Hormonal disturbances associated with obesity in dogs

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Introduction
The prevalence of obesity in dogs is on the increase in western European countries because of modifications in feeding behaviour and a higher frequency of neutering. As in other species, obesity in dogs results from an imbalance between energy intake and energy expenditure. Although diet is the main aspect of any weight loss programme in dogs, in practice the long-term follow up of treatment is often poor because some dogs do not lose weight despite a low calorie diet or tend to regain lost weight after a period of energy restriction. Such observations suggest that obesity is associated with an adaptive downregulation of metabolic rate and perhaps with other hormonal changes. There is evidence that the mechanisms controlling energy homeostasis and adiposity are incompletely understood.

For many years, the literature has emphasized that obesity is associated with multiple endocrine alterations and changes in the serum concentration of circulating hormones (Smith, 1996). Obesity in humans and rodents is associated with disturbances of the main hormonal axes, i.e. pituitary/adrenal, pituitary/gonadal, pituitary-growth hormone (GH)—insulin-like growth factor (IGF)—prolactin axis and pituitary/thyroid hormones axis. The increase in body fat seems to be associated with an increase of cortisol, insulin, IGF-1 secretion and a decrease of GH secretion (Smith, 1996).

Thyroid hormones are among the main factors involved in the regulation of energy homeostasis.
and increase the basal metabolism rate (BMR) (Krotkiewski, 2000). Previous results have indicated that changes in energy metabolism in response to energy restriction are also of considerable importance in antagonizing weight loss and promoting weight regain. The concentrations of serum thyroid hormone and thyroid-stimulating hormone are apparently preserved in obese subjects but decrease during caloric restriction (Roti et al., 2000). A recent study in obese dogs (Daminet et al., 2003) was focused on thyroid function. These authors concluded that obese dogs were euthyroidic and had slightly higher total T4 values than lean dogs, but similar free T4 values. However, the study was conducted in experimental conditions and did not include dogs with naturally acquired obesity.

Evidence of abnormal prolactin secretion in obese subjects has been reported in humans (Kopelman, 2000) but appears to be a consequence of hypothalamic–pituitary axis dysfunction rather than a primary event. Obesity is also an important factor predisposing to type 2 diabetes mellitus and the vast majority of obese individuals are insulin-resistant (Polonsky, 2000). The increase of insulin secretion and impairment of its action leads to an alteration in energy metabolism and contributes to chronic hyperglycaemia. Glucose intolerance and hyperinsulinaemia are claimed to be associated with obesity in dogs as in human patients (Buffington, 1994). Gayet et al. (2004) showed in dogs that during a period of overfeeding, a reduction in insulin sensitivity became apparent which predisposed obese animals to diabetes, insulin resistance being known to predict the onset of type 2 diabetes (Stumvoll et al., 2005). Loste and Marca (2001) had found a significant correlation between glucose concentration and serum fructosamine, the measurement of fructosamine could provide a simple test for evaluating the risk of diabetes mellitus in dogs.

Very few studies have been carried out in dogs to investigate spontaneous endocrine alterations in obese individuals. The aim of the present study was therefore to look at the incidence of hormonal disorders associated with naturally acquired overweight. The effect of naturally acquired obesity on cortisol, IGF-1 and prolactin secretion in dogs was investigated. Hypothalamic–pituitary axis dysfunction was explored by prolactin measurement. The impairment of insulin secretion and chronic hyperglycaemia was assessed by fructosamine measurement. Also as thyroid hormones are strong regulators of basal metabolism, the thyroid status of obese dogs was assessed by a combined assay of thyroid-stimulating hormone (TSH) and baseline fT4.

**Material and methods**

**Animals**

Overweight dogs with no apparent clinical signs of hormonal disorders except obesity were enrolled in the trial after complete agreement of the owners. The dogs were not receiving any medication at the time of the study. Overweight dogs with any sign of clinical abnormalities or receiving any medication were excluded from the study.

Each dog was weighed and the target body weight, based on the breed, height at shoulder and general shape of the animal, was determined (Buffington and Toll, 2000).

**Methods**

Blood samples for the determination of plasma hormones and fructosamine were collected from all dogs by jugular venipuncture directly into 5-ml glass tubes containing lyophilized heparin (Venoject; Terumo Europe, Leuven, Belgium) and centrifuged within 1 h. Plasma aliquots were frozen at −20 °C in plastic tubes until assayed.

