.59 ± 1.57	2.01 ± 1.23	2.05 ± 0.85	1.5–7.0
Monocytes ($x10^9$/L)	0.29 ± 0.18	0.19 ± 0.13	0.26 ± 0.22	0–0.85
Eosinophils ($x10^9$/L)	0.49 ± 0.30	0.52 ± 0.29	0.49 ± 0.46	0–1.5
Basophils ($x10^9$/L)	0 ± 0	0 ± 0	0 ± 0	Rare
TPP (g/dL)	7.62 ± 0.58	7.72 ± 0.53	7.90 ± 0.57	6–8
Icterus index (U)	2 ± 0	2 ± 0	2 ± 0	0–5
PLT (10^9/L)	348.0 ± 112.0	413.8 ± 197.2	319.5 ± 123.1	300–800
MPV (fL)	12.97 ± 1.26	13.12 ± 1.91	12.66 ± 1.25	12–17
Biochemical profile				
Albumin (g/dL)	2.71 ± 0.21	2.86 ± 0.35	2.88 ± 0.35	2.1–3.3 [1]
ALP (IU/L)	30.5 ± 12.5	43.5 ± 20.4	37.1 ± 12.5	25–93 [1]
ALT (IU/L)	62.7 ± 39.9	62.4 ± 39.3	49.5 ± 33.9	6–83 [1]
Amylase (IU/L)	2472 ± 805	2062 ± 474	2340 ± 851	
AST (IU/L)	52.4 ± 22.7	49.0 ± 19.3	44.0 ± 8.6	26–43 [1]
Calcium (mg/dL)	9.6 ± 1.05	9.4 ± 0.94	9.8 ± 0.83	6.2–10.2 [1]
Creatinine (mg/dL)	1.37 ± 0.36	1.34 ± 0.26	1.47 ± 0.39	0.8–1.8 [1]
Fructosamine (µmol/L)	220.3 ± 40.5	238.6 ± 39.9	212.1 ± 39.4	146–271 [2]
GGT (IU/L)	0.51 ± 0.53 ab	0.86 ± 0.45 b	0.40 ± 0.51 a	1.3–5.1 [1]
Globulin (g/dL)	4.10 ± 0.75	4.09 ± 0.55	4.21 ± 0.74	2.6–5.1 [1]
Glucose (mg/dL)	90.3 ± 18.0	95.6 ± 22.9	92.0 ± 24.5	73–134 [1]
HDL Cholesterol (mg/dL)	58.8 ± 15.7 b	63.3 ± 18.2 ab	74.0 ± 14.8 a	
Lipase (IU/L)	13.9 ± 4.9	13.8 ± 5.5	14.7 ± 3.9	0–83 [1]
Phosphate (mg/dL)	5.5 ± 1.46	4.9 ± 1.01	4.5 ± 0.84	4.5–8.1 [1]
Total cholesterol (mg/dL)	119.9 ± 31.3	138.8 ± 41.8	118.2 ± 28.9	95–130 [1]
Total protein (g/dL)	6.81 ± 0.77	6.94 ± 0.71	7.09 ± 0.83	5.4–7.8 [1]

Table 1 (continued)

Parameter	Control	Overweight	Obese	Reference interval
Triglycerides (mg/dL)	48.5 ± 17.3 b	63.0 ± 31.5 ab	74.5 ± 27.0 a	10–114 [1]
Urea (mg/dL)	56.7 ± 11.7	55.2 ± 8.6	57.0 ± 9.9	42–64 [1]
Uric acid (mg/dL)	0.38 ± 0.13	0.47 ± 0.18	0.41 ± 0.17	0–1.0 [1]

Different letters on the same line indicate a statistically significant difference ($p < 0.05$) according to Kruskal-Wallis and Dunn's multiple comparisons test (RDW and GGT) or ANOVA with Tukey's multiple comparisons test (MCV, HDL Cholesterol and Triglycerides)

Reference intervals for the feline species: Hematology: Rizzi et al. (2010); Biochemistry [1]Kaneko et al. (2008) and [2]Thoresen 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

