Seasonal Changes in Serum Total Thyroxine, Free Thyroxine, and Canine Thyroid-Stimulating Hormone in Clinically Healthy Beagles in Hokkaido

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ABSTRACT. The purpose of this study was to evaluate seasonal influences on thyroid hormone levels of healthy outdoor dogs in Hokkaido. We surveyed serum basal total thyroxine (tT₄), free thyroxine (fT₄), and canine thyroid-stimulating hormone (cTSH) levels, and tT₄ levels after administration of TSH for a year. Basal tT₄ levels decreased in January, and increased in August and September. fT₄ levels increased in January and November. No significant seasonal variation was found in cTSH. tT₄ levels after administration of TSH in August and November increased. These results suggested that the thyroid gland may have been activated in November. We should take seasonal variation into consideration when thyroid function is tested.

KEY WORDS: canine, season, thyroid hormone.

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Recent progress in the development of hormone assays has made thyroid function testing for dogs more precise [1, 8]. Advances in the ability to measure serum free thyroxine (fT₄) by dialysis [6, 9] and serum canine thyroid-stimulating hormone (cTSH) [9] levels have contributed to this. By using these tests in combination with the thyrotropin-releasing hormone (TRH)-stimulating test [14] and the TSH-stimulating test [5], researchers can now examine in detail the influence of the hypothalamus-pituitary-thyroid axis on thyroid function. The above tests make it possible to examine previously undetectable minute physiological changes in thyroid function. Many studies have reported that thyroid function in humans adapts to environmental conditions such as ambient temperature, photoperiod, and season, but the results have not been consistent because more than one parameter may mediate thyroid function [2–4, 10]. Although similar findings were reported in rat [11, 13], sheep [15], and dog [12], the only hormones measured were serum total thyroxine (tT₄), triiodothyronine (tT₃), and TSH. There has been only one report about seasonal changes in thyroid function in the dog [12], but the details of such changes have remained unclear because only tT₄ and tT₃ levels were measured.

The purpose of the present study was to evaluate the influence of the season on the thyroid hormone levels of healthy outdoor dogs. We surveyed basal tT₄, fT₄, cTSH levels, and tT₄ levels after administration of TSH for a year. Subject dogs - Ten clinically healthy beagles were used, ranging in age from 2 to 7 years and weighing 8.0 to 15.0 kg. There were 7 males and 3 females. The dogs were separated into two groups by sex, and had been raised outdoors since

puppyhood, fed a standard commercial dry dog food once daily at 4 p.m., and given water freely at the Laboratory of Veterinary Internal Medicine, Obihiro University of Agriculture and Veterinary Medicine. Exposure to sunlight was good even though the outdoor runs had roofs. Wind did not blow strongly because of surrounding buildings. The history and results of routine physical examinations and routine laboratory tests (CBC, total cholesterol, ALT, ALP, BUN, and creatinine) were unremarkable for all dogs at each blood sampling during the entire survey period.

Measurement items: cTSH levels were measured in all dogs. In 6 of the 10 dogs, fT₄ and basal tT₄ levels were measured and a TSH-stimulating test was performed. In each of the 6 dogs, 0.1 U/kg bovine-TSH (Sigma Chemical Co., U.S.A.) was administered IV, and a measurement sample of serum tT₄ was obtained 6 hr after TSH administration [8].

Sampling: The survey period was from December 1997 to November 1998. Blood samples were collected at 8 a.m. once a month throughout the survey period. The dogs were fasted for at least 12 hr before blood collection through a venipuncture of the cervical vein. The serum was separated and stored at –80°C until hormonal determination.

Hormone assays: Radioimmunoassay with a commercial kit (Coat-A-Count, Canine T₄®, Diagnostic Products Co., U.S.A.) and immunoradiometric assay (Coat-A-Count, Canine TSH®, Diagnostic Products Co., U.S.A.) were used for the determination of tT₄ and cTSH levels, respectively. Each determination was done in duplicate. A commercial laboratory (IDEXX Laboratories Co., Japan) measured fT₄ levels by equilibrium dialysis.

Analysis of data: The data were analyzed by Two-way ANOVA or the Friedman test after the Bartlett test. Multiple comparison test was applied with Fisher's PLSD to assess statistical significance. A value of P<0.05 was considered statistically significant.

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The monthly average ambient temperatures in our institution during the study were as follows: December -5.0°C , January -11.7°C , February -8.7°C , March -0.7°C , April $+7.2^{\circ}\text{C}$, May $+11.1^{\circ}\text{C}$, June $+12.7^{\circ}\text{C}$, July $+17.0^{\circ}\text{C}$, August $+17.9^{\circ}\text{C}$, September $+16.4^{\circ}\text{C}$, October $+10.1^{\circ}\text{C}$, and November $+1.1^{\circ}\text{C}$. The average tT_4 , fT_4 , and cTSH values are listed in Table 1. As shown in Table 1, the basal tT_4 level in January was the lowest, and was significantly lower than in the nine other months. Basal tT_4 levels in March, August, and September were significantly higher than in the seven to nine other months. Basal fT_4 levels in January and November were significantly higher than in the eight to ten other months. No significant variation was found in serum cTSH levels among the twelve months. Table 2 summarizes tT_4 levels after administration of TSH. The tT_4 average values after administration of TSH in both August and November were significantly higher than in June and July, and the level in November was also significantly higher than in December.

