Thyroid evaluation in suspicious hypothyroid adult dogs before and after treatment

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Abstract

The purpose of this study was to measure circulating TSH, T₄ and fT₄ concentrations in dogs submitted to a clinical visit for general symptoms (weight gain, polyuria and polydipsia, changes in hair coat). Twenty-eight dogs, 14 cross-breed and 14 purebreds (Golden Retriever, Labrador, Doberman), of both sexes (14 males and 14 females), aged 8 to 14 years, were assessed. No significant differences of circulating TSH, T₄, fT₄ concentrations between the baseline and after therapeutic treatment nor between intact and neutered females were observed. Compared to baseline values, intact males showed higher TSH concentrations (p<0.01), and castrated males lower TSH concentrations (p<0.01) after therapeutic treatment. Compared to intact males, castrated males showed baseline TSH concentrations higher (p<0.01), but lower (p<0.01) after therapeutic treatment. No significant differences of T₄ and fT₄ concentrations between baseline conditions and after therapeutic treatment, nor between intact and castrated males, were observed. The experimental sample considered in this study falls within that casuistry involving elevated TSH concentrations but low serum T₄ and fT₄ concentrations or close to the minimum physiological cut-off, in which the common clinical signs suggestive of hypothyroidism was, essentially, overweight and neglected appearance of the hair.

Key words: dog, hypothyroidism treatment, free Thyroxine, Thyroxine, TSH
Introduction

Thyroid hormones (THs) are essential for survival, regulating the metabolism of all tissues, so their circulating concentrations change to adapt to the different metabolic needs (Reimers et al. 1984, 1986, 1990). Changes in circulating THs and thyroid stimulating hormone (TSH) concentrations according to breed, body size, age, and sex have been previously recorded (Shiel et al. 2007, 2010, Piechotta et al. 2010, Hegstad-Davies et al. 2015, Shiel et al. 2021, Taszkun et al. 2021). For this reason, breed-specific laboratory reference intervals, for assessing the dog’s thyroid functionality in both physiological or pathological condition, could be considered (Daminet et al. 2003, Segalini et al. 2009, Köhler et al. 2012, Oikonomidis et al. 2021). Moreover, there are, therefore, numerous physiological conditions that can alter especially circulating thyroxine (T4) and free thyroxine (fT4), along with TSH concentrations in euthyroid dogs. However, circulating THs of individuals belonging to some breeds may be physiologically lower than those of other breeds. Thus, concentrations of T4 in euthyroid dogs of the Greyhound (Gaughan and Bruyette 2001, Hill et al. 2001, Shiel et al. 2007, Pinilla et al. 2009), Alaskan and Siberian Husky (Panciera et al. 2003, Evason et al. 2004, Lee et al. 2004), Whippet (van Geffen et al. 2006), Basenji (Seavers et al. 2008) and Sloughi (Panakova et al. 2008) breeds may be lower than the reference ranges of most laboratories. In general, higher T4 concentrations have been observed in dogs of small breeds than those of medium to large breeds (Schachter et al. 2004).

With regard to age, T4 concentration is higher in newborn puppies, and decreases with age, with a decrease of 50% of T4 concentrations in dogs 6-11 years old in comparison to dogs 1-6 weeks of age (Reimers et al. 1988, 1990).

Relative to sex and reproductive status, T4 concentrations differ between male and female dogs only in the case of diestrus or pregnant females, which exhibit higher serum T4 concentrations, in baseline conditions and after stimulation with TSH, than both males and females in proestrus, anestrus, or lactation (Reimers et al. 1984). Moreover, castration of male dogs does not change T4 and TSH concentrations (Günzel-Apel et al. 2009). Thyroid gland disorders may sometimes affect fertility in female and male dogs (Panciera et al. 2007, Segalini et al. 2009, Sontaset al. 2014); nevertheless, no significant differences in T4 concentrations between fertile and hypofertile subjects were recorded (Segalini et al. 2009), and correlation between thyroid profile, testosterone concentration and semen parameters were recently observed in dogs. (Quartuccio et al. 2021).