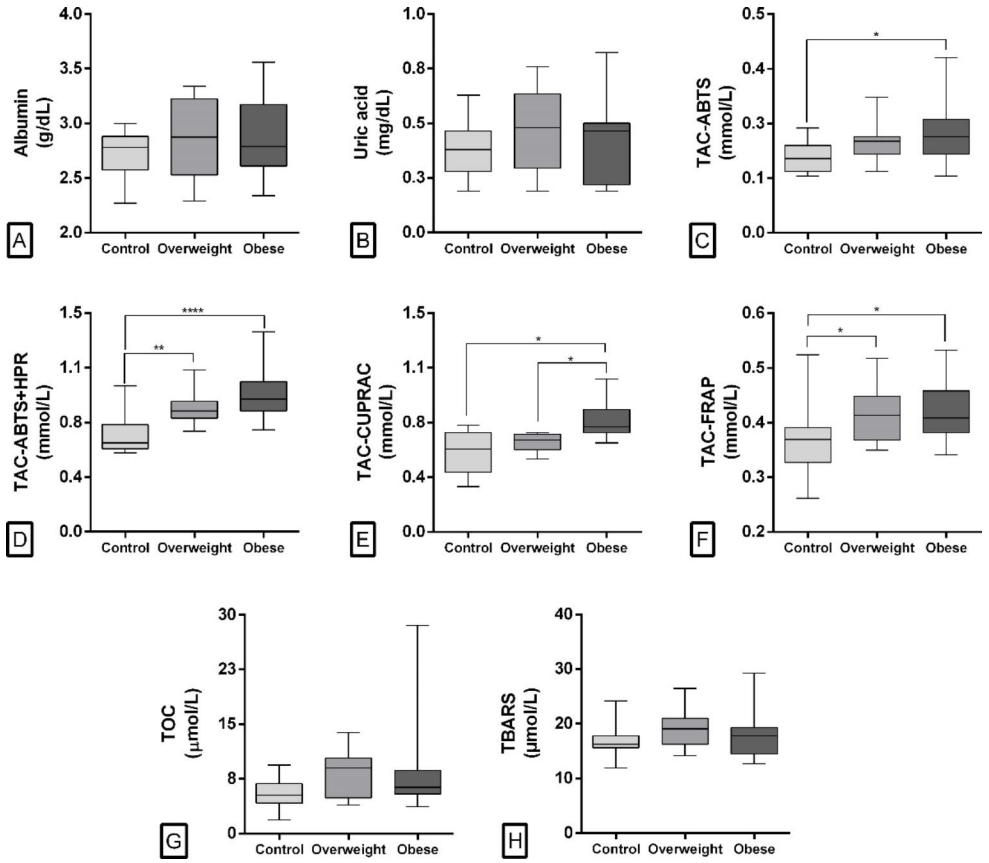

Fig. 1 Oxidative stress markers albumin (A), uric acid (B), total antioxidant capacity (TAC) determined by ABTS cation inhibition method alone (ABTS, C) or in association with peroxidase (ABTS + HRP, D), cupric reducing antioxidant capacity assay (CUPRAC, E) and by ferric reducing antioxidant power assay (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). Bars indicate minimum and maximum values and boxes represent the first and third quartiles. Statistically significant difference is indicated by * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$) or **** ($P < 0.0001$) according to Kruskal-Wallis and Dunn's multiple comparisons test (TAC-CUPRAC) or ANOVA with Tukey's multiple comparisons test (TAC-ABTS, TAC-ABTS + HRP and TAC-FRAP)

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 (Colliard et al. 2009; Courcier et al. 2010; Laflamme 2012) states 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 as soon as 3 years old 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 (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 (Lund et al. 2005; Robertson 1999; Russell et al. 2000). In dogs, obesity is usually more common among neutered females, as males have a higher resting metabolic rate than females (Courcier et al. 2010; German 2006; Kil and Swanson 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, although within the reference ranges for the feline species (Rizzi et al. 2010). To date, we have not found studies that have related the increased RDW with obesity in cats. In humans, the relationship between obesity with metabolic syndrome (MS) and RDW alterations has been explored (Tsuda et al. 2001; Fujita et al. 2013; Laufer Perl et al. 2015; Vayá et al. 2015; Farah and Khamisy-Farah 2015; Yan et al. 2019). Sanchez-Chaparro et al. (2010) observed that the inflammatory state in human MS impaired erythrocyte maturation, leading to anisocytosis and increased RDW. Farah and Khamisy-Farah et al. (2015) reported a positive correlation between RDW and MS, and RDW increased with the severity of MS. Fujita et al. (2013) observed higher RDW in overweight adolescents than in normal-weight, with no significant difference in the mice experimental model. Other study reported that high RDW was associated with increased risk of MS and long-term mortality (Laufer Perl et al. 2015). It is possible that, in humans, changes in lipid, glucose and insulin concentrations lead to reduced RBC deformability through the impact on the erythrocyte membrane, which could predispose to a higher erythrocyte replacement rate (Tsuda et al. 2001; Vayá et al. 2015; Yan et al. 2019). Furthermore, changes in these constituents would cause greater morphologic alterations (shape/volume), leading to an increase in RDW (Vayá et al. 2015). 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 cats.

As for the WBC count, considering the groups examined in this pilot study 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 still within the reference ranges for the feline species (Kaneko et al. 2008), as described in the literature of different species (Alberti et al. 2006; Hoenig 2006; Mori et al. 2012; Vasan 2003). 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 (Bailhache et al. 2003; Barrie et al. 1993; Chikamune et al. 1995; Jeusette et al. 2005; Johnson 2005; Hoenig 2006). However, in the present study, all the owners reported feeding their cats solely with commercial cat food in the amount indicated by each manufacturer. In addition, considering the cats selected in our study there was no significant change of total cholesterol levels between obesity levels, corroborating the findings of previous studies that found no change in cholesterol levels in feline obesity (Aguiar et al. 2018; de Freitas et al. 2017). 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. 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 in this pilot study. On the other hand, overweight cats showed an increase in TAC only by the ABTS + HRP and FRAP methods. Thus, oxidative stress markers varied according to animal weight, with increased TAC concentration 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 (Matsuura et al. 1998), 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 (Bełtowski 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 (Almeida et al. 2013; 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 cats, 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 (Konukoglu et al. 2006; Ozata et al. 2002) and in rodents (Bełtowski 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 were not suitable markers for the evaluation of oxidative stress in natural feline obesity in this experiment.

The main limitations of the present study include the low number of animals used in each experimental group, the impossibility of standardizing the food of the selected cats and the innate feline characteristic of showing high excitability and not tolerating prolonged manipulation, which yielded a small sample. Furthermore, the influence that excitation can exert on oxidative stress parameters is not known in the feline species. It is essential for further research to evaluate the role of oxidative stress, inflammation and metabolic syndrome 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 cats 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.

Conclusions

Natural obesity and overweight in cats alter their hematological and biochemical parameters, even if inside the reference interval of cats, which occurs together with alteration of oxidative stress parameters. Furthermore, the best way to assess oxidative stress in feline obesity is by determining the TAC, preferably using multiple methods.

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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

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

Declarations

Conflict of interest The authors declare no conflict of interest.

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

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.

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.

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