www.czasopisma.pan.pl PAN www.journals.pan.pl

Polish Journal of Veterinary Sciences Vol. 26, No. 1 (2023), 99–108

DOI 10.24425/pjvs.2023.145011

Original article

Thyroid evaluation in suspicious hypothyroid adult dogs before and after treatment

O. Bucalo[1], K. Satué[2], P. Medica[3], C. Cravana[3], E. Fazio[3]

[1] Pomezia Veterinary Hospital, Via dei Castelli romani 54, 00071 Roma, Italy
[2] Department of Animal Medicine and Surgery, CEU-Cardenal Herrera University, Tirant lo Blanc, 7, Alfara del Patriarca, 46115 Valencia, Spain
[3] Department of Veterinary Sciences, Veterinary Physiology Unit, Messina University, Polo Universitario Annunziata, Viale Palatucci 13, 98168 Messina, Italy

Abstract

The purpose of this study was to measure circulating TSH, T_4 and fT_4 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, T_4, fT_4 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 T_4 and fT_4 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 T_4 and fT_4 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

Correspondence to: P. Medica, e-mail: pmedica63@unime.it

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 (T_4) and free thyroxine (fT_4), 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 T_4 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 T_4 concentrations have been observed in dogs of small breeds than those of medium to large breeds (Schachter et al. 2004).

With regard to age, T_4 concentration is higher in newborn puppies, and decreases with age, with a decrease of 50% of T_4 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, T_4 concentrations differ between male and female dogs only in the case of diestrus or pregnant females, which exhibit higher serum T_4 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 changeT_4 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 T_4 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 T_4 and fT_4 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'-iodothyronineadeiodinase responsible for the conversion of T_4 to T_3. It has, in fact, been documented that dogs fed for more than a year on soybeans high in phytoestrogens can have increased T_4 concentrations (Cerundolo et al. 2009).

It is well known, however, that concentrations of TSH, T_4 and fT_4 vary throughout the day (circadian variations), with the highest T_4 and fT_4 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, T_4 and fT_4 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 weigh increase. Samples were tested for TSH, T_4 and fT_4 concentrations and analyses were performed in duplicate on serum.

Serum TSH concentrations were assayed using a homologous solid-phase, two-site chemiluminescent immunometric assay (Immulite VR 2000 Canine TSH, Siemens Medical Solutions-Diagnostics-USA), according to the manufacturer's instructions. Intra-assay CVs were 5%, 4% and 3.8% at TSH concentrations of 0.2, 0.5 and 2.6 ng/mL^{-1}, respectively. The inter-assay CVs were 6.3% and 8.2% at TSH concentrations of 0.16 and 2.8 ng/mL^{-1}, respectively. The sensitivity of the assay was 0.03 ng/mL^{-1}.

Serum T_4 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 T_4 concentrations of 0.65 and 3.84 mg/dL^{-1} respectively. The lowest detectable amount of T_4 was 0.12 mg/dL^{-1}.

Serum fT_4 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 fT_4 concentrations of 2.0 ng/dL determined in canine serum. The inter-assay CVs were 8.7% and 6.9% at fT_4 concentrations of 1.8 and 6.1 ng/dL, respectively, for canine serum. As determined by the manufacturer, the lowest detectable amount of fT_4 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 Corpuscolar Volume, MCH – Mean Corpuscolar Haemoglobin, MCHC – Mean Corpuscolar Haemoglobin Concentration) and the hematocrit value (PCV – Packed Cell Volume) were calculated.

Subsequently, subjects who exhibited TSH, T_4 and fT_4 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

