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Original article

Serum tT4, fT4 and TSH concentrations in German Shepherd dogs depending on age and type of work

I. Taszkun[1], A. Milczak[2], G. Kalisz[3], P. Wilkołek[1], J. Zwolska[4], M. Szczepanik[1]

[1] Subdepartment of Clinical Diagnostics and Veterinary Dermatology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głeboka 30, 20-612 Lublin, Poland
[2] Department and Clinic of Animal Internal Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głeboka 30, 20-612 Lublin, Poland
[3] Department of Biopharmacy, Faculty of Pharmacy, Medical University of Lublin, ul. W. Chodzki 4a, 20-093 Lublin, Poland
[4] Department and Clinic of Animal Surgery, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głeboka 30, 20-612 Lublin, Poland

Abstract

Serum concentration of thyroid hormones in healthy dogs varies according to age, sex, breed or professional activity. The aim of this study was to determine the influence of both age and dogs' work involvement on TSH and thyroid hormones values. Thyroid-stimulating hormone (TSH), total thyroxine (tT4) and free thyroxine (fT4) were tested in the serum of 57 healthy, German Shepherd dogs. The dogs were divided into study groups according to age: dogs aged 3 to 6 years (A), dogs over the age of 6 years (B) and involvement: police-working dogs (C) and accompanying animals (D). Mean values of TSH, tT4 and fT4 ranged from 0.19 to 0.31 ng/ml, 15.58 to 17.25 nmol/L and 11.83 to 17.89 pmol/L, respectively. The highest values of TSH were in group B and the lowest were in group A, while there was an inverse dependence in case of fT4 concentration. The highest mean values of tT4 were in dogs in group C and the lowest in group B. There were statistically significant differences in TSH (p=0.007) and fT4 (p=0.003) concentrations between the age groups. The results indicate that a dog's age is an important factor in the case of thyroid profile results interpretation.

Key words: canine hypothyroidism, thyroid, dog, thyroxine concentration

Correspondence to: M. Szczepanik e-mail: kryll@poczta.onet.pl, tel.: 814456186, fax: 814456598

Introduction

One of the factors that ensure proper metabolism of the body is the efficient functioning of the thyroid gland. The main task of thyroid cells is the production and secretion of two hormones: triiodothyronine-T3 and tetraiodothyronine-T4 (thyroxine) with an accelerating effect on metabolism. The most common endocrinopathy in dogs over the age of 5 is hypothyroidism, with a breed predilection in Golden Retriever, Doberman, Labrador, Hovawart, German Shepherd, Cocker Spaniel, English Setter (Dixon et al. 1999, Ślebodziński 2001, Boretti et al. 2003, Kennedy et al. 2006, Graham et al. 2007, Miller et al. 2013, Bianchi et al. 2015, Ahlgren and Uimari 2016, Böhm et al. 2017). Diagnosis of this disease in dogs is based on medical history, clinical signs and serum tests performed to establish specific markers of thyroid gland function (van Dijl et al. 2014, Hegstad-Davies et al. 2015, Randolph et al. 2015). Diagnosis of hypothyroidism made only on the basis of serum thyroid hormone concentrations is constantly being revised in the last years, as the results obtained by different authors are not always convergent and sufficiently reliable to determine the standards of laboratory tests (Peterson et al. 1997, Kemppainen and Birchfield 2006, Böhm et al. 2017). Published research results indicate that serum concentration of thyroid hormones in healthy dogs physiologically varies depending on age, sex and breed (Gaughan and Bruyette 2001, Lee et al. 2004, Shiel et al. 2007, Hegstad-Davies et al. 2015). A variety of different laboratory techniques and equipment used for examination of thyroid hormones in serum (radioimmunoassay, immunoradiometric, immunochemistry and immunoenzymatic) also seems still to be a substantial issue for fair serum test results (Shiel et al. 2007, Higgs et al. 2014). Thus, it is an important issue to determine relative physiological reference values depending on various factors e.g. age and way of use, due to their important influence on the concentration of thyroid hormones and related thyroid-stimulating hormone (TSH). The aim of the present study was to determine the correlation of chosen factors with serum concentrations of the aforementioned hormones.

