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Effects of thyroxin therapy on different analytes related to obesity and inflammation in dogs with hypothyroidism

A. Tvarijonaviciute [a,1], L. Jaillardon [b,1], J.J. Cerón [a,*], B. Siliart [b]

[a] Department of Animal Medicine and Surgery, Veterinary School, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30100 Murcia, Spain
[b] Department of Biology, Pathology and Food Sciences, Nantes Atlantic College of Veterinary Medicine, Food Science and Engineering, F-44307 Nantes, France

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ABSTRACT

Hypothyroidism in dogs is accompanied by changes in intermediary metabolism including alterations in bodyweight (BW), insulin resistance, and lipid profile. In this study, changes in selected adipokines (adiponectin, leptin), butyrylcholinesterase (BChE), and acute phase proteins, including C-reactive protein, haptoglobin (Hp) and serum amyloid A (SAA), were studied in dogs with hypothyroidism under thyroxin therapy. Blood samples were collected when hypothyroidism was diagnosed (before treatment) and after treatment with thyroxin.

Twenty-eight of 39 dogs exhibited a good therapeutic response (group A), whereas the remainder were considered to have been insufficiently treated (group B). Following treatment, group A dogs demonstrated a statistically significant decrease in canine thyroid stimulating hormone (c-TSH) ($P < 0.001$) and an increase in free thyroxine (fT4) ($P < 0.001$) concentrations, associated with a significant decrease in BW ($P < 0.05$), leptin ($P < 0.01$), and adiponectin, ($P < 0.001$) and an increase in BChE ($P < 0.01$) and Hp ($P < 0.05$). Group B dogs showed no statistically significant changes in c-TSH, but had a significant increase in fT4 ($P < 0.001$) accompanied by a significant decrease in adiponectin ($P < 0.05$) of lower magnitude than group A. No significant changes in the mean circulating levels of APPs were observed in both groups, with the exception of an increase in Hp ($P < 0.05$) in group A. In summary, the successful treatment of hypothyroidism reduces circulating levels of adiponectin and leptin, while increasing BChE activity in dogs. The mean increase in Hp values and decrease in SAA for some of the dogs after treatment warrants further investigation.

Introduction

Thyroid abnormalities, hyperthyroidism and hypothyroidism are accompanied by changes in intermediary metabolism, including alterations in bodyweight (BW), insulin resistance, and plasma lipid profile in humans (Heimberg et al., 1985; Pucci et al., 2000) and dogs (Dixon et al., 2002; Hofer-Inteeworn et al., 2012). Hypothyroidism is associated with decreased concentrations of triiodothyronine (T3), thyroxine (T4), and increased thyroid stimulating hormone (TSH). These changes lead to increased BW with increased plasma lipids and lipoproteins, and are associated with alterations in glucose and insulin metabolism (Diekman et al., 2000; Pucci et al., 2000; Hofer-Inteeworn et al., 2012).

Adipose tissue secretes a variety of active biological substances called adipocytokines, such as adiponectin and leptin, which among others are involved in appetite control, thermogenesis and thyroid function. Butyrylcholinesterase (BChE) is a non-specific choline esterase and although its biological role has yet to be clearly established (Kutty et al., 1995), BChE has been associated both with different parameters of both adiposity and inflammation in humans, rats and dogs (Antopol et al., 1973; Jain et al., 1983; Magarian and Dietz, 1987; Tvarijonaviciute et al., 2011b). Measurements of acute phase proteins (APPs) can be used in dogs as a screening test for gauging the systemic response to an inflammatory stimulus and are considered as the most accurate markers of inflammation. Positive APPs, such as C-reactive protein (CRP), haptoglobin (Hp) and serum amyloid A (SAA) increase their rate of synthesis in the liver and are released into the blood when inflammation occurs (Eckersall, 2000).

Some adipocytokines and inflammatory proteins have been studied in human hypothyroidism (Lee et al., 2004; Yu et al., 2006; Kokkinos et al., 2007) but information about canine hypothyroidism is more limited (Dixon et al., 2002; Mazaki-Tovi et al., 2010; Jaillardon et al., 2011). Moreover, to the best of our knowledge, no data have been reported about the possible influence of thyroxin therapy on analytes related to obesity and inflammation in dogs with hypothyroidism. Therefore, the aim of the present study was to study the concentration of selected adipokines (such

* Corresponding author. Tel.: +34 68 364722.
E-mail address: jjceron@um.es (J.J. Cerón).
[1] These authors equally contributed to this work.