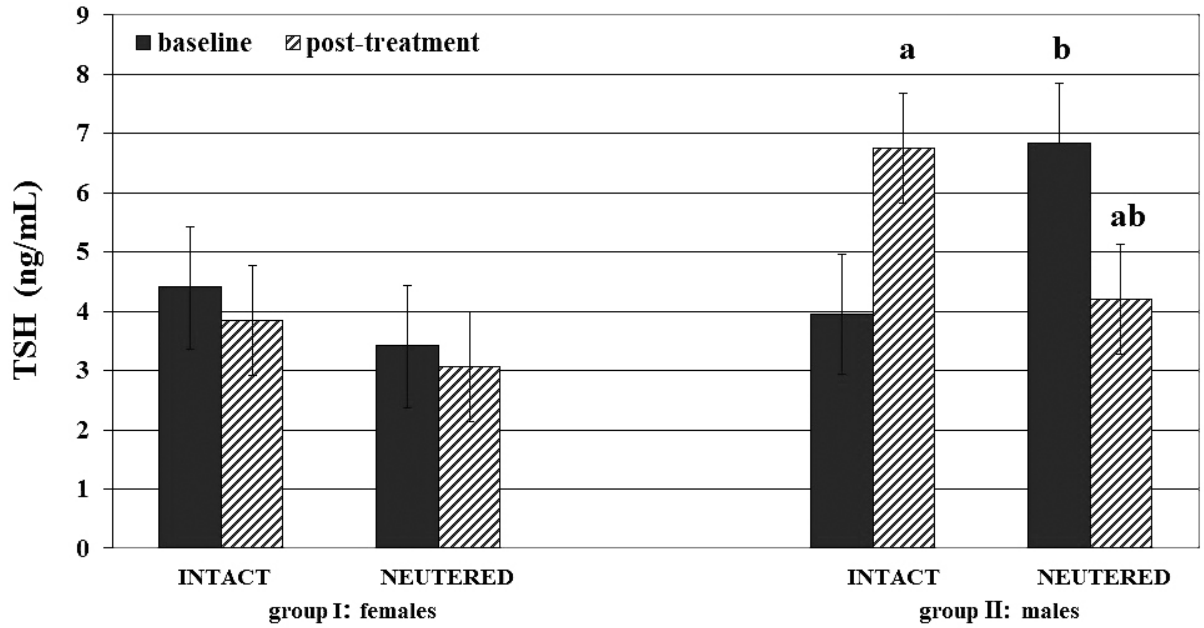

Fig. 1. Circulating TSH concentrations in two dog groups in baseline conditions and after therapeutic treatment with Levothyroxine sodium/Leventa.
Different superscripts indicate significant differences (*$p<0.01$) vs baseline conditions and vs whole.

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±0.84 (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, whole males 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).

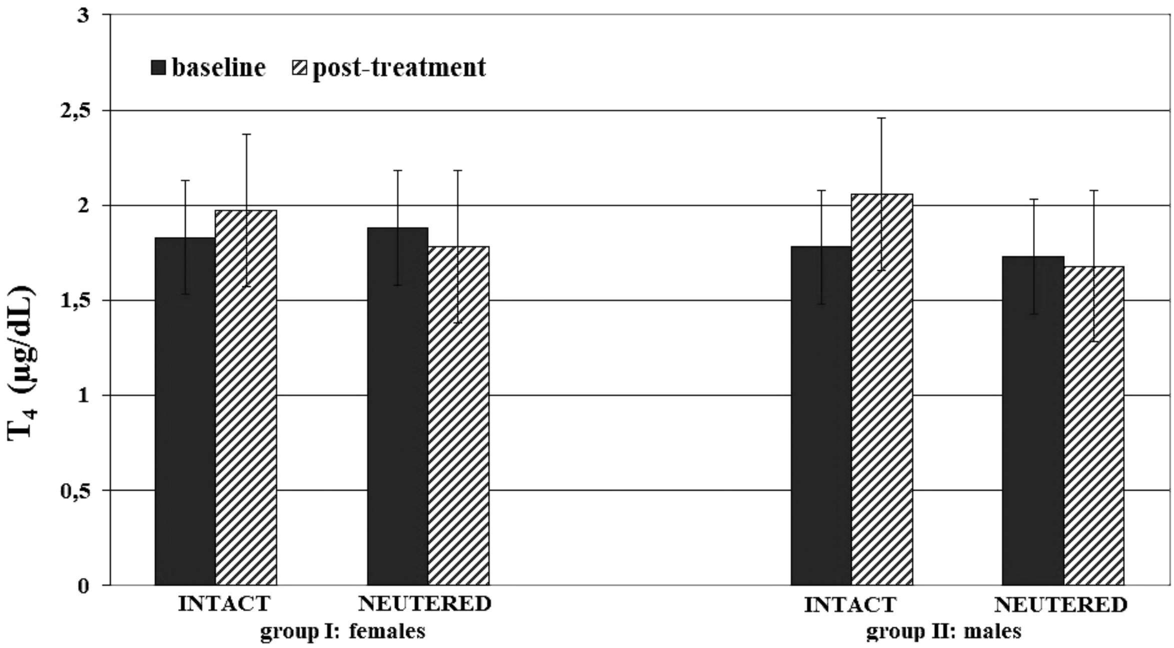

Fig. 2. Circulating T_4 concentrations in two dog groups in baseline conditions and after therapeutic treatment with Levothyroxine sodium/Leventa.

