Effects of administration of a low dose of frozen thyrotropin on serum total thyroxine concentrations in clinically normal dogs

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Abstract

Thyroid function was evaluated in 18 healthy dogs by thyrotropin (TSH) stimulation. Two dose regimens were used in each dog: 0.1 IU/kg body weight of freshly reconstituted lyophilized TSH and 1 IU/dog of previously frozen and stored TSH (up to 200 days), both given intravenously. Blood samples were collected prior to and at four and six hours after TSH administration. Serum was evaluated for total thyroxine concentrations by radioimmunoassay. All dogs were classified as euthyroid on the basis of response to 0.1 IU/kg body weight of freshly reconstituted TSH at four and six hours. The 1 IU dose of TSH, previously frozen for up to 200 days, induced increases in serum total thyroxine concentration over baseline at four and six hours that were not significantly different from those resulting from the use of the higher dose of fresh TSH. In all test groups, there were no statistically significant differences between total thyroxine concentrations at four and six hours post-TSH administration.

It was concluded that an adequate TSH response can be achieved with the use of 1 IU of TSH/dog for clinically normal dogs between 29.0 kg and 41.6 kg body weight, even if this TSH has been frozen at −20°C for up to 200 days. Further, blood collection can be performed at any time between four and six hours. Similar studies are needed to evaluate this new protocol in hypothyroid dogs and euthyroid dogs suffering nonthyroidal systemic diseases.

Résumé

Effet de l'administration d'une faible dose de thyrotropine congelée sur la concentration sérique de thyroxine totale chez des chiens cliniquement normaux.

La fonction thyroïdienne de 18 chiens normaux fut évaluée à l'aide du test de stimulation à la thyrotropine (TSH). Deux procédures ont été utilisées pour chaque chien : 0,1 IU/kg de TSH lyophilisée, fraîchement reconstituée et 1 IU/chien de TSH congelée (jusqu'à 200 jours). Les prises de sang ont été effectuées avant et à quatre et six heures après l'administration intraveineuse de TSH. Les sérums ont été évalués quant à la concentration de thyroxine totale (TT_4) par radio-immuno-assay. Tous les chiens ont été classifiés comme étant euthyroïdiens en se basant sur la réponse à 0,1 IU/kg de TSH fraîchement reconstituée à quatre et six heures. La dose de 1 IU/chien de TSH, congelée jusqu'à 200 jours, a induit une augmentation de la concentration sérique de TT_4 à quatre et six heures (par rapport à la TT_4 de base) qui n'était pas différente de façon significative de celle résultant de la dose élevée de TSH fraîche aux temps correspondants. De plus, il n'y avait pas de différences significatives entre la TT_4 à quatre et six heures après l'administration de TSH.

Il a été conclu que pour les chiens normaux entre 29,0 et 41,6 kg, l'on peut obtenir une réponse à la TSH adéquate avec 1 UI de TSH/chien, et ce, même si la TSH était congelée à −20 °C pour une période allant jusqu'à 200 jours. De plus, le sang peut être collecté n'importe quand entre quatre et six heures après l'administration de TSH. Des études semblables sont requises chez les chiens hypothyroïdiens et euthyroïdiens souffrant de maladies systémiques pour évaluer ce nouveau protocole.

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Introduction

Hypothyroidism is one of the most frequent endocrinopathies in the dog (1–5). A deficiency in circulating iodinated thyroid hormone affects the metabolic function of all organ systems. Therefore, clinical signs are numerous, variable, often nonspecific, and rarely pathognomonic for hypothyroidism. Consequently, it is important that diagnosis of hypothyroidism be based on reliable biochemical tests. Currently, the best method for confirming a clinical diagnosis of canine hypothyroidism is the thyrotropin (thyroid stimulating hormone, (TSH)) stimulation test. Bovine TSH is available commercially (Thytropar, Rorer Canada Inc., Bramalea, Ontario); however, its cost often precludes its use in a clinical setting.

A number of TSH stimulation protocols have been proposed (1–9). Currently, the most frequently recommended TSH doses are 0.1 IU/kg body weight (BW) (1,3,5) or 5 IU/dog (7–9), both administered intravenously (IV). To minimize the expense, the lowest dose of TSH that can provide a diagnostically useful stimulation of the thyroid gland should be employed. However, it has been suggested that a total dose of 1 IU/dog of TSH can be used to evaluate thyroid function in dogs (2,10), and in a recent study evaluating the effectiveness of reduced doses of TSH in assessing thyroid function in healthy dogs (11), it was concluded that injection of 1 IU of TSH will meet with the criterion of an adequate response to TSH, in the form of serum total thyroxine (TT_4) concentration >32 nmol/L at 4 h after its administration.