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as adiponectin and leptin), BChE, and various APPs (such as CRP, SAA, and Hp) in dogs with hypothyroidism under thyroxin therapy.

Material and methods

Animals

Hypothyroid client-owned dogs of different breeds ($n = 39$) presented at the Veterinary University Hospital in Nantes were included in the study. The median age of the 39 dogs in the study was 5 years (range, 2.5–10 years); 19 of the dogs were females (10 spayed) and 20 were males (7 castrated). Median BW prior to treatment was 37 kg (range, 6–69 kg). Primary hypothyroidism was diagnosed based on clinical and laboratorial signs of primary hypothyroidism, i.e. lethargy, BW gain, dermatologic signs, high cholesterol (>6.5 mmol/L), high c-TSH (>0.5 ng/mL) and low free T4 (fT4) values (⩽12 pmol/L) (Tyler, 2007). All dogs were subjected to thyroxin therapy (mean ± standard deviation, 14.4 ± 5.7 μg/kg/day) for 3 months. Good therapeutic response was considered if the dogs exhibited a significant clinical improvement (including BW loss, increase activity, improvement of the skin) and at least a 30% decrease in c-TSH values.

Blood samples were collected by jugular venepuncture when hypothyroidism was diagnosed (before treatment, T1) and after treatment with thyroxin over 3 months (T2). All blood samples were taken after a fast of at least 12 h, and in T2 4 h after thyroxin administration. Immediately after collection, samples were centrifuged and the plasma was harvested and frozen at −20 °C. Samples were subsequently shipped on dry ice to the University of Murcia. Immediately upon arrival, samples were transferred to a freezer at −20 °C and subsequently analysed in batches.

Assays

fT4 was measured by radioimmunoassay (Immunotech kit 1363) and c-TSH by chemiluminescence (Siemens Immulite CanineTSH) (Jaillardon et al., 2011). Leptin concentrations were measured with a canine leptin enzyme-linked immunosorbent assay (ELISA) (Millipore) (Tvarijonaviciute et al., 2011a). Adiponectin concentration was determined by ELISA (Human Adiponectin ELISA, High Sensitivity Kit, BioVendor-Labaratorni medicine) (Tvarijonaviciute et al., 2010a). BChE activity was measured as previously reported (Tecles et al., 2000) and adapted for an automated analyser (Olympus AU2700, Olympus Diagnostica).

High sensitivity CRP (hs-CRP) was measured with a time-resolved immunofluorometric assay (TRFIA) previously validated for canine samples using goat anti-canine polyclonal antibodies (Parra et al., 2006). Haptoglobin (Hp) concentration was measured by a commercially available colorimetric method (Tridelta Phase range haptoglobin kit, Tridelta Development). SAA concentrations were measured using the Tridelta Phase Range assay. Final absorbance of the samples was measured by use of a microtitre plate reader (Powerwave XS, Biotek Instruments) at 450 nm using 630 nm as the reference (Martinez-Subiela and Cerón, 2005).

Statistical analysis

Results are expressed as the median (range) except where indicated. The D'Agostino and Pearson omnibus normality test was used to assess normality. A paired Student's t test was used to compare changes for data that were normally distributed and Wilcoxon signed rank test was used to compare changes for data that were not normally distributed. Analytes that were shown to be more significantly affected by hypothyroidism treatment (leptin, adiponectin, and BChE) were used to set a predictive model by binary logistic regression analysis and to perform a Spearman correlation test. Statistical significance was defined as $P < 0.05$ on two-tailed testing for all tests.

Results

Twenty-eight of 39 dogs exhibited a good therapeutic response with a decrease of more than 30% in c-TSH value, which was associated with a satisfactory clinical improvement. These dogs were assigned to group A, whereas the remainder of the dogs ($n = 11$) were considered to have been insufficiently treated and were assigned to group B. No significant differences were observed in baseline BW between the two groups of dogs. However, dogs in group A (5.75 years; range, 3–10 years) were significantly older than group B dogs (3.50 years; range, 2.5–6 years) ($P = 0.005$).