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 (T_4)

Results obtained (Fig. 2) showed that mean serum T_4 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 T_4 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 T_4 concentrations of 1.97±0.55 (µg/dL), while neutered females exhibited concentrations of 1.78±0.65 (µg/dL).

No significant differences between T_4 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 T_4 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 T_4 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 T_4 concentrations between baseline conditions and after therapeutic treatment were observed.

Free Thyroxine (fT_4)

Results obtained (Fig. 3) showed mean serum fT_4 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 fT_4 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 fT_4 concentrations paired to 1.48±0.37 (ng/dL) and 1.50±0.43 (ng/dL), respectively.

No significant differences of fT_4 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 fT_4 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 fT_4 concentrations of 2.39±0.55 (µg/dL), while neutered males exhibited concentrations paired to 1.36±0.42 (µg/dL).

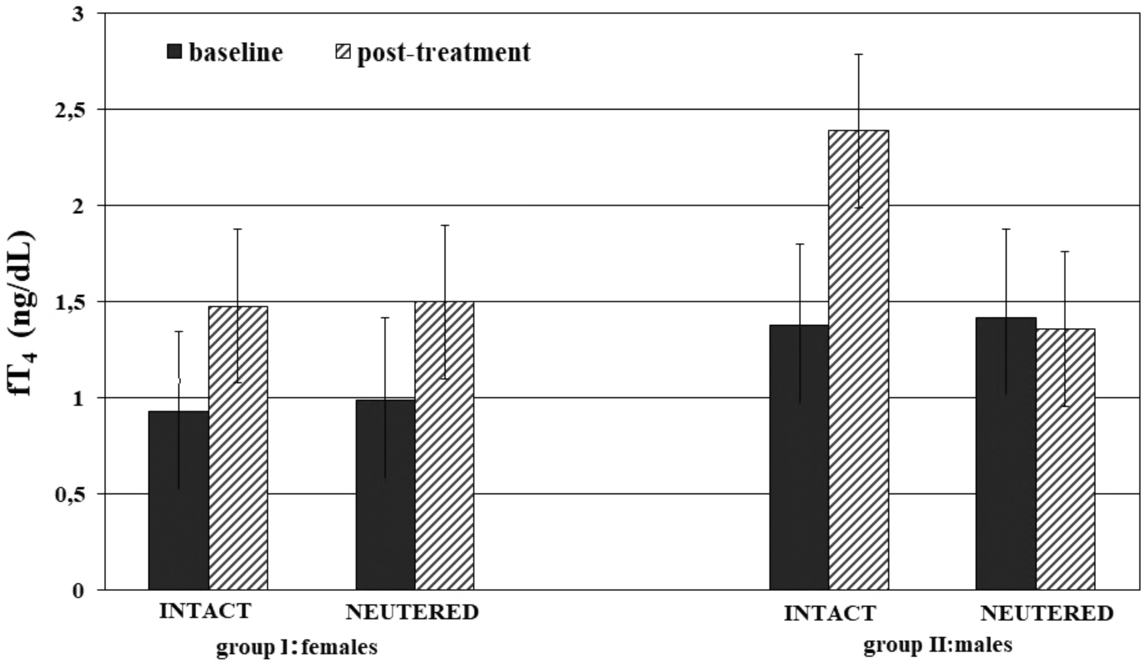

Fig. 3. Circulating fT_4 concentrations in two dog groups in baseline conditions and after therapeutic treatment with Levothyroxine sodium/Leventa.

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 more restrained thyroid response, described in these subjects, could find an explanation in a possible inhibition, probably a negative feed-back, of thyroid gland activity, documented precisely by significantly reduced concentrations or very close to the minimum physiological limits, reported for T_4 and fT_4 in the dog.