It is also important to assess and know the iodine content of commercial and home diets of dogs with reduced thyroid function. Indeed, excess iodine (>150 μg/day) inhibits TH synthesis and secretion (Wolff-Chaikoff effect), resulting in reduced circulating T4 and fT4 concentrations associated with increased TSH concentrations, i.e., a condition of primary hypothyroidism (Castillo et al. 2001). In contrast, less well known are the effects on thyroid function tests in dogs related to the administration of a prolonged diet of soybeans high in phytoestrogens that can inhibit 5'-iodothyronineadidoxidase responsible for the conversion of T3 to T4. It has, in fact, been documented that dogs fed for more than a year on soybeans high in phytoestrogens can have increased T4 concentrations (Cerundolo et al. 2009).

It is well known, however, that concentrations of TSH, T4, and fT4 vary throughout the day (circadian variations), with the highest T4 and fT4 concentrations in euthyroid dogs between 11 a.m. and 2 p.m. (Hoh et al. 2006). The pulsatile nature of TSH secretion is more evident in hypothyroid dogs than in euthyroid dogs, with concentrations within the reference range at certain times of the day (Kooistra et al. 2000).

In the presence of heterogeneous and often generic data, mainly related to an almost asymptomatic course of some dysendocrinias, it seemed, therefore, interesting to provide an additional scientific contribution, directed to the comparison of changes of TSH, T4, and fT4 profile in suspicious hypothyroid dogs housed in a domestic environment, according to age, sex (male/female, intact/neutered subjects), pure or crossbreed and Body Condition Score (BCS).

Materials and Methods

Animals

The research complied with the guidelines for Good Clinical Practices (EMEA 2000) and the Italian and European regulations on animal welfare (D.L. 26/2014; Directive 2010/63/EU 2020). Ethical approval was not required for this type of study as animals submitted for medical examination were used. Written informed consent was obtained from the owners for the participation of their animals in this study. Owner consent was obtained prior to sample collection, and owners received a copy of all laboratory test results.

The study was conducted on a total of 28 dogs, 14 cross-breeds and 14 purebreds (Golden Retriever, Labrador, Doberman), of both sexes (14 males and 14 females), aged 8 to 14 years, referred for suspected hypothyroidism. All owners had first noted clinical signs 2 and 4 months before examination. Clinical signs
reported by owners included weight gain, polyuria and polydipsia, changes in hair coat (dryness, dandruff, excessive shedding, non-pruritic hair thinning).

The recruitment, enrolment, sample collection and analysis carried out during the routine investigation of clinical cases at the “Veterinary Wellness Center – My Sweet Pets”, for companion and unconventional animals, Aci S. Antonio, Catania, Italy. Animal inclusion criteria were based on animal’ history, physical examination to exclude pathological conditions, and biochemical and haematological tests were previously performed in all subjects to exclude systemic alterations. None of the dogs were receiving any drug supplements administered within 3 months prior to the blood sampling, and management factors such as diet, veterinary and husbandry cares were similar for all dogs to minimise the impact of heterogeneous factors.

The subjects were divided into two groups according to gender. Group I included 14 females (7 intact and 7 neutered) and Group II included 14 males (7 intact and 7 neutered). Particular attention was paid to changes in body weight and BCS, since the study subjects examined in this experimental study had, as a common symptom, mild to moderate body weight gain, so much so that owners brought their pets in for examination, along with polyuria, polydipsia and hair opacity.

All subjects had free access to water, and food, represented exclusively by commercial “Adult” dog-size-specific feed, was given twice daily; in addition, they were given only fruit, especially apples, as a reward or “snack”. The subjects lived in a home environment without cohabitation with other animals and were taken outside 4 times a day for about 30 minutes.

**Blood samples, analyses, BCS and body weight evaluation**

Blood samples were collected from all dogs by syringe from the cephalic or radial vein, under baseline conditions, in the morning from 9.00 to 10.00 during the routine veterinary check-up, as all subjects exhibited mild to moderate body weight increase. Samples were tested for TSH, T₄ and fT₃ concentrations and analyses were performed in duplicate on serum.

Serum TSH concentrations were assayed using a homologous solid-phase, chemiluminescent enzyme immunoassay (Immulite VR 2000 Canine Total Thyroxine, Siemens Medical Solutions-Diagnostics-USA), according to the manufacturer’s instructions. The intra-assay coefficient of variation (CV) and inter-assay CV were 10.8% and 4.4%, respectively, 13.8% and 6.8% at TSH concentrations of 0.65 and 3.84 mg/dL respectively. The lowest detectable amount of TSH was 0.12 mg/dL.

