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Concurrent hyperadrenocorticism and diabetes mellitus in dogs

D.D. Miceli[a,b], O.P. Pignataro[b], V.A. Castillo[a,*]

[a] Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Cátedra de Clínica Médica de Pequeños Animales y Hospital Escuela de Medicina Veterinaria, Unidad de Endocrinología, Av. Chorroarín 280, Buenos Aires CP 1427, Argentina

[b] Laboratorio de Endocrinología Molecular y Transducción de Señales, Instituto de Biología y Medicina Experimental – CONICET, Vuelta de Obligado 2490, Buenos Aires CP 1428, Argentina

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ABSTRACT

Hyperadrenocorticism (HAC) and diabetes mellitus (DM) are two diseases that can occur concurrently in dogs. The objective of this study was to evaluate the coexistence of HAC and DM, and the risk factors involved that could contribute to the development of DM in dogs with HAC. A total of 235 dogs with HAC were studied and, according to their fasting glycemia, they were divided into three groups: < 5.6 mmol/L, between 5.6 and 10.08 mmol/L and > 10.08 mmol/L. The following parameters were evaluated: age, gender, cause of HAC, body condition, glycemia, total cholesterol, triglycerides, urinary cortisol:creatinin ratio (UCCR) and survival time. A 13.61% concurrence of HAC and DM was observed. Dogs with a fasting glycemia > 5.6 mmol/L, with dislipemia, with Pituitary-Dependent Hyperadrenocorticism, UCCR > 100×10^{-6} and non-castrated females showed a higher risk of developing DM. The development of DM in dogs with HAC reduces the survival time.

1. Introduction

Hyperadrenocorticism (HAC) and diabetes mellitus (DM) are two well-documented diseases in dogs (Kooistra and Galac, 2012; Nelson and Reusch, 2014). Both are expressed with greater frequency in middle aged to elderly dogs and share some clinical signs such as polydypsia, polyuria and polyphagia, which can make diagnosis and eventual treatment difficult. Although both diseases can appear independently, they can also be manifested concurrently (Hess et al., 2000; Nichols, 1997). Not only has this condition been reported in dogs, but also in cats, horses, monkeys and humans (Fey et al., 1998; Schäcke et al., 2002; Valentin et al., 2014; Wilkinson et al., 1999).

Various studies have been carried out on the association between HAC and DM in dogs and on the response to different treatments (Blaxter and Gruffydd-Jones, 1990; McLauchlan et al., 2010; Peterson et al., 1981). Nevertheless, it is a complex subject that requires more research to understand the nature of this coexistence to best be able to prevent or manage it.

Although it is well established that HAC predisposes to DM, for the authors' knowledge, to date there are no reports describing risk factors (such as age, gender, cause of HAC, body condition, urinary cortisol:creatinin ratio (UCCR), glycemia, total cholesterol, triglycerides), that could predispose to DM in dogs with HAC, nor any reports that consider the importance of controlling fasting hyperglycemia, lower than 10.08 mmol/L (180 mg/dL), that could precede clinical DM. In a previous study (Miceli et al., 2012), we observed that dogs with HAC and fasting blood glucose levels > 5.88 mmol/L (105 mg/dL) at the time of diagnosis, have a higher risk of developing DM than dogs with HAC and blood glucose levels lower than 5.88 mmol/L. In addition, we observed that the preventive use of insulin detemir in dogs with HAC and blood glucose levels > 5.88 mmol/L contribute to control the metabolic disorders of HAC and to prevent the development of DM.

Dogs with HAC can show a tendency to high fasting blood glucose levels (Elliot et al., 1997) and lipid disorders (Jericó et al., 2009), that in some cases progress toward DM. Cortisol regulates carbohydrate and lipid metabolism, countering many of the functions of insulin (Andrews and Walker, 1999). Hyperadrenocorticism, either iatrogenic or spontaneous (such as HAC), induces a state of insulin resistance, a condition in which the peripheral tissues have a lower response to insulin (Miceli et al., 2014; Schäcke et al., 2002). Basically it favors an increase in hepatic gluconeogenesis and a decrease in the peripheral use of glucose, all of which increases blood glucose levels (Peterson et al., 1984; Vegiopoulos and Herzig, 2007). The antagonism of cortisol to insulin is so potent that glucocorticoid effects are referred to as “diabetogenic” (Van Raalte et al., 2009).

The objective of this study was to examine the concurrence between HAC and DM in a case series and evaluate the risk factors that could predispose dogs with HAC to concurrently develop DM.

* Corresponding author.
E-mail address: vcastill@fvet.uba.ar (V.A. Castillo).

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2. Materials and methods

2.1. Population under study

Between January 2011 and December 2015, 235 dogs with HAC, 32 of which presented with concurrent DM, were included in a longitudinal prospective study at the Endocrinology Unit of our Institution.

2.2. HAC and DM diagnosis and treatment

The diagnosis of Pituitary-Dependent Hyperadrenocorticism (PDH) was based on clinical signs and increased UCCR in two consecutive morning urine samples collected at home. After collection of the second urine sample, 3 doses of dexamethasone (0.1 mg/kg) were administered PO at 8-hour intervals, and the next morning a third urine sample was collected, according to Kooistra and Galac (2012). The diagnosis of Adrenal-Dependent Hyperadrenocorticism (ADH) was based on clinical signs, increased UCCRs, resistance to suppression by a high dose of dexamethasone. ADH was differentiated from non-suppressible forms of PDH by measuring the plasma ACTH concentration. The diagnosis was further supported by visualization of the adrenal glands by ultrasonography and pituitary gland using nuclear magnetic resonance imaging.

In dogs suspected of HAC, and with previously diagnosed DM, studies to confirm HAC were not carried out until the DM was controlled (Kaplan et al., 1995), blood glucose levels were stable and within the therapeutic aim and were without ketone bodies in blood or urine. All doubtful cases in which diagnosis of HAC was not conclusive (Ristic et al., 2002) and all dogs that had another concurrent disease (systemic infection, neoplasia –except pituitary or adrenal neoplasia- or any other sever illness) that was not HAC or DM were discarded.

All 235 dogs received routine HAC treatment. In the ADH cases, adrenalectomy was carried out while in the PDH cases, a frequent therapy used in Argentina was administered: combined retinoic acid and cabergoline, 2 mg/kg every 24 h and 0.07 mg/kg/week, divided into doses every 48 h, respectively (Castillo et al., 2006, 2008).