Regarding the sex variable, the results obtained for total and free thyroxine concentrations confirm the physiological reference ranges, which are almost superimposable between females and males (Reimers et al. 1984, 1986). 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 anaestrus and lactation, and also higher than males; moreover, the intact females in this study were all in anaestrus (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).

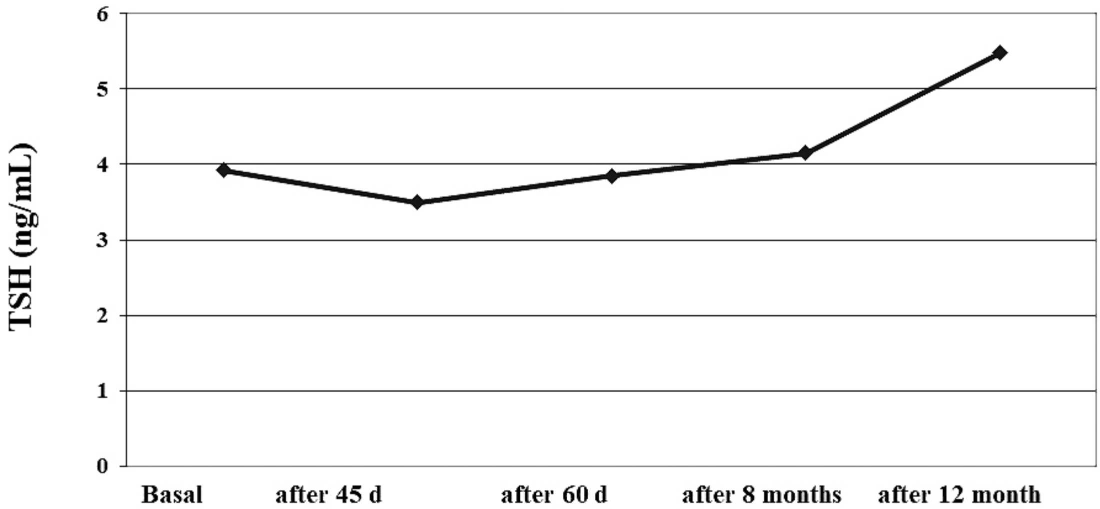

Fig. 4. Circulating TSH concentrations in dogs at follow-up.

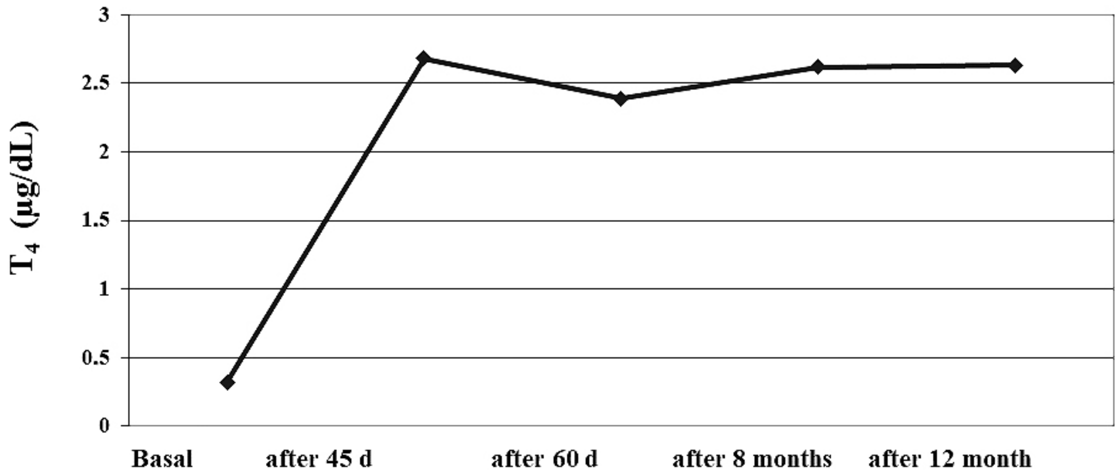

Fig. 5. Circulating T_4 concentrations in dogs at follow-up.