Serum T₄ concentrations were assayed using equilibrium dialysis, Nichols Institute Diagnostic, San Clemente, CA, according to the manufacturer’s instructions. The intra-assay CV was 8.3% at T₄ concentrations of 2.0 ng/dL determined in canine serum. The inter-assay CVs were 8.7% and 6.9% at T₄ concentrations of 1.8 and 6.1 ng/dL, respectively, for canine serum. As determined by the manufacturer, the lowest detectable amount of T₄ was 0.15 ng/dL.

Hematochemical examination was performed on plasma by colorimetric reading with a spectrophotometer (Sirio S, Radim/Seac Co., Rome, Florence, Italy).

Hemochromocytometric examination on EDTA-supplemented whole blood was performed with the aid of an automatic cell counter with impedance or volumetric principle (SEAC) for the determination of direct parameters (RBC – Red Blood Cell, WBC – White Blood Cell, Plt – Platelet). Total hemoglobin (Hb) was determined by photometric reading. In addition, the main erythrocyte constants (MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentration) and the hematocrit value (PCV – Packed Cell Volume) were calculated.

Subsequently, subjects who exhibited TSH, T₄ and fT₃ concentrations not within the physiological ranges for the dog for whom a diagnosis of primary hypothyroidism was issued, were administered adequate replacement therapy with Levothyroxine sodium/Leventa – a veterinary specialty, once a day and their response to treatment was evaluated. The dose recommended was paired to 20µg/kg every 24 hours, 2/3 hours before meals for liquid formulation of Leventa, or 15-20 µg/kg every 12 hours: once good disease control is achieved, one can switch to administration every 24 hours this applies to tablets. The therapy was last a lifetime. Although routine follow-up was required after 15-20 days of therapy to adjust and correct the dosage if necessary, it was possible to follow up only some of the above patients after 45 and 60 days, and again after 8 and 12 months, thanks to the owners’ willingness and cooperation. Afterwards, blood sampling
and follow-up were performed after 6 months. All treated subjects returned to a healthy weight, and therapy was modified according to the norm weight achieved.

The BCS was assessed in order to evaluate the current state and changes over time, together with body weight, assessed using an electronic digital scale (Wunder, Monza, Italy). The determination of body weight (BW), with median values paired to 25±5 kg, was measured on fasted animals, in the morning at 9:00 am, using a digital scale.

Although different scoring systems may have specific merits for different situations, it was preferred to choose a single system followed by all doctors and facility staff, recording the total points on which it is based on a 9-point scale system. The Body Condition Score (BCS) ranged between 7 and > 8, was evaluated by a visual assessment and palpation adopting a 9-point scale system. Four classes of BCS were considered: BCS 1 to 3 – lean dog, BCS 4 and 5 – ideal dog, BCS 6 and 7 – overweight dog, and BCS > 8 – obese dog (Laflamme et al. 1997, Ricci et al. 2007).

The goal for most companion animals is a BCS of 4-5 out of 9; however, these values may appear to be associated with a perception of excessive thinness for some owners, which is why it is important to educate the owners.

Statistical analysis

Statistical analysis of observed changes for comparison between the two groups under study (females and males), and between whole and castrated and sterilized subjects was performed by t-test for paired data using SAS/STATVR software (SAS Institute 2017) and differences were considered significant if p<0.05. Results were reported as means ± standard deviation of the mean.

Results

TSH

Results obtained (Fig. 1) showed that the mean serum TSH concentrations were higher than physiological ranges recorded in the literature for the dog (Kaneko et al. 1997, Greco et al. 2004).

Specifically, intact females (Group I) exhibited baseline mean serum TSH concentrations of 4.42±1.06 (ng/mL), while neutered females exhibited lower values of 3.43±0.80 (ng/mL). At follow-up after 6 months of treatment intact females showed mean serum TSH concentrations of 3.85±1.05 (ng/mL), while neutered females exhibited lower values paired to 3.07±1.01 (ng/mL). No significant differences of TSH concentrations between baseline and after therapeutic treatment nor between intact and neutered females were observed.