Diagnosis of DM was carried out based on the typical clinical signs of the disease, the persistent high fasting blood glucose levels and glucosuria (Nelson and Reusch, 2014). All dogs with DM were treated with insulin (porcine lente insulin or detemir) twice daily (doses varied according to the requirements of each animal) and commercial food for diabetic dogs (Monroe et al., 2005; Sako et al., 2011).

2.3. Groups division

According to the fasting blood glucose levels measured at the first consultation, dogs were distributed into 3 groups (see Table 1):

- *Group A*: Dogs with HAC and fasting blood glucose levels < 5.6 mmol/L (< 100 mg/dL) (n = 146).
- *Group B*: Dogs with HAC and fasting blood glucose levels > 5.6 mmol/L and < 10.08 mmol/L (100–180 mg/dL) (n = 70) (Tvarijonaviciute et al., 2012). These dogs did not receive insulin or any oral treatment to lower blood glucose levels.
- *Group C*: Dogs with HAC and fasting blood glucose levels > 10.08 mmol/L (> 180 mg/dL), i.e., dogs with DM (n = 19).

2.4. Hormone and biochemical determinations

Glucose, triglycerides, total blood cholesterol and UCCR were analyzed. Blood samples to measure the different parameters were taken at the same time and with 12 h fast of solid food. Blood glucose was assessed using an automated laboratory method (Metrolab Autoanalizer Merck) according to the manufacturer's instructions. Blood for glucose analysis was collected in glass tubes with sodium fluoride and EDTA as the anticoagulant (Anticoagulant G; Wiener Laboratory, Argentina).

Table 1
Comparison of age, gender, cause of HAC, body condition and breed between the different groups of dogs with HAC, distributed according to their fasting glucose levels at the first consultation (Group A < 5.6 mmol/L, Group B 5.6–10.08 mmol/L, Group C > 10.08 mmol/L).

	Group A (n = 146)	Group B (n = 70)	Group C (n = 19)
Age (years)	10 (4–16)	9.75 (4–15)	9.4 (2–15)
Gender: Female:			
Castrated	61/101 (60.4%)	21/47 (44.68%)	4/11 (36.36%)
Non-castrated	40/101 (39.6%)	26/47 (55.32%)	7/11 (63.64%)
Male:			
Castrated	10/45 (22.22%)	10/23 (43.48%)	2/8 (25%)
Non-castrated	35/45 (77.78%)	13/23 (56.52%)	6/8 (75%)
Cause of HAC: PDH	117/146 (80.13%)	59/70 (84.28%)	17/19 (89.47%)
ADH	29/146 (19.87%)	11/70 (15.72%)	2/19 (10.53%)
Body condition:			
Normal weight	56/146 (38.35%)	12/70 (17.14%)	8/19 (42.1%)
Overweight	52/146 (35.61%)	33/70 (47.14%)	7/19 (36.84%)
Obese	38/146 (26%)	25/70 (35.71%)	4/19 (21.05%)
Breed:			
Crossbreeds	43/146 (29.45%)	21/70 (30%)	4/19 (21.05%)
Purebreds	103/146 (70.55%)	49/70 (70%)	15/19 (78.95%)

Age is expressed as the median and range. The rest of the values are expressed as a percentage.

Triglyceride and total cholesterol were collected in another tube and measured by a laboratory-automated method (Metrolab Autoanalizer Merck), according to the manufacturer's instructions.

UCCR was measured by means of radioimmunoassay (RIA) using a commercial kit (DPC Corporation, San Diego, California, USA). The inter- and intra-assay coefficients of variation for cortisol were 8% and 5%, respectively. Creatinine was measured by an automated method (Metrolab Autoanalizer Merck, Germany) according to the manufacturer's indications.

2.5. Body condition

Dogs were classified using the 9 points body condition score (BCS) (Jeusette et al., 2010; Laflamme, 1997). We considered dogs with 5/9 as “normal weight”, those with 6/9 or 7/9 as “overweight” and those with a score of 8/9 or 9/9 as “obese”.

2.6. Statistical analysis

Statistical analysis (GraphPad Prism 6, USA) was carried out using the following non parametric tests: Mann-Whitney test and an ANOVA-Kruskal-Wallis followed by Dunn's test for multiple comparisons of medians, according to whichever was appropriate. By means of Chi-square test and two-way ANOVA the homogeneity of the groups (effect of breeds, gender and gonadal status) were first analyzed. To evaluate the statistical association between the cut-off point determined for each variables and the progress or not to DM, a contingency table was performed by the Fisher's exact test. Subsequently the Relative Risk (RR) and Positive Predictive Value (PPV) were calculated. Survival time was analyzed by the Long-Rank (Mantel-Cox) test, in which each step represents the death of the individual cases. The hazard ratio (HR) was calculated by Mantel-Haenszel method. The significance level was set at $P < 0.05$, and results are expressed as medians and interquartile ranges.

2.7. Ethical approval

The Ethics Committee of the Faculty of Veterinary Science (CICUAL) and the Office of Science at the University of Buenos Aires approved the present study, according to the laws on experimentation in animals in Argentina and World Health Organization recommendations. Signed consent was obtained from the dogs' owners for participation in the

present Project.

3. Results

3.1. Age, gender, breed, causes of HAC, body condition

Group A: median age was 10 years (range of 4 to 16 years); 101/146 (69.17%) were female (39.6% non-castrated and 60.4% castrated) and 45/146 (30.82%) were male (77.77% non-castrated and 22.22% castrated). *Breed*: 43 crossbreed; 36 poodles; 7 Labrador Retriever; 6 Miniature Schnauzer; 5 Boxer, Dachshund; 4 Bichón Frisé, Beagle, Pitbull Terrier; 3 Pekinese, West Highland Terrier, 2 English Cocker Spaniel, German Shepherd, Breton, Shar pei, Shitzu, Siberian Husky, Yorkshire Terrier; 1 Akita, Doberman, Lasha apso, Pincher, Rottweiler, Staffordshire Bull Terrier, Maltese, Golden Retriever, Jack Russell, Belgian Sheepdog, Scottish Terrier, Standard Schnauzer. *Causes of HAC:* 117/146 (80.13%) of the dogs had PDH, while for 29/146 (19.86%) had ADH. *Body condition:* 56/146 normal weight (38.35%), 52/146 overweight (35.61%) and 38/146 obese (26.02%) (Table 1).