Materials and Methods

The research was carried out in winter season in a group of German Shepherd-type dogs, aged from 3 to 12 years and weighing from 25 to 45 kg (average 30kg), with Body Condition Score assessed from 3/5 to 3.5/5 points (median 3). All the dogs were neutered/ /castrated and clinically healthy at the time of testing. The animals received prophylactic fipronil (Frontline spot on L, Merial, Poland) against fleas and antiparasitic tablets with febantel, pyrantel embonate and praziquantel (Drontal plus flavour, Bayer, Poland) regularly.

The 57 dogs were divided into four study groups. Group A consisted of 24 individuals aged 3-6 (5 neutered females and 19 castrated males), group B included 33 individuals over the age of 6 (including 12 neutered females and 21 castrated males), group C consisted of 37 dogs working in the police service of the Lubelskie Voivodeship (including 8 neutered females and 29 castrated males), and group D included 20 dogs being companion animals (8 neutered females and 12 castrated males). The examination was carried out as part of routine veterinary-medical activities during periodic health assessment of patients from the University of Life Sciences in Lublin Veterinary Clinic and did not require special permission from the local ethics committee. Prior to entry into the study, the owner's consent and willingness to comply with all study requirements was obtained.

After an overnight fast, venous blood was collected from the cephalic vein in each dog into appropriate tubes. Blood intended for haematological examinations was collected into single-use sterile EDTA K2 tubes (Medlab Products Sp.z o.o., Raszyn, Poland), while for biochemical and hormonal tests into single-use sterile Vacuette tubes with clotting activator (Cormay, Lublin, Poland). The collected venous blood was centrifuged within 2 hours of collection.

Both haematological and biochemical blood tests were performed within no more than 4 hours from the sample collection for each individual. Haematological analysis was performed with impedance and spectrophotometry method using the ScilVet ABC plus haematology analyser (Horiba Medical, France). Biochemical tests including total protein (TP), total cholesterol (TC), alanine transaminase (ALT), alkaline phosphatase (AP) and creatinine concentration were measured by an enzymatic method according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommendations with a compact biochemical analyser ABX Pentra 400 (Horiba Medical, France).

Determination of the thyroid hormones: tT4, fT4 and TSH concentrations was performed using chemiluminescence analyser Immulite 1000 with PMT photomultiplier (Siemens Healthcare Diagnostics, Germany).

The results obtained were subjected to statistical analysis using Microsoft Excel 2010 for Windows 7 and Statistica 13.0 PL. Mean values (x), standard deviation (±SD) and median (M) were determined. The results were tested for distribution normality with the use of Shapiro-Wilk test. In order to reveal statistically significant differences between the parameters examined, the Mann-Whitney test was performed and the Spear-

Table 1. Serum levels of TSH, fT4 and tT4 in dogs in study groups.

Parameter	Mean and standard deviation (x+SD) ABCD				Median (M)			
	A	B	C	D	A	B	C	D
TSH (ng/ml)	0.19±0.08	0.31±0.22	0.26±0.2	0.24±0.12	0.17	0.25	0.2	0.21
fT4 (pmol/L)	17.89±6.32	11.83±6.41	14.04±6.75	16.09±11.6	19.56	10.2	14.16	14.54
tT4 (nmol/L)	15.7±8	15.58±5.76	17.28±5.96	15.99±8.15	15.31	14.54	17.37	15.57

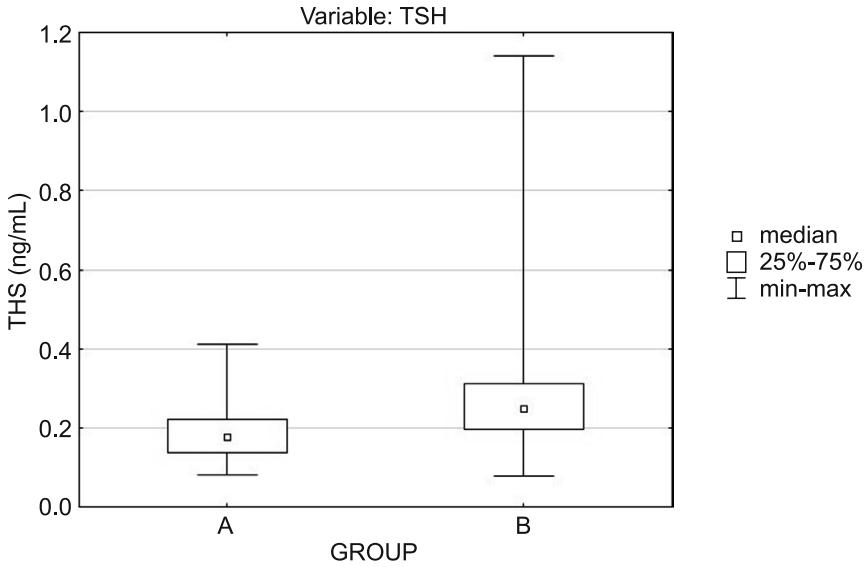

Fig. 1. Comparative analysis of TSH results in the age groups A and B of dogs, where statistically significant differences were found at $p<0.05$.