Intact males (Group II) exhibited baseline mean serum TSH concentrations of 3.95±1.06 (ng/mL), while neutered males exhibited almost doubled values, with concentrations of 6.83±1.96 (ng/mL). At follow-up after 6 months of treatment intact females showed mean serum TSH concentrations of 6.76±1.25 (ng/mL), while castrated males exhibited the lowest values paired to 4.20±0.93 (ng/mL).
Compared to baseline values, intact males showed higher TSH concentrations (p<0.01), and neutered males lower showed TSH concentrations (p<0.01) after therapeutic treatment.

Compared to intact males, neutered males showed baseline TSH concentrations higher (p<0.01), but lower (p<0.01) after therapeutic treatment.

**Total Thyroxine (T₄)**

Results obtained (Fig. 2) showed that mean serum T₄ concentrations were within the physiological reference ranges reported for the dog (Kaneko et al. 1997, Feldman and Nelson, 2004).

More specifically, intact and neutered females (Group I) exhibited almost overlapping baseline mean serum T₄ concentrations of 1.83±0.82 (µg/dL) and of 1.88±0.74 (µg/dL), respectively.

At follow-up after 6 months of treatment, intact females exhibited serum T₄ concentrations of 1.97±0.55 (µg/dL), while neutered females exhibited concentrations of 1.78±0.65 (µg/dL).

No significant differences between T₄ concentrations in baseline conditions and after therapeutic treatment, nor between intact and neutered females, were observed.

Intact and neutered males (Group II) exhibited almost overlapping mean serum T₄ concentrations of 1.78±0.60 (µg/dL) and 1.73±0.51 (µg/dL), respectively.

After 6 months of treatment, intact dogs exhibited serum T₄ concentrations paired to 2.06±0.46 (µg/dL), while neutered dogs exhibited values paired to 1.68±0.45 (µg/dL), and no significant differences in T₄ concentrations between baseline conditions and after therapeutic treatment were observed.

**Free Thyroxine (fT₄)**

Results obtained (Fig. 3) showed mean serum fT₄ concentrations within the physiological reference ranges recorded for the dog (Kaneko et al. 1997, Feldman and Nelson 2004).

More specifically, intact and neutered females (Group I) exhibited mean serum fT₄ concentrations of 0.93±0.58 (ng/dL), and of 0.99±0.33 (ng/dL), respectively.

At follow-up, performed after 6 months of treatment, both intact and neutered females exhibited almost overlapping mean serum superimposed fT₄ concentrations paired to 1.48±0.37 (ng/dL) and 1.50±0.43 (ng/dL), respectively.

No significant differences of fT₄ concentrations between baseline conditions and after therapeutic treatment nor between intact and neutered females, were observed.

Related males (Group II), intact and neutered dogs exhibited superimposed baseline serum fT₄ concentrations paired to 1.38±0.53 (ng/dL) and 1.42±0.40 (ng/dL), respectively.

At follow-up after 6 months of treatment, intact males exhibited serum fT₄ concentrations of 2.39±0.55 (µg/dL), while neutered males exhibited concentrations paired to 1.36±0.42 (µg/dL).
No significant differences of $fT_4$ concentrations between baseline conditions and after therapeutic treatment, nor between intact and neutered males, were observed.

Figures 4, 5 and 6 showed the trend of TSH, $T_4$ and $fT_4$ of patients in baseline conditions and after different time points of therapy.

**Discussion**

On the basis of the results obtained, it is possible to exclude a possible influence of circadian rhythm on TH trends, having carried out blood sampling always in the morning, from 9.00 to 10.00 a.m., both in baseline conditions and after therapeutic treatment.

The higher serum TSH concentrations detected, regardless of sex, confirm data previously reported in dogs with primary (Castillo 2001) and spontaneous hypothyroidism (De Lucia et al. 2010), with lower concentrations of $T_4$ and $fT_4$. In addition, the fact that TSH concentrations were not correlated to severity of illness is not surprising, confirming previous results (Kantrowitz et al. 2001, Money et al. 2008). Moreover, the most significant differences have been reported in the literature for females in diestrus and pregnancy, exhibiting higher $T_4$ concentrations than females in anoestrus and lactation, and also higher than males; moreover, the intact females in this study were all in anoestrus (Reimers et al. 1984).