Group B: median age was 9.75 years (range between 4 and 15 years), 47/70 (67.14%) were female (55.31% non-castrated and 44.68% castrated) and 23/70 (32.85%) were male (56.52% non-castrated and 43.48% castrated). *Breed:* 21 crossbreed; 17 poodles; 4 Beagle, Maltese; 3 Boxer, Yorkshire Terrier; 2 Breton, Dalmatian, Fox Terrier, Labrador Retriever, Miniature Schnauzer; 1 Basset Hound, English Cocker Spaniel, Golden Retriever, Pincher, Shitzu, Welsh Terrier, Shetland sheepdog, Scottish Terrier. *Causes of HAC:* 59/70 (84.28%) of the dogs had PDH, while 11/70 (15.71%) had ADH. *Body condition:* 12/70 normal weight (17.14%), 33/70 overweight (47.14%) and 25/70 obese (35.71%) (Table 1).

Group C: median age was 9.4 years (range between 2 and 15 years); 11/19 (57.89%) were female (63.63% non-castrated and 36.36% castrated) and 8/19 (42.1%) were male (75% non-castrated and 25% castrated). *Breed:* 10 Poodle; 4 Crossbreed; 2 Beagle; 1 Doberman, Breton, Vizsla. *Causes of HAC:* 17/19 (89.47%) of the dogs had PDH, while 2/19 (10.5%) had ADH. *Body condition:* 8/19 normal weight (42.1%), 7/19 overweight (36.84%) and 4/19 obese (21.05%) (Table 1).

All groups showed a similar proportion of crossbreeds and purebreds dogs, males and females. These proportions were similar to a previous reports in Buenos Aires, Argentina (Gallelli et al., 2009, 2010). There were not interaction or association ($P > 0.05$, both 2-way ANOVA and chi square test) between the three groups and age, breeds and gender, therefore the groups were considered homogeneous.

3.2. Clinical characteristics and diagnostic steps for DM

At the time of the first consultation, 12/32 dogs (37.5%) presented a confirmed diagnosis of concurrent HAC and DM. In all cases, the diagnosis of both diseases was confirmed using the methods previously detailed. Strong suspicion of HAC was based on: the excessive requirements of insulin needed to control blood glucose levels (> 1.5 IU insulin/kg/12 h); alterations of hair coat (alopecia); a prominent abdomen; thin, dry and inelastic skin in the abdominal/inguinal region, exposing the superficial blood vessels. Another common piece of information in non-castrated females was a persistent anestrus (greater than two years).

Likewise, in 7/32 dogs (29.7%) DM developed before HAC (at least in terms of diagnosis and treatment). Nevertheless, in all cases HAC was suspected right from the first consultation. Therefore, after the necessary time to control the DM, tests were carried out to diagnose HAC. Suspicion of HAC was based on: insulin resistance (insulin dose > 1.5 IU insulin/kg/12 h), presence of mild clinical signs of HAC (mild alopecia and prominent abdomen) and persistent anestrus. As HAC progressed in these dogs, prominent abdomen, alterations of hair coat and others dermatological signs became more evident.

Lastly, HAC was diagnosed before DM in 13/32 dogs (40.6%). In the course of two to eleven months following the first consultation, clinical DM became manifest, persistently reaching fasting blood glucose levels > 10.08 mmol/L (180 mg/dL) and showing glucosuria, with a recrudescence of polydipsia, polyuria and polyphagia. In these 13 dogs, median age was 9.6 years (range between 7 and 15 years), 9 were female (7 non-castrated and 2 castrated) and 4 males (3 non-castrated and 1 castrated). *Breed:* 6 Poodle; 3 Crossbreed; 2 Maltese; 1 Fox Terrier, Miniature Schnauzer. A progressive decrease in body weight was observed, reaching the lowest values at the time of DM diagnosis. At the time of the first consultation, in 3/13 dogs fasting blood glucose levels were < 5.6 mmol/L (dogs from Group A); while in 10/13 dogs they were > 5.6 mmol/L (dogs from Group B). In 2/10 cases with glycemia > 5.6 mmol/L, DM was expressed acutely, developing diabetic ketoacidosis. The response to treatment of PDH was good in almost all of these dogs (11/13), with clinical improvement, decrease in UCCR and other biochemical parameters, except for the two dogs that developed diabetic ketoacidosis acutely (two or three months after the first consultation).

In summary, of the 235 dogs with HAC, 13.61% presented DM ($n = 32$). Median age was 9.4 years (range between 2 and 15 years), 20 of which were females (20/32 = 62.5%): 14 non-castrated (14/20 = 70%) and 6 castrated (6/20 = 30%); 12 were males (12/32 = 37.5%): 9 non-castrated (9/12 = 75%) and 3 castrated (3/12 = 25%). *Breed:* 16 Poodle (50%); 7 Crossbreed (21.87%); 2 Beagle and Maltese; 1 Doberman, Miniature Schnauzer, Breton, Vizsla, Fox Terrier (data summarized in Table 2).

3.3. Blood glucose, total cholesterol and triglycerides

Of the 70 dogs with blood glucose levels > 5.6 mmol/L and < 10.08 mmol/L, 24 presented blood glucose levels > 6.72 mmol/L (34.29%), in other words, 24/235 (10.21%) of all dogs with HAC had high fasting hyperglycemia without reaching a clinical DM, considering 6.72 mmol/L (120 mg/dL) as the upper limit of the blood glucose reference range in dogs (Kaneko et al., 2008; Torre et al., 2007; Peterson et al., 1986). When comparing the variations of blood glucose levels, total cholesterol and triglycerides between males and female and between castrated and non-castrated animals within each group, no significant differences were observed.

Comparing the variations in total cholesterol and triglycerides between the three groups, significant differences (ANOVA $P < 0.001$) were observed. To be precise, between groups A and C ($P < 0.001$) and between groups B and C ($P < 0.05$), being greater in group C; with

Table 2
Age, gender, cause of HAC, body condition and breed of dogs with HAC and DM.

	Dogs ($n = 32$)
Age (years)	9.4 (2–15)
Gender:	
Female: Castrated	6/20 (30%)
Non-castrated	14/20 (70%)
Male: Castrated	3/12 (25%)
Non-castrated	9/12 (75%)
Cause of HAC: PDH	30/32 (93.75%)
ADH	2/32 (6.25%)
Body condition:	
Normal weight	15/32 (46.87%)
Overweight	11/32 (34.38%)
Obese	6/32 (18.75%)
Breed: Crossbreeds	7/32 (21.87%)
Purebreds	25/32 (78.13%)

Age is expressed as median and range. The rest of the values are expressed a percentage. The dogs included in this table are all dogs with HAC and DM, i.e. those that already came with HAC and DM at the first consultation and all dogs with HAC that developed DM over the months.