All assays used had been previously validated for use in dogs in our laboratory and the assay procedures were performed according to the manufacturer’s instructions. Reference ranges were established by using plasma samples from clinically normal dogs.

An adrenocorticotropic hormone (ACTH) stimulation test was performed in each dog by collecting blood samples for determination of plasma cortisol concentration before and 1 h 30 min after IM injection of 0.25 mg of ACTH (Synacthen®; Novartis Pharma, Malmaison, France). Cortisol concentration was determined by using a radioimmunoassay (RIA) method (Clinical Assay Gamma coat Cortisol 125I RIA kit, Diasorin, Stillwater, MN, USA) before (basal cortisol) and after ACTH injection. A cortisol concentration of >450 nm in the sample collected 90 min after ACTH administration was considered abnormal and consistent with hyperadrenocorticism.

Free thyroxine (fT4) concentration was determined by use of a previously validated immunoradiometric assay (Immunotech, Marseille, France). Free thyroxine was assayed with two kits: a direct RIA method and the modified equilibrium dialysis from Nichols (Free T4 by Equilibrium Dialysis, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), which is considered the gold standard but time-consuming
and expensive for routine tests. Blood samples were collected from 46 clinically healthy beagles (21 males, 25 females), between 1 and 5 years old. Results showed an exact parallelism between the fT4 assayed by both direct RIA and the modified dialysis methods. The correlation between the two tests was significant ($r = 0.88$ and $p < 0.01$). The RIA test was therefore chosen to measure fT4 concentration.

The TSH concentration was determined with a RIA method validated for use in dogs (Immagine Canine TSH, Diagnostic Product Corporation, Los Angeles, CA, USA). Thyroid disorders were assessed by considering a baseline fT4 concentration of <15 pm associated with a TSH concentration >0.5 ng/ml as abnormal and consistent with hypothyroidism (Siliart and Stambouli, 1997; Kemppainen and Behrend, 2001).

The IGF-1 concentration was assayed from plasma samples using a non-specific immunoradiometric kit based on two region-restricted affinities purified polyclonal antibodies (IGF-1 Irma, Nichols Institute Diagnostics). A concentration in the range of 60–200 ng/ml was considered normal for dogs for which the estimated optimal body weight was <15 kg, 110–290 ng/ml for an optimal body weight between 15 and 25 kg and 140–290 ng/ml for an optimal body weight between 26 and 40 kg (Gayet et al., 2003).

The serum prolactin concentration in the sample was measured using a highly specific canine enzyme immunoassay (EIA) method (Milenia Biotech, Bad Nauheim, Germany). A prolactin concentration below 6 ng/ml was considered normal (Siliart, 1998).

Fructosamine concentration was measured with a commercial kit (Sigma Diagnostics, Chimie SARL, St Quentin Fallavier, France). The normal reference range for fructosamine concentration was set at 207–340 µM (Reusch et al., 2002).

Calculations and statistical analysis

Dogs were divided into groups on the basis of their relative body weight (RBW) that is the current weight divided by the estimated optimal weight (Burkholder and Toll, 2000). In this 5-point scale, when the RBW ranged from 1.1 to 1.19, the corresponding body condition score (BCS) was 4 (obese dog) and when the RBW was >1.2, the corresponding BCS was 5 (massively obese dog). The dogs were also categorized according to age (C1: dogs <5 years old, C2: dogs between 5 and 7 years old, C3: dogs >7 years old) and hormonal status (see Results).

Possible hormonal disturbances were diagnosed by comparing the hormone measurements with the reference ranges.

Statistical analysis was performed with statistical software (Statview 4.1; SAS Abacus Concept, Inc, Berkeley, CA, USA). All data were expressed as mean ± SEM. The Mann–Whitney non-parametric U-test was used to examine the effect of overweight on the measured variables. The chi-square test was used for the categorical variables. P-values of <0.05 were considered significant in all the analyses.

Results

Thirty-one dogs were included in the study. The mean estimated excess weight was 27 ± 12% of initial body weight and the mean age was 7 ± 3 years (Fig. 1). The enrolled dogs were of various breeds. About 74% were females and 58% were castrated animals of either sex (Table 1).