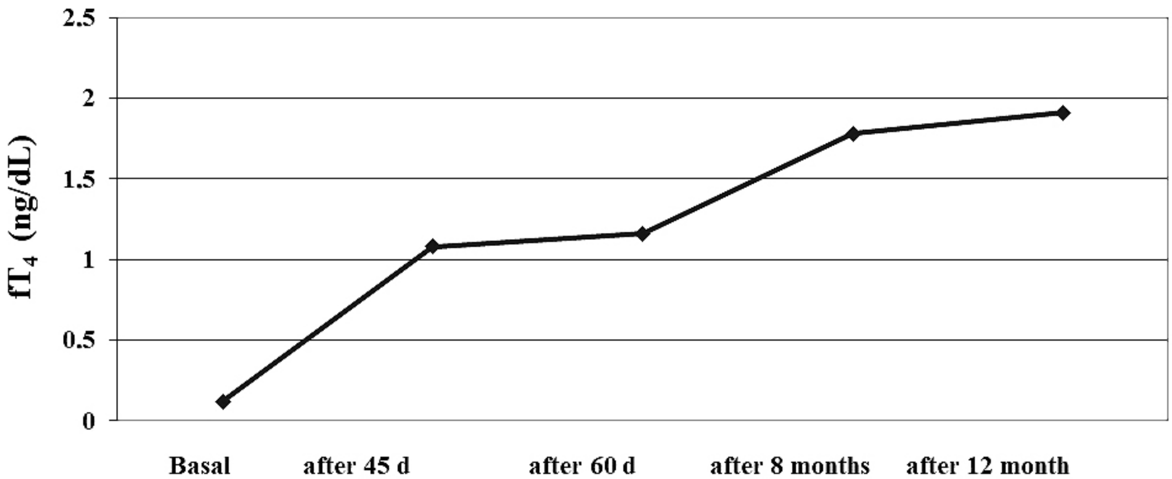

Fig. 6. Circulating fT_4 concentrations in dogs at follow-up.

Regarding the age variable, based on the knowledge that the highest TH concentrations, especially T_4, 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, T_4, and fT_4 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 T_4 and fT_4 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 fT_4 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 T_4 and fT_4 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 T_4 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 T_4 concentrations in the diagnosis of hypothyroidism. We suggest using an arbitrary value of serum T_4 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.

Acknowledgements

The authors would like to thank Dr. Francesca Aveni and Dr. Santo Fragalà, Medical Director of the "Veterinary Wellness Center - My Sweet Pets" for companion and unconventional animals, Aci S. Antonio, Catania, Italy, for the cooperation granted to the study and for the technical and laboratory assistance provided to the authors during the sampling.

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Castillo VA, Lalia JC, Junco M, Sartorio G, Marquez A, Rodriguez MS, Pisarex MA **(2001)** Changes in thyroid function in puppies fed a high iodine commercial diet. Vet J 161: 80-84.

Cerundolo R, Michel KE, Court MH, Shrestha B, Refsal K, Oliver JW, Biourge V, Shofer FS **(2009)** Effects of dietary soy isoflavones on health, steroidogenesis, and thyroid gland function in dogs. Am J Vet Res 70: 353-360.

Daminet S, Jeusette I, Duchateau L, Diez M, Van de Maele I, De Rick A **(2003)** Evaluation of thyroid function in obese dogs and in dogs undergoing a weight loss protocol. J Vet Med A Physiol Pathol Clin Med 50: 213-218.

De Lucia M, Caldin M, Fondati A **(2010)** Serum thyroxine (tT4), free thyroxine (fT4) and thyrotropin (TSH) concentrations in dogs: changes not related to spontaneous hypothyroidism. Veterinaria 24: 47-54.

Directive 2010/63/EU. **2020**. The European Parliament and of the Council of 22 September 2010. Protection of animals used for scientific purposes. OJL 2010, 276, 33-79. Available online: http://data.europa.eu/eli/dir/. 2010/63/oj (accessed on 20 July 2020).

Evason MD, Carr AP, Taylor SM, Waldner CL **(2004)** Alterations in thyroid hormone concentrations in healthy sled dogs before and after athletic conditioning. Am J Vet Res 65: 333-337.

Feldman EC and Nelson RW **(2004)** Canine and Feline endocrinology and reproduction. 3rd Edition, St. Louis, Saunders, pp. 135-136.

Ferguson DC **(2007)** Testing for Hypothyroidism in dogs. Vet Clin North Am Small Anim Pract 37: 647-669.