After treatment, group A dogs demonstrated a statistically significant decrease in c-TSH (>30%) and an increase in fT4 levels (~60%), whereas group B dogs demonstrated a significant increase in fT4 (~40%), and no statistically significant changes were observed in c-TSH (Tables 1 and 2). In addition, group A dogs showed a decrease in BW, leptin, and adiponectin concentrations ($P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively), and an increase in BChE activity ($P < 0.01$) and in Hp concentrations ($P < 0.05$). In contrast, group B dogs showed only a significant decrease ($P < 0.05$) in adiponectin, which was of lower magnitude than that of group A dogs (14% vs. 30%).

Although no statistically significant changes were observed, CRP concentrations were >20 mg/L in two dogs, while SAA > 5 mg/L was observed in 14 dogs before treatment; with the exception of two animals, all of these dogs showed a decrease in these APPs after therapy.

When initial values of the different analytes in both groups were compared, group A had higher leptin and lower adiponectin values than group B ($P < 0.05$ for both parameters). No significant differences were found in BChE and APPs between the two groups.

Binary logistic regression analysis indicated that pre-treatment adiponectin levels can be a predictor of successful/failed response to treatment (lower pre-treatment adiponectin = more likely to obtain successful treatment), whereas pre-treatment leptin levels showed only a trend (higher pre-treatment leptin = more likely to obtain successful treatment) (Table 3).

Table 1
Median (range) data of variables of successfully treated dogs (group A).

	T1	T2	P
BW, kg	35.50 (6.00–69.00)	33.45 (15.00–70.00)	0.023
c-TSH, ng/mL	1.55 (0.60–8.30)	0.40 (0.03–4.80)	<0.0001
FT4, pmol/L	10.00 (⩽2.4–16.00)	16.50 (10.00–29.00)	<0.0001
Leptin, ng/mL	14.20 (⩽3.6–92.80)	6.90 (⩽3.6–21.80)	0.003
Adiponectin, μg/mL	13.30 (5.23–23.78)	9.48 (0.43–27.92)	0.0006
BChE, μmol/mL/min	5.20 (2.90–9.50)	5.65 (2.70–10.70)	0.009
hs-CRP, mg/L	1.17 (0.00–29.00)	1.16 (0.00–17.35)	NS
Hp, g/L	2.15 (0.09–7.4)	2.4 (0.28–9.20)	0.0393
SAA, μg/mL	5.04 (0.68–116.00)	1.83 (0.20–24.88)	NS

BW, bodyweight; c-TSH, canine thyroid stimulating hormone; fT4, free thyroxine; BChE, butyrylcholinesterase; hs-CRP, high sensitivity C-reactive protein; Hp, haptoglobin; SAA, serum amyloid A.

Table 2
Median (range) data of variables of unsuccessfully treated dogs (group B).

	T1	T2	P
BW	39.00 (26.00–54.00)	38.50 (26.00–51.00)	NS
c-TSH, ng/mL	1.00 (0.60–4.30)	0.90 (0.50–4.20)	NS
FT4, pmol/L	10.00 (7.00–11.00)	14.00 (12.00–17.00)	<0.0001
Leptin, ng/mL	6.60 (⩽3.60–26.30)	5.50 (⩽3.60–31.70)	NS
Adiponectin, μg/mL	16.97 (8.02–29.87)	14.53 (5.92–23.64)	0.0383
BChE, μmol/mL/min	6.80 (1.90–12.30)	6.10 (2.40–10.60)	NS
hs-CRP, mg/L	1.13 (0.00–10.54)	1.18 (0.00–4.95)	NS
Hp, g/L	2.11 (0.05–4.03)	2.17 (0.20–3.72)	NS
SAA, μg/mL	2.21 (0.20–90.60)	3.57 (0.84–33.95)	NS

BW, bodyweight; c-TSH, canine thyroid stimulating hormone; fT4, free thyroxine; BChE, butyrylcholinesterase; hs-CRP, high sensitivity C-reactive protein; Hp, haptoglobin; SAA, serum amyloid A.

Table 3
Coefficients of the model of binary logistic regression analysis.

Variable	B	P	Exp(B)	CI 95.0% for Exp(B)	
				Lower	Higher
Adiponectin	−0.164	0.049	0.849	0.72	1.001
BChE	−0.376	0.131	0.687	0.422	1.118
Leptin	0.116	0.071	1.123	0.99	1.275
Constant	4.174	0.05	65.001		

B, coefficient of the logistic regression model; Exp(B), exponential B (odd ratio); CI, confidence intervals of exponential B.