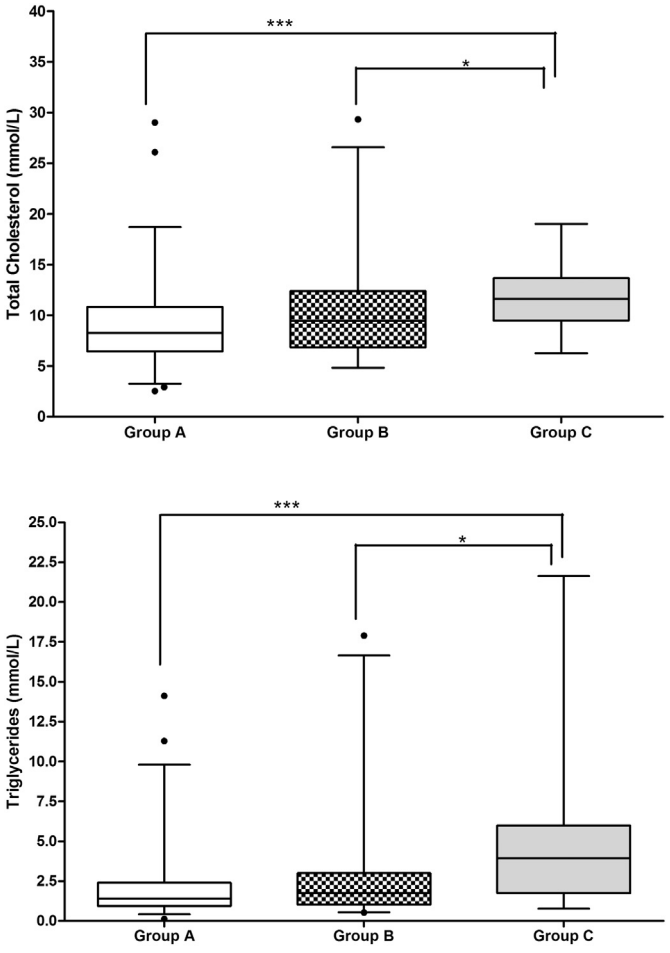

Fig. 1. (a) Comparison of the total cholesterol in dogs with HAC: Group A (glycemia < 5.6 mmol/L), Group B (glycemia > 5.6–10.08 mmol/L), Group C (glycemia > 10.08 mmol/L). Reference value for total cholesterol: up to 6.5 mmol/L. (b) Comparison of the triglycerides levels in dogs with HAC between the different groups. ***$P < 0.001$, *$P < 0.01$. Triglycerides reference value: up to 2.5 mmol/L.

no differences between groups A and B (Fig. 1).

3.4. UCCR

When comparing the variations within each group, UCCR was significantly higher in non-castrated females than in castrated ones ($P < 0.05$).

Significant differences (ANOVA $P < 0.05$) were observed between groups when comparing the variations of UCCR. To be precise, groups B and C were significantly higher than group A ($P < 0.05$) and no differences were observed between groups B and C (Fig. 2).

3.5. Risk factors for the development of DM in dogs with HAC

When analyzing the risk of developing DM in dogs with HAC, we observed a significant association between dogs with blood glucose levels > 5.6 mmol/L ($P < 0.001$), with PDH ($P < 0.05$), with UCCR > 100×10^{-6} ($P < 0.05$), total cholesterol > 9.1 mmol/L (350 mg/dL) ($P < 0.01$), triglycerides > 2.5 mmol/L (250 mg/dL) ($P < 0.05$) and in non-castrated females ($P < 0.05$) (Table 3). Risk factors were evaluated in all dogs with HAC that developed DM and in those that did not develop DM, excluding dogs with HAC that already had it.

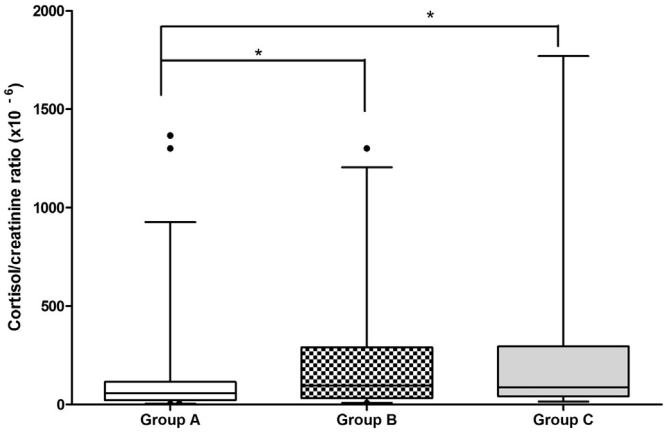

Fig. 2. Comparison of the UCCR in dogs with HAC: Group A (glycemia < 5.6 mmol/L), Group B (glycemia > 5.6–10.08 mmol/L), Group C (glycemia > 10.08 mmol/L). *$P < 0.05$.

Table 3
Risk factors involved in the development of DM in dogs with HAC.

	RR (95% CI)	PPV (95% CI)	*P*
Glycemia > 5.6 mmol/L	7.6 (2.2–26.5)	0.16 (0.08–0.26)	< 0.001
Triglycerides > 2.5 mmol/L	3.8 (1.4–10.7)	0.18 (0.08–0.34)	< 0.05
T. Cholesterol > 9.1 mmol/L	6.6 (1.5–28.9)	0.14 (0.07–0.23)	< 0.01
UCCR > 100×10^{-6}	3.5 (1.2–9.9)	0.15 (0.07–0.27)	< 0.05
PDH	2.8 (1.3–8.6)	0.2 (0.15–0.26)	< 0.05
Non-castrated females	2.2 (1.05–4.67)	0.22 (0.13–0.33)	< 0.05

Risk factors were evaluated in all dogs with HAC that developed DM and in those that did not develop DM, excluding dogs with HAC that already had it.
RR: Relative Risk; PPV: Positive Predictive Value.

3.6. Survival time

Median survival time showed significant differences ($P < 0.001$) between dogs with HAC without DM (28 months, ratio survival 2.4; 95% CI 1.3–4.2) and dogs with HAC and concurrent DM (14 months, ratio survival 0.4; 95%CI 0.2–0.8) (Fig. 3). The HR was 0.22 (95% CI 0.1–0.5) for dogs with HAC without DM and 4.4 (95% CI 1.9–9.8) for dogs with HAC and concurrent DM.