Nevertheless, whichever dose of TSH is employed, only a portion of the vial is utilized to execute a single TSH stimulation test. In most instances, more than one

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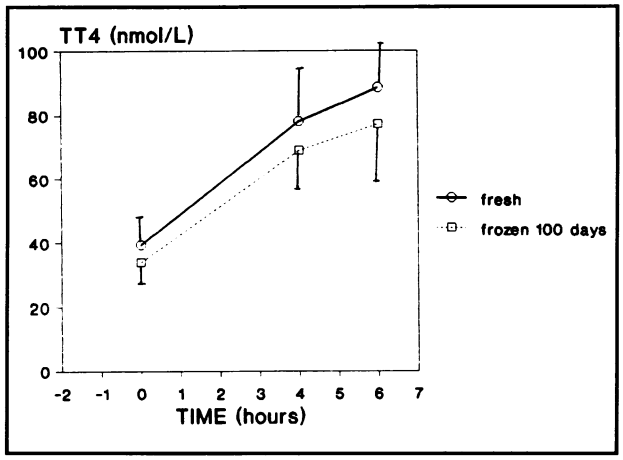

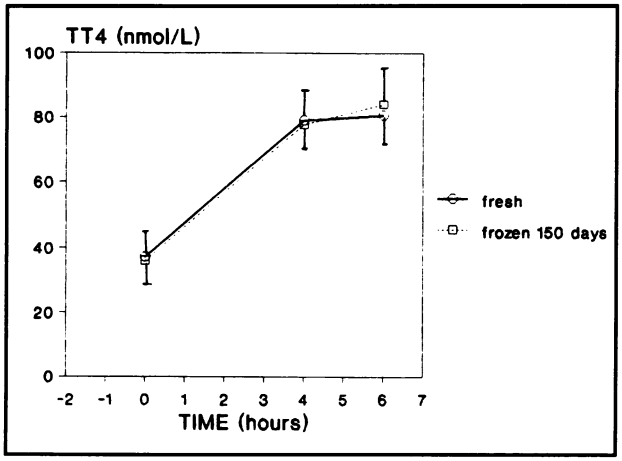

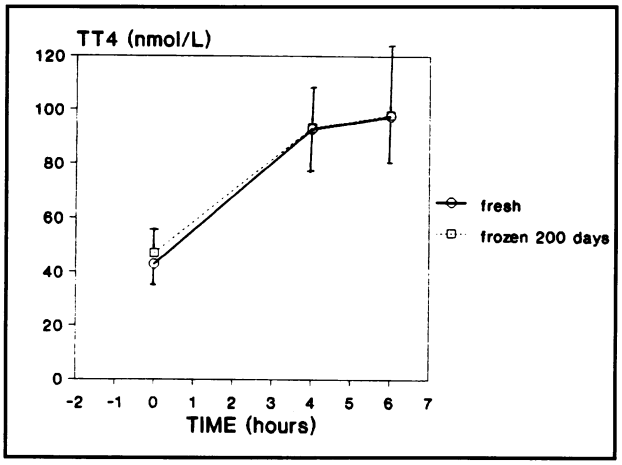

Figure 1. Comparison of TSH stimulation test protocols in healthy dogs.
Graph A: Serum total thyroxine (TT_4) concentrations (± = SD) before and after intravenous (IV) administration of 0.1 IU/kg body weight (BW) of freshly reconstituted TSH (⊖) or IV administration of 1.0 IU/dog of TSH frozen and stored 100 d (⊟) (n = 6).
Graph B: Serum TT_4 concentrations (± = SD) before and after IV administration of 0.1 IU/kg BW of freshly reconstituted TSH (⊖) or IV administration of 1.0 IU/dog of TSH frozen and stored 150 d (⊟)(n = 6).
Graph C: Serum TT_4 concentrations (± = SD) before and after IV administration of 0.1 IU/kg BW of freshly reconstituted TSH (⊖) or IV administration of 1.0 IU/dog of TSH frozen and stored 200 d (⊟) (n = 6).

test can be performed from the content of a single vial. The suppliers have traditionally advised that TSH be utilized within 48 h following its reconstitution. However, two recent studies, both utilizing 0.1 IU/kg BW of TSH, showed that TSH maintains an adequate biological activity for at least three weeks when refrigerated at 4°C (12), and for up to three months when frozen at −20°C (13).