man's correlation test was used to determine the correlation coefficient between selected indicators by assessing the correlation strength according to the Guilford classification, where r=0.6-0.8 was considered a high correlation, r=0.8-0.9 very high and r>0.9 a full correlation. Statistical tests were performed at the significance level of $p<0.05$.

Results

In all groups of the dogs examined the haematological and biochemical parameters were within normal limits. There were no significant differences between the groups .

In the studied groups, the mean serum TSH concentrations ranged from 0.19±0.08 ng/ml in group A to 0.31±0.22 ng/ml in group B. The highest levels were in group B (median 0.25 ng/ml), and the lowest were in group A with median 0.19 ng/ml (Table 1). The mean serum fT4 concentrations ranged from 11.83±6.41 in group B to 17.89±6.32 pmol/L in group A. The highest levels were in the group A (median 19.56 pmol/L), and the lowest were in a group B (median 10.2 pmol/L). Whereas the mean serum tT4 concentrations varied from 15.58±5.76 (B) to 17.28±5.96 nmol/L (C). The highest results were in group C, (median 17.37 nmol/L). The lowest concentrations were obtained in group B (median 14.54 nmol/L).

Comparing the findings of different age groups: in groups A and B statistically significant differences were found in TSH (p=0.007) and fT4 (p=0.003) concentrations, whereas for tT4 there was no statistical difference (p=0.885). The TSH values obtained in group A were significantly lower than in group B (Fig. 1), and fT4 were significantly higher in group A than in group B (Fig. 2). A comparative analysis of the results obtained in the groups of working and accompanying dogs (groups C and D) did not show statistically significant differences in the TSH (p=0.955), tT4 (p=0.456) and fT4 (p=0.874) concentrations.

The analysis of all the findings revealed a very high positive correlation of fT4 and tT4 results, (Fig. 3). In group A, the value of the coefficient r=0.863, in group B r=0.891, in group C r=0.835, and in group D r=0.936. There was no significant correla-

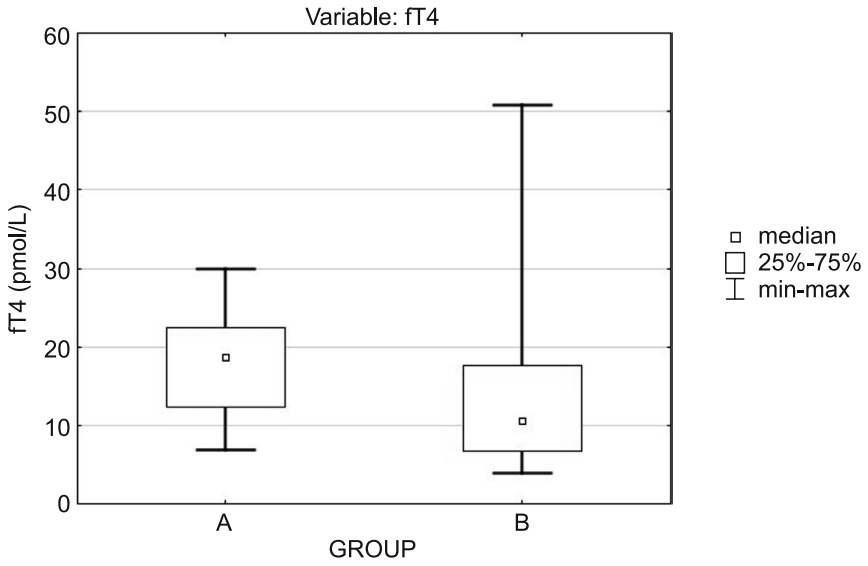

Fig. 2. Comparative analysis of fT4 results in the age groups A and B of dogs, where statistically significant differences were found at $p < 0.05$.

tion between TSH levels and thyroid hormones tested in any of the groups.