4. Discussion

The concurrence of HAC and DM is a well-studied phenomenon both

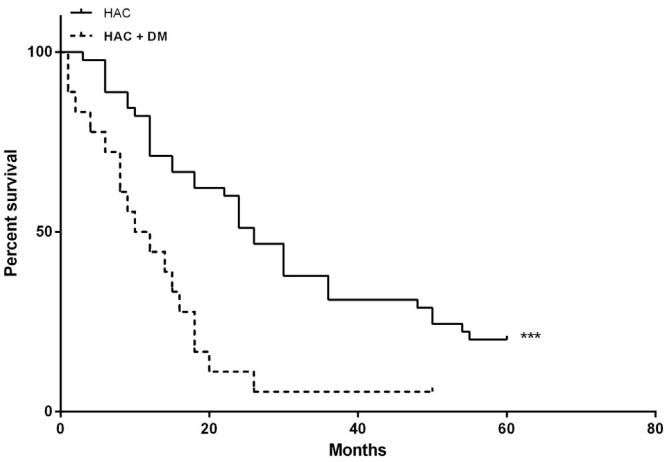

Fig. 3. Life expectancy in dogs with HAC: comparison between dogs with HAC and dogs with concurrent HAC and DM. The average life expectancy in dogs with HAC was 28 months, while dogs with concurrent HAC and DM it was 14 months. **$P < 0.01$.

in veterinary and human medicine (Arnaldi et al., 2012; Eigenmann and Peterson, 1984; Resmini et al., 2009). Glucocorticoids antagonize the effects of insulin, inducing a sustained increase in blood glucose levels and, in the most severe cases, the development of DM (Di Dalmazi et al., 2012). In humans, the incidence of DM in patients with HAC is 20–50% (Biering et al., 2000; Pivonello et al., 2010); likewise, it is known that in women that receive glucocorticoids, gestational DM increases from 4% to 23.8% (Fisher et al., 1997) and that glucocorticoids are the main responsible for the development of DM post-transplant (Onwubalili and Obineche, 1992). The incidence of DM in felines with HAC is approximately 80–90% (Niessen et al., 2013; Valentin et al., 2014). Some authors sustain that approximately 16.6–22% of dogs with HAC present concurrent DM (Gomes Pöppl et al., 2016; Peterson et al., 1981), while others affirm that this association is around 5–10% (Ishino et al., 2010; Nichols, 1997) and that the overvaluation of this prevalence is due to diagnostic errors, as a result of the difficulty in interpreting the tests for HAC in special cases such as dogs with uncontrolled DM (Behrend, 2015; Kaplan et al., 1995). In the present study, we observed that the concurrence of HAC and DM in the city of Buenos Aires, during 2011 and 2015, was 13.61%, which was close to the value we had previously reported (Miceli et al., 2012). Hess et al. (2000) carried out the analysis inversely and observed that 23% of the dogs with DM presented HAC. Hence, at least in our environment, an important proportion of dogs with HAC present DM simultaneously. It should be clarified that neither retinoic acid nor cabergoline predispose to DM as a side effect (Castillo et al., 2006, 2008; Lau et al., 2015).

It is not the purpose of this study to physiopathologically define which of the two diseases appears first, but various reasons permit the assumption that HAC predisposes to DM. It is well known that cortisol antagonizes the functions of insulin and that hypercortisolism induces a state of insulin resistance (Andrews and Walker, 1999; Vegiopoulos and Herzig, 2007). Dogs with HAC have a lowered peripheral sensitivity to insulin and produce high levels of insulin to compensate (Miceli et al., 2014; Montgomery et al., 1996). It is also known that dogs with HAC express less internal insulin signaling molecules (Nozawa et al., 2014). In other words, dogs with HAC can present fasting high blood glucose level, hyperinsulinism and lower insulin sensitivity, which, added to the dyslipidemia, present a state of “pre-diabetes” (Feldman and Nelson, 2004; Miceli et al., 2014). In humans it is known that hyperinsulinism stimulates the adrenal axis (Fruehwald-Schultes et al., 1999), which could favor maintaining hypercortisolism. It is possible that in dogs with HAC, pancreatic β cells become exhausted after the overexertion of trying to compensate insulin resistance, thus establishing DM (Miceli et al., 2014). The confusion over which disease is diagnosed first can present itself because DM is easier to detect than HAC, which doesn't necessarily mean that it chronologically appeared first. In fact, all diabetic dogs enrolled in this study had other signs that increase HAC suspicion (high insulin requirements, alopecia, prominent abdomen, thin inelastic skin and persistent anestrous). Another controversial point is “the moment of diagnosis” as this depends on the moment that the owners bring the dog to the hospital, the severity of the case and course of the disease. Some owners take their dog to consultation shortly after perceiving the first abnormality (for example: polydipsia and polyuria), while others wait various months before making an endocrinological consultation.

The American Diabetes Association (ADA) establishes that people with fasting blood glucose levels between 5.6 and 7 mmol/L (100–125 mg/dL) are at risk of developing DM and as a result are included in the “pre-diabetic” category (ADA, 2016). There is still no consensus on this in veterinary medicine, and the parameters that define the pre-diabetic condition are not yet established. Tvarijonaviciute et al. (2012), compared Obesity-related Metabolic Dysfunction in dogs with the Human Metabolic Syndrome, and established a cut-off value for fasting blood glucose levels at 5.6 mmol/L. In this study, we used this cut-off value and observed that the risk of developing DM in dogs with HAC and blood glucose levels > 5.6 mmol/L, is greater than in dogs with HAC and blood glucose levels lower than 5.6 mmol/L.

Dogs with HAC present disturbances in lipid metabolism: either high circulating levels of cholesterol or of triglycerides or of both (Jericó et al., 2009). In this study we observed that dogs with HAC and presenting total cholesterol values > 9.1 mmol/L or triglycerides > 2.5 mmol/L have a greater risk of developing DM, which could support the hypothesis that dogs with HAC present a pre-diabetic state that can progress to clinical diabetes.

Galac et al. (1997) reported that dogs with HAC with a UCCR $> 100 \times 10^{-6}$ have an almost exclusive origin in PDH and not ADH. Taking this value as a reference, in this study we observed that dogs with HAC with a UCCRs $> 100 \times 10^{-6}$ show a greater predisposition to develop DM, probably because of the difficulty they have to normalize those hypercortisolism levels as they are perpetuated by the insulin resistance. It is also interesting to highlight that 93.75% of the dogs with HAC and DM presented PDH, observing that dogs with PDH showed a greater predisposition to develop DM than those with ADH. There is not much evidence to explain this association, but it is possible that in the case of not carrying out a hypophysectomy, PDH hypercortisolism is more prolonged than that produced by ADH where the adrenalectomy, being a more accessible surgery, allows a considerable reduction of this time. In fact, in both dogs with ADH and DM the adrenalectomy could not be performed because the owners refused to do the surgery.