Gaughan KR, Bruyette DS **(2001)** Thyroid function testing in Greyhounds. Am J Vet Res 62: 1130-1133.

German AJ, Holden SL, Moxham GL, Holmes KL, Hackett RM, Rawlings JM **(2006)** A simple, reliable tool for owners to assess the body condition of their dog or cat. J Nutr 136 (Suppl 7): 2031S-2033S.

Greco DS **(2004)** Laboratory evaluation on of the thyroid, parathyroid, adrenal, and pathology glands. In: Thrall MA, Backer DC, Campbell TW, DeNicola D, Fettman MJ, Lassen ED, Rebar A, Weiser G (eds) Veterinary haematology and clinic chemistry. Wiley-Blackwell, Baltimora, USA, pp 445-456.

Günzel-Apel AR, Seefeldt A, Eschricht FM, Urhausen C, Kramer S, Mischke R, Hoppen HO, Beyerbach M, Koivisto M, Dieleman SJ **(2009)** Effects of gonadectomy on prolactin and LH secrection and the pituitary-thyroid axis in male dogs. Theriogenology 71: 746-753.

Hegstad-Davies RL, Torres SM, Sharkey LC, Gresch SC, Muñoz-Zanzi CA, Davies PR **(2015)** Breed-specific reference intervals for assessing thyroid function in seven dog breeds. J Vet Diagn Invest 27: 716-727.

Hill RC, Fox LE, Lewis DD, Beale KM, Nachreiner RF, Sundstrom DA, Jones GL, Butterwick RF **(2001)** Effects of racing and training on serum thyroid hormone concentrations in racing Greyhounds. Am J Vet Res 62: 1969-1972.

Hoh WP, Oh TH **(2006)** Circadian variations of serum thyroxine, free thyroxine and 3,5,3'triiodothyronine concentrations in healthy dogs. J Vet Sci 7: 25-29.

Kaneko J, Harney JW, Bress ML **(1997)** Blood analyte reference values in small and same laboratory animals (Appendix IX). In: Kaneko J, Harney JW, Bress ML (eds) Clinical biochemistry of domestic animals. Acad. Press, San Diego, p 896.

Kantrowitz LB, Peterson ME, Melian C, Nichols R (**2001**) Serum total thyroxine, total triiodothyronine, free thyroxine, and thyrotropin concentrations in dogs with nonthyroidal disease. J Am Vet Med Assoc 219: 765-769.

Kooistra HS, Diaz-Espineira M, Mol JA, van den Brom WE, Rijnberk A **(2000)** Secretion pattern of thyroid-stimulating hormone in dogs during euthyroidism and hypothyroidism. Domest Anim Endocrinol 18: 19-29.

Köhler B, Stengel C, Neiger R **(2012)** Dietary hyperthyroidism in dogs. J Small Anim Pract 53: 182-184.

Laflamme D **(1997)** Development and validation of a body condition score system for dogs. Canine Pract 22: 10-15.

Lee JA, Hinchcliff KW, Piercy RJ, Schmidt KE, Nelson S Jr **(2004)** Effects of racing and nontraining on plasma thyroid hormone concentrations in sled dogs. J Am Vet Med Assoc 15: 226-231.

Mooney CT, Shiel RE, Dixon RM (**2008**) Thyroid hormone abnormalities and outcome in dogs with non-thyroidal illness. J Small Anim Pract 49: 11-16.

Oikonomidis IL, Theodorou K, Papaioannou E, Xenoulis PG, Adamama-Moraitou KK, Steiner JM, Kritsepi-Konstantinou M, Suchodolski JS, Rallis T, Soubasis N **(2021)** Serial measurement of thyroid hormones in hospitalised dogs with canine parvoviral enteritis: Incidence of non-thyroidal illness syndrome and its association with outcome and systemic inflammatory response syndrome. Vet J 274: 105715.

Panakova L, Koch H, Kolb S, Mueller RS **(2008)** Thyroid testing in Sloughis. J Vet Intern Med 22: 1144-1148.

Panciera DL, Hinchcliff KW, Olson J, Constable PD **(2003)** Plasma thyroid hormone concentrations in dogs competing in a long distance sled dog race. J Vet Intern Med 17: 593-596.