However, the results obtained in males under baseline conditions, with significantly higher serum TSH concentrations in neutered than in intact subjects, do not fully confirm literature data in which the castration of males does not change circulating TSH and $T_4$ concentrations (Gunzel-Apel et al. 2009). Furthermore, again in males, the TSH trend exhibited an inverse trend after therapeutic treatment, with significantly higher serum TSH concentrations in intact than in neutered males. This trend could, however, be explained, and therefore be justified, by the daily administration of sodium Levothyroxine, which, after 6 months, evidently established and ensured positive feed-back in whole dogs, and negative feed-back in castrated dogs, which, under baseline conditions, had exhibited, respectively, lower values in the former and almost doubled TSH concentrations in the latter. This wide variability, found between the two groups, confirms the widely accepted finding that the activation of the hypothalamic-pituitary-thyroid axis also in the dog, as already documented in the cat, more so than in the human, depends on various intrinsic and extrinsic factors that can differentiate and modify these values (Panciera et al. 2003, Ferguson 2008, Seavers et al. 2008).
Regarding the age variable, based on the knowledge that the highest TH concentrations, especially T4, are described in puppies, with lower levels, up to 50% in adults, aged 6 to 11 years, when compared with 1- to 6-week-old puppies (Reimers et al. 1990), it should be pointed out that the data reported are exclusively for adult subjects, thus for a very homogeneous age group and, therefore, considered as a one group.
Finally, with regard to the breed variable, results obtained did not show statistically significant differences in THs between purebred and crossbred dogs, thus confirming that the reference ranges for THs are not entirely specific, as, for example, those for Greyhound dogs, which exhibit, instead, lower ranges than the physiological species ranges (Gaughan and Bruyette 2001); rather, often more than breed it is the size of the subject that affects and modifies the physiological ranges of total and free iodothyronines and, in particular, small dogs show higher TH concentrations than subjects of medium-large sizes (Schachter et al. 2004).

Results obtained and reported in this study met the proposed objectives and allow some observational and general considerations to be drawn about the relationships between thyroid function, suspicion of hypothyroidism, and the presence of symptomatology that is often too generalized, vague, and inconclusive, and not necessarily pathognomonic of a condition.

Moreover, the adjustments of TSH, T₄, and fT₄ profile in the dog, together with the mechanisms underlying their onset, can contribute to making diagnosis difficult, as soon as the non-thyroidal illness syndrome or the euthyroid sick syndrome. It is necessary, therefore, to cautiously evaluate the laboratory investigations that most frequently result in decreased serum T₄ and fT₄ concentrations, associated with physiologic TSH concentrations, in 20-40% of cases. In these cases, a diagnosis of hypothyroidism can be assumed only when striking symptoms such as hypercholesterolemia, arigenerative anemia, obesity, skin affections, etc. are present.

On the other hand, it cannot be ruled out that dogs with increased serum TSH, and nearly physiological fT₄ values, will experience a decline in thyroid function. These dogs, moreover, are not to be considered hypothyroid, but may become hypothyroid over time; in this case, it would be desirable to repeat thyroid function tests after 3-6 months.

Conclusions

The experimental sample considered in this study falls within that casuistry involving elevated TSH concentrations, and contemplating low serum T₄ and fT₄ values or close to the minimum physiological cut-off, in which the common symptomatology was, essentially, overweight and neglected appearance of the hair.

The final consideration of the results allows, therefore, to admit the existence of a differentiated thyroid response in the two dogs’ groups to the study, in relation to sex for serum TSH concentrations only, with a probable greater functional involvement in males than in females and, again, in intact males than in neutered ones after therapeutic treatment.

Unfortunately, the range of serum T₄ concentrations in hypothyroid dogs overlaps with that of healthy subjects, and this overlap becomes more apparent in euthyroid dogs with concomitant disease. The extent of residual thyroid function at the time of specimen collection, the suppressive effects of extraneous factors on serum thyroid hormone concentrations, and the presence of circulating anti-thyroid hormone antibodies are all factors that affect the sensitivity and specificity of serum T₄ concentrations in the diagnosis of hypothyroidism. We suggest using an arbitrary value of serum T₄ concentrations to distinguish euthyroidism from hypothyroidism. We commend that such a value be evaluated in the context of the history of the dog, clinical findings and other laboratory data so that an index of suspicion for euthyroidism or hypothyroidism can be developed.

It is more likely that the clinical signs and corresponding endocrinological tests need to be thoroughly evaluated in these patients to better understand whether integration of therapy is necessary, considering the costs for integration monitoring and daily medication. In all this, the cooperation of the owners is also of paramount importance for the successful outcome of the patient.

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