It is known that in humans, an increase in visceral adipose tissue decreases the peripheral sensitivity to insulin (Abate et al., 1995; Ye, 2013). Fatty acids reach the liver via the portal system and decrease the use of glucose (Randle cycle) (Martins et al., 2012). In turn, adipose tissue releases cytokines that modify tissue response to insulin (Makki et al., 2013). Cabrera Blatter et al. (2012) report that the levels of adiponectin in dogs with HAC are decreased thus affecting their insulin sensitizing effect. In this study, we observed that approximately 77.5% of dogs with HAC and blood glucose levels > 5.6 mmol/L were overweight or obese, while in dogs with HAC and blood glucose levels < 5.6 mmol/L the percentage was 62%. Nevertheless, we did not observe a significant association between overweight/obesity and DM. Dogs with HAC that went on to develop DM presented overweight or obesity at the first consultation and once DM initiated, they evidenced a considerable weight loss. Such weight loss could be explained by the success of the HAC treatment (Castillo et al., 2006, 2008), or by the loss of the anabolic effect of insulin, once the DM is initiated (Dimitriadis et al., 2011). In other words, in the period prior to clinical DM, insulin levels are excessive due to insulin resistance, favoring the weight increase, but once clinical DM is declared, insulin levels decrease, producing weight loss (ADA, 2016; Nelson and Reusch, 2014).

With regard to racial predisposition, it is interesting to mention that the association between HAC and DM is presented as much in crossbreeds as in breed dogs. Because of the type of study performed, it is not possible to argue for a genetic predisposition for some breed. Nevertheless, Peterson et al. (1981) highlight that 56% of dogs with HAC and DM are poodles; likewise, in this study, we observed that 50% were poodles (in Argentina, predominant are the *toy* and *dwarf* varieties).

Castration of bitches is not frequent in Argentina, as in other countries. Taking this particularity into account, we observed that non-castrated females with HAC present a greater predisposition to develop DM than those that are castrated. In the study by Peterson et al. (1981), 75% of the bitches that developed DM and HAC were non-castrated. It is difficult to determine whether this is a consequence of the circulating sexual hormones, as at the moment of diagnosis all the non-castrated bitches were in a prolonged anestrous as a result of the inhibition of the gonadal axis generated by the chronic hypercortisolism (Meij et al., 1997).

As we observed in this study, concurrence of HAC and DM shortens life expectancy in dogs. Although the fact that dogs with HAC are usually adults or old should be considered, concurrent development of

DM significantly reduces the expected lifespan. In 2/32 dogs, DM appeared as the terminal stage of an uncontrolled HAC, with a diabetic ketoacidosis that was difficult to revert, and both dogs died a few days after hospitalization (Peterson et al., 1981).

5. Conclusions

The percentage of dogs with HAC and DM, over the period of 2011–2015 in the city of Buenos Aires, was 13.61%. Dogs with fasting blood glucose levels > 5.6 mmol/L, with dyslipidemia (either high triglycerides levels above > 2.5 mmol/L or hypercholesterolemia > 9.1 mmol/L), with a UCCR > 100×10^{-6} have a higher risk of developing DM. Likewise, dogs with PDH and non-castrated females have a greater predisposition to develop DM. Appearance of DM in dogs with HAC shortens life expectancy. For future research, rapid detection and control of the risk factors analyzed in this study would permit implementation of preventive therapy to reduce the incidence of DM in dogs with HAC.

Conflict of interest

The Author(s) declare(s) that there is no conflict of interest.

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References

Abate, N., Garg, A., Peshock, R.M., Stray-Gundersen, J., Grundy, S.M., 1995. Relationship of generalized and regional adiposity to insulin sensitivity in men. J. Clin. Invest. 96, 88–98.

American Diabetes Association, 2016. Standards of medical care in diabetes – 2016. Diabetes Care 39, 13–22.

Andrews, R., Walker, B., 1999. Glucocorticoids and insulin resistance. Old hormones, new targets. Clin. Sci. 96, 513–523.

Arnaldi, G., Mancini, T., Tirabassi, G., Trementino, L., Boscaro, M., 2012. Advances in the epidemiology, pathogenesis, and management of Cushing's syndrome complications. J. Endocrinol. Investig. 35, 434–448.

Behrend, E.N., 2015. Canine hyperadrenocorticism. In: Feldman, E.C., Nelson, R.W., Reusch, C.E., Scott-Moncrieff, J.C.R., Behrend, E.N. (Eds.), Canine and Feline Endocrinology, Fourth edition. Elsevier Saunders, USA, pp. 377–451.

Biering, H., Knappe, G., Gerl, H., Lochs, H., 2000. Prevalence of diabetes in acromegaly and Cushing syndrome. Acta Med. Austriaca 27, 27–31.

Blaxter, A.C., Gruffydd-Jones, T.J., 1990. Concurrent diabetes mellitus and hyperadrenocorticism in the dog: diagnosis and management of eight cases. J. Small Anim. Pract. 31, 117–122.

Cabrera Blatter, M.F., del Prado, B., Miceli, D.D., Gomez, N., Ivanic, J., Di Tollo, B., Gallelli, M.F., Castillo, V.A., 2012. Interleukin-6 and insulin increase and nitric oxide and adiponectin decrease in blind dogs with pituitary-dependent hyperadrenocorticism. Res. Vet. Sci. 93, 1195–1202.

Castillo, V.A., Giacomini, D.P., Páez-Pereda, M., Stalla, J., Labeur, M., Theodoropoulou, M., Holsboer, F., Grossman, A., Stalla, G., Arzt, E., 2006. Retinoic acid as a novel medical therapy for Cushing's disease in dogs. Endocrinology 174, 4438–4444.

Castillo, V.A., Gomez, N.V., Lalia, J.C., Cabrera Blatter, M.F., García, J.D., 2008. Cushing's disease in dogs: cabergoline treatment. Res. Vet. Sci. 85, 26–34.