Discussion

The literature data indicate that the most useful for assessment of thyroid function in dogs are serum total and free thyroxine (tT4, fT4) and thyrotropin (TSH) concentrations, while the determination of antithyroid antibodies is less important (Daminet and Paradis 2000, Boretti et al. 2003, Diaz Espineira et al. 2007, Randolph et al. 2015). The "golden standard" for diagnostic procedures in dogs with suspected hypothyroidism nowadays is the TSH stimulation test, however the results of simultaneous measurement of tT4 and TSH concentration is the best indicator of overall thyroid functional status and thus gives the best chance of diagnosing this disease (Boretti and Reusch 2004, Diaz Espineira et al. 2007, Higgs et al. 2014, Bianchi et al. 2015, Hare et al. 2018).

Numerous authors emphasize, that the results of serum thyroxine and TSH concentrations, may vary depending on the research technique used, size, age or dog breed (Gaughan and Bruyette 2001, Lee et al. 2004, Shiel et al. 2007, Hegstad-Davies et al. 2015). These factors make comparability of acquired results challenging in clinical practice. Published results of thyroid hormone level examinations in dogs by an immunochemical method with chemiluminescence indicate that in healthy dogs serum tT4 concentration should be 2-18 nmol/L (average 8 nmol/L), fT4 from 1.9 to 13.1 pmol/L (median 9.3 pmol/L), while the concentration of TSH is 0.03-0.98 mg/L, and the median 0.2 mg/L (Diaz Espineira 2007). The present results are within those limits, however, the median values of tT4 (14.54 nmol/L in group B to 17.37 nmol/L in group C) and fT4 (10.2 pmol/L in group B to 19.56 pmol/L in group A) were different. These values can be explained by a low number of animals in groups used in our study.

In addition, the published data point to significant differences in concentrations of thyroid hormones between different dog breeds (Shiel et al. 2007, Hegstad-Davies et al. 2015, Ziener et al. 2015). There was, however, a proven correlation between the decrease in T3 and T4 levels in castrated dogs, reported by Taheri et al (2019). It was connected with inhibited metabolism of studied stray dogs. Such metabolic changes, like decreased relative volume densities of the follicles, colloid and epithelium, were not found in this study. The dissimilarity may be a result of a different method used in the cited paper, also indicated as a factor in several other contributions (Madiyal et al. 2016, Chen et al. 2018).

In large dog breeds (mean body weight 25 kg), such as Alaskan Malamute, Golden Retriever, Collie Sheepdog, English Setter, Samoyed, Spotted Husk and Husky, tT4 threshold reference values >12.9 nmol/L are found in over 80% dogs (Hegstad-Davies et al. 2015). These results are similar to those obtained in our German Shepherd trial (results ranging from 15.58 to 17.4 nmol/L). The fT4 values from published data were reported higher than 10.9 pmol/L, which was found in about 75% of dogs. The fT4 results obtained in this study ranged from 11.83 to 17.89 pmol/L, and are similar to those reported by Hegstad-Davies et al. (2015). According to the published data, TSH concentration below 0.6 ng/ml is found in 96% of dogs, while in dogs of particular breeds (Collie, Samoyed, Keenshond) physiological values were higher than