Panciera DL, Purswell BJ, Kolster KA **(2007)** Effect of short-term hypothyroidism on reproduction in the bitch. Theriogenology 68: 316-321.

Piechotta M, Arndt M, Hoppen HO **(2010)** Autoantibodies against thyroid hormones and their influence on thyroxine determination with chemiluminescence immunoassay in dogs. J Vet Sci 11: 191-196.

Pinilla M, Shiel RE, Brennan SF, McAllister H, Mooney CT **(2009)** Quantitative thyroid scintigraphy in Greyhounds suspected of primary hypothyroidism. Vet Radiol Ultrasound 50: 224-229.

Quartuccio M, Fazio E, Medica P, Cristarella S, Emmanuele G, Sinagra L, Liotta L **(2021)** Correlation between sperm parameters and circulating thyroid hormones and testosterone concentrations in Labrador Retriever dog. Ital J Anim Sci 20: 947-954.

Reimers TJ, Lawler DF, Sutaria PM, Correa MT, Erb HN **(1990)** Effects of age, sex, and body size on serum concentrations of thyroid and adrenocortical hormones in dogs. Am J Vet Res 51: 454-457.

Reimers TJ, McGarrity MS, Strickland D **(1986)** Effect of fasting on thyroxine, 3,5,3'-triiodothyronine, and cortisol concentrations in serum of dogs. Am J Vet Res 47: 2485-2490.

Reimers TJ, Mummery LK, McCann JP, Cowan RG, Concannon PW **(1984)** Effects of reproductive state on concentrations of thyroxine, 3,5,3'-triiodothyronine and cortisol in serum of dogs. Biol Reprod 31: 148-154.

Ricci R, Gottardo F, Ferlito JC, Stefani A, Ravarotto L, Andrighetto I **(2007)** Body condition score (BCS) and metabolic status of shelter dogs. Ital J Anim Sci 6: 859-861.

SAS Institute **2017**. SAS/STATVR Users Guide, Version 9.3; SAS Institute Inc., Cary, NC, USA.

Schachter SS, Nelson RW, Scott-Moncrieff C, Ferguson DC, Montgomery T, Feldman EC, Neal L, Kass PH **(2004)** Comparison of serum free-thyroxine concentrations determined by standard equilibrium dialysis, modified equilibrium dialysis, and 5 radioimmunoassays in Dogs. J Vet Intern Med 18: 259-264.

Seavers A, Snow DH, Mason KV, Malik R **(2008)** Evaluation of the thyroid status of Basenji dogs in Australia. Aust Vet J 86: 429-434.

Segalini V, Hericher T, Grellet A, Rosenberg D, Garnier F, Fontbonne A **(2009)** Thyroid function and infertility in the dog: a survey in five breeds. Reprod Domest Anim 44 (Suppl 2): 211-213.

Shiel RE, Brennan SF, Omodo-Eluk AJ, Mooney CT **(2007)** Thyroid hormone concentrations in young, healthy, pretraining greyhounds. Vet Rec 161: 616-619.

Shiel RE, Nolan CM, Nally JE, Refsal KR, Mooney CT **(2021)** Qualitative and semiquantitative assessment of thyroid hormone binding proteins in greyhounds and other dog breeds. Domest Anim Endocrinol 76: 106623.

Shiel RE, Sist MD, Nachreiner RF, Ehrlich CP, Mooney CT **(2010)** Assessment of criteria used by veterinary practitioners to diagnose hypothyroidism in sighthounds and investigation of serum thyroid hormone concentrations in healthy Salukis. J Am Vet Med Assoc 236: 302-308.

Sontas BH, Schwendenwein I, Schaefer-Somi S **(2014)** Primary anestrus due to dietary hyperthyroidism in a miniature pinscher bitch. Can Vet J 55: 781-785.

Taszkun I, Milczak A, Kalisz G, Wilkołek P, Zwolska J, Szczepanik M **(2021)** Serum tT4, fT4 and TSH concentrations in German Shepherd dogs depending on age and type of work. Pol J Vet Sci 24: 63-68.

van Geffen C, Bavegems V, Duchateau L, De Roover K, Dainet S **(2006)** Serum thyroid hormone concentrations and thyroglobulin autoantibodies in trained and non-trained healthy whippets. Vet J 172: 135-140.