Di Dalmazi, G., Pagotto, U., Pasquali, R., Vicennati, V., 2012. Glucocorticoids and type 2 diabetes: from physiology to pathology. Mediterr. J. Nutr. Metab, 525093. http://dx.doi.org/10.1155/2012/525093.

Dimitriadis, G., Mitrou, P., Lambadiari, V., Maratou, E., Raptis, S.A., 2011. Insulin effects in muscle and adipose tissue. Diabetes Res. Clin. Pract. 93, S52–59.

Eigenmann, J.E., Peterson, M.E., 1984. Diabetes mellitus associated with other endocrine disorders. Vet. Clin. North Am. Small Anim. Pract. 14, 837–858.

Elliot, D., Nelson, R., Feldman, E., Leal, L., 1997. Glycosylated hemoglobin concentrations in the blood of healthy dogs and dogs with naturally developing diabetes mellitus, pancreatic beta-cell neoplasia, hyperadrenocorticism, and anemia. J. Am. Vet. Med. Assoc. 211, 723–727.

Feldman, E.C., Nelson, R.W., 2004. Canine hyperadrenocorticism (Cushing's syndrome). In: Feldman, E.C., Nelson (Eds.), Canine and Feline Endocrinology, Third edition. Elsevier Saunders, USA, pp. 252–357.

Fey, K., Jonigkeit, E., Moritz, A., 1998. Equine Cushing syndrome (ECS). Case report, review of its diagnosis and therapy and substantial differences from Cushing syndrome in dogs. Tierarztl. Prax. Ausg. G Grosstiere Nutztiere 26, 41–47.

Fisher, J.E., Smith, R.S., Lagrandeur, R., Loren, R.P., 1997. Gestational diabetes mellitus in women receiving beta-adrenergics and corticosteroids for threatened preterm delivery. Obstet. Gynecol. 90, 880–883.

Fruehwald-Schultes, B., Kern, W., Bong, W., Wellhoener, P., Kerner, W., Born, J., Fehm, H.I., Peters, A., 1999. Supraphysiological hyperinsulinemia acutely increases hypothalamic-pituitary-adrenal secretory activity in humans. J. Clin. Endocrinol. Metab. 84, 3041–3046.

Galac, S., Kooistra, H.S., Teske, E., Rijnberk, A., 1997. Urinary corticoid:creatinine ratios in the differentiation between pituitary-dependent hypercortisolism and hypercortisolism due to adrenocortical tumor in the dog. Vet. Q. 19, 17–20.

Gallelli, M.F., Miceli, D.D., Cabrera Blatter, M.F., Brañas, M.M., Castillo, V.A., 2009. The occurrence of corticotrophinoma in cross-breed and breed dogs. Slov. Vet. Res. 46, 115–121.

Gallelli, M.F., Cabrera Blatter, M.F., Castillo, V.A., 2010. A comparative study by age and gender of the pituitary adenoma and ACTH and a-MSH secretion in dogs with pituitary-dependent hyperadrenocorticism. Res. Vet. Sci. 88, 33–40.

Gomes Pöppl, A., Comparsi Coelho, I., Alves da Silveira, C., Bianchini Moresco, M., Luiz Carvalho de Carvalho, G., 2016. Frequency of Endocrinopathies and characteristics of affected dogs and cats in Southern Brazil (2004–2014). Acta Sci. Vet. 44, 1379.

Hess, R.S., Saunders, H.M., Van Winkle, T.J., Ward, C.R., 2000. Concurrent disorders in dogs with diabetes mellitus: 221 cases (1993–1998). J. Am. Vet. Med. Assoc. 217, 1166–1173.

Ishino, H., Hara, Y., Teshima, T., Tanaka, S., Takekoshi, S., Nezu, Y., Harada, Y., Yogo, T., Sako, T., Koyama, T., Teramoto, A., Osamura, R., Tagawa, M., 2010. Hypophysectomy for a dog with coexisting Cushing's disease and diabetes mellitus. J. Vet. Med. Sci. 72, 343–348.

Jericó, M.M., De Camargo, Ch.F., Kajihara, K., Moreira, M.A., Gonzales, R., Machado, F.L., Nunes, V.S., Catanozi, S., Nakandakare, E.R., 2009. Chromatographic analysis of lipid fractions in healthy dogs and dogs with obesity or hyperadrenocorticism. J. Vet. Diagn. Investig. 21, 203–207.

Jeusette, I., Greco, D., Aquino, F., Detilleux, J., Peterson, M., Romano, V., Torre, C., 2010. Effect of breed on body composition and comparison between various methods to estimate body composition in dogs. Res. Vet. Sci. 88, 227–232.

Kaneko, J.J., Harvey, J.W., Bruss, M.L., 2008. Clinical Biochemistry of Domestic Animals, Sixth ed. Academic Press, Elsevier Inc.

Kaplan, A.J., Peterson, M.E., Kemppainen, R.J., 1995. Effects of disease on the results of diagnostic tests for use in detecting hyperadrenocorticism in dogs. J. Am. Vet. Med. Assoc. 207, 445–451.

Kooistra, H.S., Galac, S., 2012. Recent advances in the diagnosis of Cushing's syndrome in dogs. Top. Companion Anim. Med. 27, 21–24.

Laflamme, D.P., 1997. Development and validation of a body condition score system for dogs. Canine Pract. 22, 10–15.

Lau, D., Rutledge, C., Aghi, M.K., 2015. Cushing's disease: current medical therapies and molecular insights guiding future therapies. Neurosurg. Focus. 38, E11. http://dx.doi.org/10.3171/2014.10.FOCUS14700.

Makki, K., Froguel, P., Wolowczuk, I., 2013. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. ISRN Inflamm. http://dx.doi.org/10.1155/2013/139239.

Martins, A.R., Nachbar, R.T., Gorjao, R., Vinolo, M.A., Festuccia, W.T., Lambertucci, R.H., Cury-Boaventura, M.F., Silveira, L.R., Curi, R., Hirabara, S.M., 2012. Mechanisms underlying skeletal muscle insulin resistance induced by fatty acids: importance of the mitochondrial function. Lipids Health Dis. 11, 30. http://dx.doi.org/10.1186/1476-511X-11-30.

McLauchlan, G., Knottenbelt, C., Augusto, M., Helm, J., McGrotty, Y., Ramsey, I., 2010. Retrospective evaluation of the effect of trilostane on insulin requirement and fructosamine concentration in eight diabetic dogs with hyperadrenocorticism. J. Small Anim. Pract. 51, 642–648.