There is only one published report about seasonal change in thyroid function in dogs, that of Tuckova *et al.* of Slovakia [12]. Their data showed increased tT_4 levels in autumn and decreased tT_4 levels in summer, but in our present study, the tT_4 level decreased in January (midwinter) and increased in March (early spring), August (late summer), and September (early autumn). Reports about seasonal differences in human thyroid function are available. Nishi *et al.* [4] reported that TSH and fT_3 levels were higher during winter than in summer, but no such change was observed with fT_4 levels in healthy subjects in Sapporo Japan. Leppaluto *et al.*

of Finland [3] reported that fT_3 levels were lower in February than in August and TSH levels were higher in December than in other months, and that fT_4 , tT_4 , and tT_3 levels were unchanged. So that the results vary geographically, and the influences of ambient temperature and photoperiod are thought to account for these regional differences. It could be thought that the different results for dogs in Hokkaido and Slovakia were also caused by differences in ambient temperature or photoperiod. In our present study, in January, the basal tT_4 decreased and the fT_4 increased, although the cTSH and tT_4 after administration of TSH did not change. A decreased tT_4 level is not specific for a diagnosis of canine hypothyroidism. tT_4 consists of T_4 bound to plasma proteins (bT_4) and fT_4 . Although the bT_4 level in euthyroid dogs is often decreased by many factors, such as changes in hormone binding to serum carrier proteins, and changes in peripheral hormone distribution and metabolism, fT_4 is less affected by them. Because only free hormone can enter cells and bind to receptors, measurement of fT_4 should give a more accurate representation of thyroid function [8]. In our study, it was thought that thyroid function was not decreased in January, because the fT_4 level was increased and the tT_4 level post TSH was not decreased. Solter *et al.* [10] reported that, in healthy workers exposed to cold environment, the tT_4 level decreased, the fT_4 level increased or did not change, and thyroxine-binding globulin (TBG) decreased. Therefore, the higher fT_4 level noted in our present data in January may have been caused by the decreased bT_4 , although TBG was not measured at that time. The tT_4 level decreases when the cortisol level is high [8].

Table 1. Seasonal variation in serum Basal tT_4 , fT_4 and cTSH levels in healthy beagles in Hokkaido (mean $\pm$ SD)

	Winter			Spring			Summer			Autumn		
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Basal tT_4 (nmol/l)	14.4 ± 3.1	11.3 ^{a)} ± 1.7	15.1 ± 1.4	20.7 ^{b)} ± 2.5	14.6 ± 3.4	14.0 ± 1.2	15.4 ± 2.6	14.3 ± 3.4	18.7 ^{e)} ± 2.6	18.7 ^{d)} ± 3.3	17.0 ± 4.0	17.1 ± 1.2
Basal fT_4 (pmol/l)	18.0 ± 8.4	44.5 ^{e)} ± 20.8	24.6 ± 6.6	30.1 ± 5.2	17.3 ± 3.2	16.5 ± 3.1	17.6 ± 1.6	17.2 ± 4.8	18.6 ± 4.0	26.3 ± 7.8	19.2 ± 5.2	39.0 ^{f)} ± 31.5
Basal cTSH (ng/ml)	0.18 ± 0.22	0.26 ± 0.14	0.26 ± 0.14	0.36 ± 0.25	0.36 ± 0.20	0.38 ± 0.54	0.41 ± 0.81	0.30 ± 0.62	0.38 ± 0.71	0.33 ± 0.23	0.34 ± 0.48	0.17 ± 0.12

a) Basal tT_4 level in January is significantly lower than in Dec, Feb, Mar, Apr, Jun, Aug, Sep, Oct, and Nov.

b)-d) Basal tT_4 levels in March, August, and September are significantly higher than in Dec, Jan, Feb, Apr, May, Jun, and Jul. Basal tT_4 level in March is also significantly higher than in Oct and Nov.

e), f) Basal fT_4 levels in both January and November are significantly higher than in Dec, Feb, Apr, May, Jun, Jul, Aug, and Oct. Basal fT_4 level in January is also significantly higher than in Mar and Sep.

Table 2. Seasonal variation in serum tT_4 levels 6 hr after administration of 0.1 U/kg TSH in healthy beagles in Hokkaido (mean $\pm$ SD)

	Winter			Spring			Summer			Autumn		
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
tT_4 post TSH (nmol/l)	20.8 ± 7.1	22.0 ± 2.8	22.8 ± 9.2	28.0 ± 8.8	23.9 ± 11.2	22.1 ± 8.8	20.1 ± 8.2	20.2 ± 7.9	30.4 ^{a)} ± 12.1	28.4 ± 7.9	26.6 ± 9.8	31.9 ^{b)} ± 7.8

a) b) tT_4 post TSH levels in both August and November are significantly higher than in Jun and Jul. tT_4 post TSH level in November is also significantly higher than in Dec.

Exposure to cold in January might create a condition like Cushing's syndrome because of stress, although the cortisol level was not measured in the present study. Many reports about the influence of environmental temperature--specifically, exposure to cold--on the thyroid hormones of the human being [2, 7] and rat [11, 13] suggest that cold exposure activates pituitary and thyroid functions and increases the basal metabolic rate. In our present study, the response to the TSH-stimulating test was increased in August and November. The thyroid gland may have reacted against the progressive decrease in ambient temperature in order to increase the basal metabolic rate in November (late autumn) because the fT_4 level increased. It was not possible to know the exact reasons for increased tT_4 response to TSH in August, and those for increased tT_4 levels in March, August and September in this survey.

These results suggest that thyroid hormone levels vary according to the season in healthy outdoor dogs. We should take seasonal variation into consideration when thyroid function is tested. It might be necessary to investigate other factors, such as the photoperiod or regional differences, and to do other thyroid function tests such as measurements of triiodothyronine or TRH stimulation test, to evaluate the cause of the seasonal change in detail.

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