The body weight excess varied significantly with age ($p = 0.02$; Fig. 1) and gender (male, female or neutered dogs; $p = 0.05$; Table 1). The RBW excess was higher in castrated animals than in entire ones (1.32 ± 0.03 vs. 1.22 ± 0.03 and $p = 0.02$). The fT4, TSH, cortisol, IGF-1, fructosamine and prolactin concentrations did not vary significantly with body weight excess (Table 2). The mean values of IGF-1 and prolactin did not vary according to gender.

Five of 31 dogs exhibited some adrenocortical disturbances. Only four of 31 dogs showed hyperreactivity of the adrenals after ACTH injection. The cortisol concentration was below the limit of detection of the test in one dog.

An increase of prolactinaemia was observed in seven of 31 dogs, and of IGF-1 in six of 31 dogs. The fructosamine concentration in 20 of 31 dogs was >340 µM.

Fig. 1 Boxplots of obese dogs. For each boxplot, T-bars represent the main body of data, which is equal to the range; the horizontal bar represents the median. BCS4: $n = 11$ dogs; BCS5: $n = 20$ dogs, $p = 0.01$. 
Thirteen dogs exhibited normal thyroid function (diagnosis based on both TSH and fT4 values; Table 3). The TSH concentration in 15 of 31 dogs was >0.5 ng/ml (Table 3). Eleven of these dogs showed hyposecretion of fT4, whereas in the other four, the concentrations of fT4 were normal. Another group of dogs exhibited normal TSH values but low fT4 concentrations. The fT4 concentrations for this group did not differ from those of the group of hypothyroid (HOI) dogs. Based on the combined results for TSH and fT4 the dogs could be divided into four groups: normal group (N group; normal for thyroid function, TSH < 0.5 ng/ml and fT4 > 15 pm), normal? group (N? group; normal TSH but low fT4 concentration, TSH < 0.5 ng/ml and fT4 < 15 pm), HOI group (TSH > 0.5 ng/ml and fT4 < 15 pm) and discordant group (DIS group; high TSH concentration but normal fT4 concentration, TSH > 0.5 ng/ml and fT4 > 15 pm). The N and N? groups differed statistically from the HOI and DIS groups for TSH (p < 0.01) and the N and DIS groups statistically differed from the N? and HOI groups for fT4 (p < 0.01; Table 3).

No hormonal disturbances were detected in six of 31 dogs: four castrated females, one female and one male.

**Discussion**

The aim of the study was to determine the hormonal changes associated with obesity in dogs with naturally occurring excess weight. Whether these abnormalities are primary events or not remains a matter of debate. Most studies on obese dogs have used animals that became obese after a relatively short period of time under a specific fattening protocol. In the present study, the mean age of the dogs was 7 years, with a maximum of 12 years. Ten dogs (32%) were 10–12 years old and had been obese for many years. So, the observed hormonal disturbances reflected the condition of obese dogs in clinical practice.

As in the Robertson’s (2003) study, neutering was confirmed to be an important risk factor for obesity (p = 0.05) as more than half the dogs (18) had been castrated. Neutered animals (both male and female) represent approximately 25% of the total medically treated canine population in France. The relative number of castrated animals was higher in the present study. Hypotheses about the role of neutering (which induces a defect in the metabolic actions of the sex hormones oestrogen, testosterone and progesterone) are numerous and imply an increased food consumption or decreased metabolic rate. However, the present study was not designed to elucidate the role of castration in obesity. Panciera (1994) reported that neutering was the most significant gender-associated risk factor for the development of hypothyroidism, but our results did not confirm this (chi-square, p = 0.99).

Twenty of 31 dogs (50%) showed an increase in serum fructosamine (>340 μM). The fructosamine concentration ranged from 222 to 511 μM (median 347 μM) and was similar to that found by Reusch...
et al. (2002). Our results confirm that the risk of type 2 diabetes mellitus is increased in obese dogs as in humans. However, no relationship was detected between serum fructosamine concentration and thyroid function (p = 0.92) although such a relationship had been suggested by Reusch et al. (2002). Elevated plasma fructosamine appears to be a consequence of obesity but this modification remained inconstant in our study and 35% of the dogs had normal serum fructosamine concentrations.