Meij, B.P., Mol, J.A., Bever, M.M., Rijnberk, A., 1997. Alterations in anterior pituitary function of dogs with pituitary dependent hyperadrenocorticism. J. Endocrinol. 154, 505–512.

Miceli, D.D., Gallelli, M.F., Cabrera Blatter, M.F., Martiarena, B., Brañas, M.M., Ortemberg, L.R., Gomez, N.V., Castillo, V.A., 2012. Low dose of insulin detemir controls glycaemia, insulinemia and prevents diabetes mellitus progression in the dog with pituitary-dependent hyperadrenocorticism. Res. Vet. Sci. 93, 114–120.

Miceli, D.D., Cabrera Blatter, M.F., Gallelli, M.F., Pignataro, O.P., Castillo, V.A., 2014. Involvement of glucagon-like peptide 1 in the glucose homeostasis regulation in obese and pituitary-dependent hyperadrenocorticism affected dogs. Res. Vet. Sci. 97, 211–217.

Monroe, W.E., Laxton, D., Fallin, E.A., Richter, K.P., Santen, D.R., Panciera, D.L., Towell, T.L., Williams, K.A., Hart, J.R., Hill, S., Finkler, M.R., Shinn, J.S., 2005. Efficacy and safety of a purified porcine insulin zinc suspension for managing diabetes mellitus in dogs. J. Vet. Intern. Med. 19, 675–682.

Montgomery, T.M., Nelson, R.W., Feldman, E.C., Robertson, K., Polonsky, K.S., 1996. Basal and glucagon-stimulated plasma C-peptide concentrations in healthy dogs, dogs with diabetes mellitus, and dogs with hyperadrenocorticism. J. Vet. Intern. Med. 10, 116–122.

Nelson, R.W., Reusch, C.E., 2014. Animal models of disease: classification and etiology of diabetes mellitus in dogs and cats. J. Endocrinol. 222, T1–9.

Nichols, R., 1997. Complications and concurrent disease associated with canine hyperadrenocorticism. Vet. Clin. North Am. Small Anim. Pract. 27, 309–320.

Niessen, S.J., Church, D.B., Forcada, Y., 2013. Hypersomatotropism, acromegaly, and hyperadrenocorticism and feline diabetes mellitus. Vet. Clin. North Am. Small Anim. Pract. 43, 319–350.

Nozawa, S., Oda, H., Akiyama, R., Ueda, K., Saeki, K., Shono, S., Maruyama, N., Murata, A., Tazaki, H., Mori, A., Momota, Y., Azakami, D., Sako, T., Ishioka, K., 2014. Decreased gene expressions of insulin signal molecules in canine hyperadrenocorticism. J. Vet. Med. Sci. 76, 1177–1182.

Onwubalili, J.K., Obineche, E.N., 1992. High incidence of post-transplant diabetes

mellitus in a single-centre study. Nephrol. Dial. Transplant. 7, 346–349.
Peterson, M.E., Nesbitt, G.H., Schaer, M., 1981. Diagnosis and management of concurrent diabetes mellitus and hyperadrenocorticism in 30 dogs. J. Am. Vet. Med. Assoc. 178, 66–69.
Peterson, M.E., Altszuler, N., Nichols, C.E., 1984. Decreased insulin sensitivity and glucose tolerance in spontaneous canine hyperadrenocorticism. Res. Vet. Sci. 36, 177–182.
Peterson, M.E., Winkler, B., Kintzer, P.P., Altszuler, N., 1986. Effect of spontaneous hyperadrenocorticism on endogenous production and utilization of glucose in the dog. Domest. Anim. Endocrinol. 3 (2), 117–125.
Pivonello, R., De Leo, M., Vitale, P., Cozzolino, A., Simeoli, Ch., De Martino, M.C., Lombardi, G., Colao, A., 2010. Pathophysiology of diabetes mellitus in Cushing's syndrome. Neuroendocrinology 92, 77–81.
Resmini, E., Minuto, F., Colao, A., Ferone, D., 2009. Secondary diabetes associated with principal endocrinopathies: the impact of new treatment modalities. Acta Diabetol. 46, 85–95.
Ristic, J.M.E., Ramsey, I.K., Heath, F.M., Evans, H.J., Herrtage, M.E., 2002. The use of 17-hydroxyprogesterone in the diagnosis of canine hyperadrenocorticism. J. Vet. Intern. Med. 16, 433–439.
Sako, T., Mori, A., Lee, P., Oda, H., Saeki, K., Miki, Y., Kurishima, M., Mimura, K., Nozawa, S., Mizutani, H., Makino, Y., Ishioka, K., Arai, T., 2011. Time-action profiles of insulin detemir in normal and diabetic dogs. Res. Vet. Sci. 90, 396–403.
Schäcke, H., Docke, W.D., Asadullah, K., 2002. Mechanisms involved in the side effects of glucocorticoids. Pharmacol. Ther. 96, 23–43.
Torre, D.M., deLaforcade, A.M., Chan, D.L., 2007. Incidence and clinical relevance of hyperglycemia in critically ill dogs. J. Vet. Intern. Med. 21, 971–975.
Tvarijonaviciute, A., Ceron, J.J., Holden, S.L., Cuthbertson, D.L., Biourge, V., Morris, P.J., German, A.J., 2012. Obesity-related metabolic dysfunction in dogs: a comparison with human metabolic syndrome. BMC Vet. Res. 8, 147. http://dx.doi.org/10.1186/1746-6148-8-147.
Valentin, S.Y., Cortright, C.C., Nelson, R.W., Pressler, B.M., Rosenberg, D., Moore, G.E., Scott-Moncrieff, J.C., 2014. Clinical findings, diagnostic test results, and treatment outcome in cats with spontaneous hyperadrenocorticism: 30 cases. J. Vet. Intern. Med. 28, 481–487.
Van Raalte, D.H., Ouwens, D.M., Diamant, M., 2009. Novel insights into glucocorticoid-mediated diabetogenic effects: towards expansion of therapeutic options? Eur. J. Clin. Investig. 39, 81–93.
Vegiopoulos, A., Herzig, S., 2007. Glucocorticoids, metabolism and metabolic diseases. Mol. Cell. Endocrinol. 275, 43–61.
Wilkinson, A.C., Harris, L.D., Saviolakis, G.A., Martin, D.G., 1999. Cushing's syndrome with concurrent diabetes mellitus in a rhesus monkey. Contemp. Top. Lab. Anim. Sci. 38, 62–66.
Ye, J., 2013. Mechanisms of insulin resistance in obesity. Front. Med. 7, 14–24.