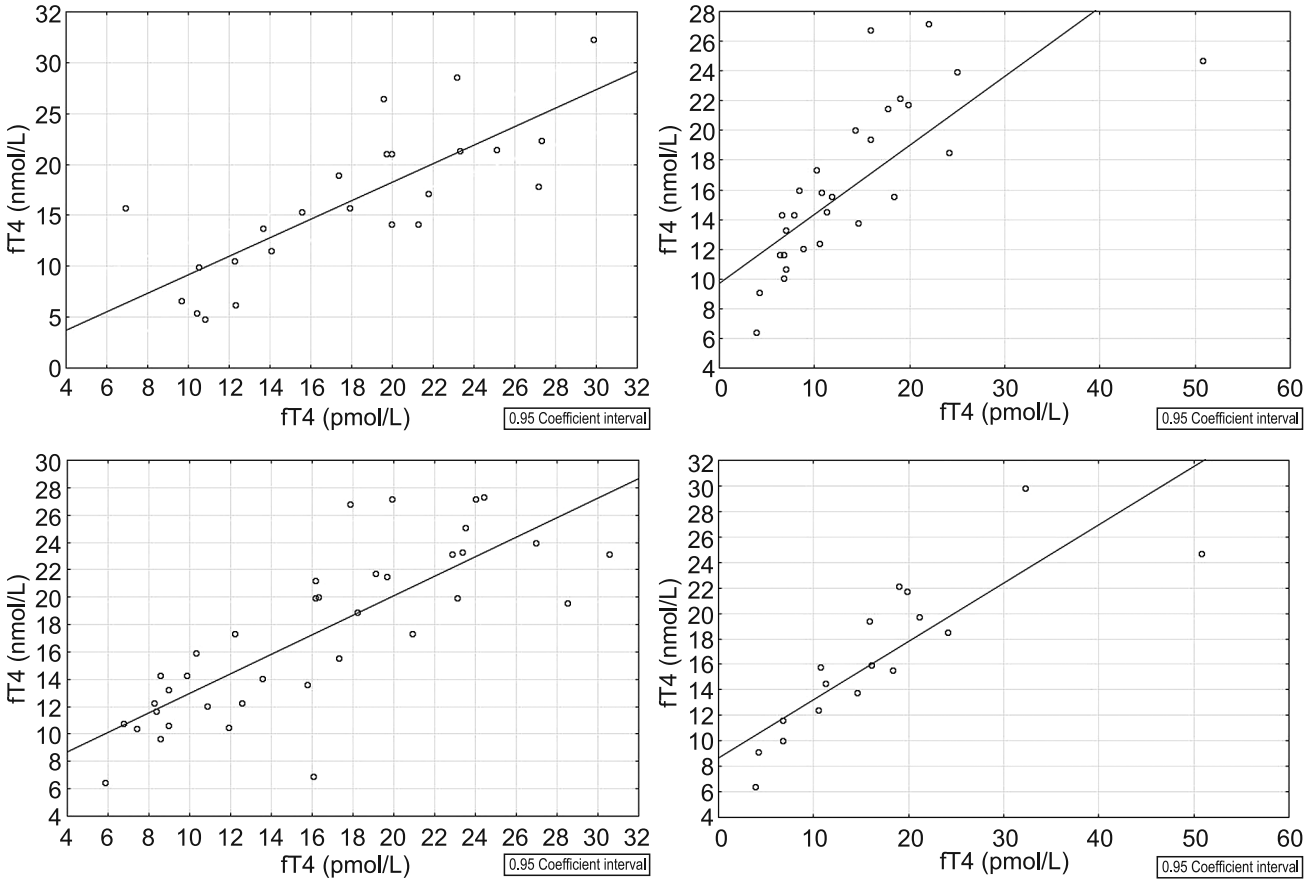

Fig. 3. A correlation between fT4 and tT4 in Group A, B correlation between fT4 and tT4 in Group B, C correlation between fT4, tT4 in Group C, D and correlation fT4 and tT4 in Group D.

0.6 ng/ml (Hegstad-Davies et al. 2015). In our study, all the dogs had serum TSH values below 0.6 ng/ml and similar to those obtained by the abovementioned authors for the overall population of dogs, without the shift of threshold regarding the body mass. However, abovementioned results indicate that in healthy dogs concentrations of tT4 and fT4 decreases and TSH increasies with age (Hegstad-Davies et al. 2015). This is also consistent with the present findings, because such differences were found in two (TSH and fT4) of three investigated hormones. In German Shepherd type dogs aged 3-6 years, the values of fT4 were significantly higher, and TSH significantly lower than in older dogs. On the contrary, the research conducted by Shiel et al. (2007) on some healthy greyhound dogs involving chemiluminescence did not show any significant differences between individuals of different ages (Shiel et al. 2007). The assessment of the correlation between individual hormones in greyhound dogs revealed a high positive correlation between fT4 and tT4, which is consistent with the results presented in this report. In addition, there was no correlation between these hormones and TSH concentration which is also consistent with our findings.

So far, there have been no studies on the concentration of thyroid hormones and TSH depending on the use of dogs, therefore it is difficult to compare the present results with those obtained by other authors. It was not confirmed that the work performed by dogs exert a significant influence on the parameters assessed.

Conclusion

The availability of modern diagnostic methods and more publications on the concentration of thyroid hormones in dogs will help to establish reliable reference values for the healthy animals considering their age, breed and technique used. The involvement in work, compared to intensive sport activity has a rather small impact on hormones concentrations in healthy dogs.

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