We had previously shown that IGF-1 increased when body weight increased in castrated male beagle dogs (p < 0.01) and was correlated to postprandial insulin (Gayet et al., 2004). We found here that serum IGF-1 was increased in only six of 31 dogs (<20%) and was not correlated with overweight (p = 0.79). The six dogs included two females, three neutered females and one castrated male. The IGF-1 values in all male dogs always remained within the normal range. Whether IGF-1 changes are gender-linked needs further investigation. The elevated plasma IGF-1 appeared to be a consequence of obesity but remained inconstant in the present study and did not confirm an effect of increased body mass (fat) on its secretion (Hausman et al., 2001). Douyon and Schteingart (2002) also reported normal IGF-1 concentrations in obese humans.

The basal plasma cortisol concentration was never increased even in massively obese dogs (p = 0.40). Only four of 31 dogs showed hypersecretion of cortisol after ACTH injection. All the animals were free of any clinical signs except for body condition. Two of four dogs with increased cortisol showed severe disturbances of thyroid function and the adrenal reactivity could be a consequence of thyroid imbalance. This finding seems to indicate that disorders of the adrenals are not dominant in obese dogs. Gayet (2004) studied the modifications occurring when body weight increased in nine neutered female beagle dogs. She also showed that basal plasma cortisol remained unchanged during the fattening period.

Thyroid function was the most affected endocrine function. Although various endocrine tests are available for the diagnosis of canine hypothyroidism there is no consensus at present. Nevertheless, the combined evaluation of fT4 and TSH has seemed useful for the diagnosis of hypothyroidism in dogs (Peterson et al., 1997; Kantrowitz et al., 2001; Kemppainen and Behrend, 2001). Our results revealed that 11 of 31 dogs suffered from hypothyroidism as diagnosed by high TSH and low fT4 measurements (Kemppainen and Behrend, 2001). All the dogs in the present study were solely obese and less active, and according to their owners, their appetite was not excessive. Nevertheless, clinical signs are associated with hypothyroidism because any thyroid hormone deficiency affects all body functions. In addition to the changes noted for thyroid hormones and TSH, laboratory investigations have revealed other alterations such as high serum cholesterol, and prolactin concentrations and abnormal serum enzymes (Surks and Ocampo, 1996). In the present study, the dogs with high TSH concentration and low baseline fT4 concentration were obese but showed no other clinical signs. Subclinical hypothyroidism is a relatively common disorder in humans that also occurs in asymptomatic individuals (Surks and Ocampo, 1996). Subclinical hypothyroidism is defined biologically by a high TSH concentration in association with either normal or decreased serum T4, serum T3 or serum fT4. The HOI and DIS groups both corresponded to the above definition, i.e. 15 dogs in all. Both had high TSH concentrations associated with normal or low serum fT4. Although the elevated TSH together with low fT4 concentration indicated a high possibility of hypothyroidism (Kemppainen and Behrend, 2001), interpretation of the DIS group results remained ambiguous. Approximately 10% of euthyroid dogs exhibit a high TSH value (Daminet et al., 2003). Confirmation of hypothyroidism will only be possible from the follow up of these dogs. The results for the N? group (three dogs) were also difficult to interpret. In this group, one dog showed hyperactivity of the adrenals after ACTH injection, which could explain the false-positive result, but adrenal function in the other two was normal. These two dogs had TSH concentrations of 0.2 and 0.3 ng/ml and low fT4 concentration of 9.0 and 4.0 μm respectively. As the canine TSH assay is known to give a significant percentage of false-positive and false-negative results, and the fT4 concentrations are less affected by non-thyroidal illness than the total T4 assay (Kemppainen and Behrend, 2001), the above results may indicate a high likelihood of hypothyroidism. We can therefore conclude that 42% of our dogs (13 of 31 dogs) suffered from hypothyroidism and that the alteration of thyroid function is a primary event in obesity. This finding suggests that dogs should be treated for this condition in addition to receiving a hypocaloric diet.

As prolactin is increased in hypothyroidism and can be abnormal in obese patients, we investigated the possible impairment of prolactin secretion in obese dogs. Six of 31 dogs presented hyperprolactinaemia
and there was no relationship between hyperprolactinaemia and hypothyroidism (chi-square, p = 0.88).

In conclusion, the above results revealed the high incidence of hormonal disturbances, especially with regard to thyroid function, in obese dogs apparently free from any other clinical signs. Some of the modifications that we observed in naturally obese dogs seemed to be primary events (hypothyroidism) and others were adaptive (elevated IGF-1 and fructosamine). These findings demonstrate the importance of investigating endocrine function during the initial evaluation of such dogs and before administering any treatment.

References