To the authors' knowledge, there have been no reports to demonstrate whether 1 IU of TSH/dog is as effective as 0.1 IU/kg BW for the evaluation of canine thyroid function. If proven so, 1 IU aliquots could be frozen for future use. But even if the IV administration of 1 IU of TSH provides adequate stimulation of the thyroid gland of dogs of any size, it remains important to learn how long this reduced dose of TSH can be stored at −20°C without losing potency.

Current recommendations for times of blood sample collection after the administration of TSH depend on the dose of TSH being used and its route of administration; blood collection times vary between 4 h and 8 h following IV administration (1–13). It is also of interest to determine the optimal blood collection time for post-TSH TT_4 concentration when using the reduced dose of frozen TSH.

The purpose of our study was to evaluate the effectiveness of a reduced dose of TSH (1 IU/dog), frozen at −20°C and stored for up to 200 d, in assessing thyroid function in healthy dogs, and to determine the optimal time for blood collection under these test conditions.

Materials and methods

Eighteen, privately owned, clinically normal dogs weighing between 29 kg and 41.5 kg ($\bar{x}$: 32.5 kg) aged between 1 yr and 7 yr ($\bar{x}$: 2.75 yr) were used for this study. They consisted of 10 males (6 intact, 4 neutered) and 8 females (4 intact, 4 ovariohysterectomized) and comprised 10 Labrador retrievers, 3 golden retrievers, 1 collie, 1 bull terrier, 1 bull mastiff, 1 Burmese mountain dog, and 1 Airedale terrier. None of the bitches showed vaginal signs or had a history of proestrus, estrus, or diestrus. None of the dogs was receiving medication known to alter serum TT_4 concentrations.

The dogs were randomly allotted to three groups of six dogs each. Each group received two stimulation tests with TSH, one with 0.1 IU/kg BW ("high dose") of TSH and the other with 1 IU/dog ("low dose"). The 10 IU vial of lyophilized TSH was reconstituted with 10 mL of sterile physiological saline. For the test using the low dose, 1 IU (1 mL) aliquots of TSH were stored frozen at −20°C in 3 mL plastic syringes with a rubber cap for 100 d (group I), 150 d (group II), and 200 d (group III). For the test using the high dose of TSH, the required dose of TSH (ie from 2.9 to 4.15 IU/dog) was administered within a few hours following its reconstitution. The test using the high dose of TSH was performed one week before (in 3 dogs of each group) or one week after (in the other 3 dogs of each group) the test using the low dose of frozen TSH.

The TSH stimulation tests were performed as follows: blood was obtained from the jugular or the cephalic vein between 8:00 AM and 10:00 AM, TSH was administered into the cephalic vein, and blood samples were

Table 1. Serum total thyroxine (TT_4) concentrations (nmol/L) before and after intravenous administration of freshly reconstituted or previously frozen thyrotropin (TSH) in 18 healthy dogs

					TSH stimulation test using 0.1 IU/kg body weight of freshly reconstituted TSH			TSH stimulation test with 1 IU/dog of previously frozen TSH			
					Time following TSH administration			Time following TSH administration			Time of TSH storage at −20°C
Dog	Age (yr)	Breed	Sex	Wt (kg)	0	4 (hours)	6	0	4 (hours)	6	(days)
1	1.0	Bull-mastiff	F	31.0	52.4	90.2	97.7	38.0	69.9	68.2	100
2	7.0	Collie-cross	FS	34.0	36.9	58.9	56.5	34.9	51.7	50.9	100
3	7.0	Airedale T	MC	30.0	35.4	69.4	84.3	36.0	72.2	80.2	100
4	4.0	Burmese-MD	M	36.0	37.9	86.6	94.5	37.4	74.7	83.3	100
5	2.5	Labrador-R	M	41.6	49.4	106.9	120.4	41.2	90.5	115.3	100
6	4.0	Labrador-R	M	34.5	23.5	56.3	77.1	14.5	54.3	64.7	100
7	2.0	Golden-R	MC	30.0	31.1	88.1	71.6	28.1	65.3	66.2	150
8	1.5	Labrador-R	FS	31.0	36.8	81.3	74.5	32.8	80.1	88.7	150
9	1.0	Labrador-R	FS	30.0	33.3	70.6	78.9	39.0	86.4	78.4	150
10	1.5	Golden-R	MC	34.5	31.0	70.6	80.0	29.4	74.3	79.4	150
11	7.0	Golden-R	FS	30.0	54.0	88.4	88.5	43.2	81.4	91.7	150
12	2.0	Labrador-R	MC	36.5	36.2	74.6	90.2	44.1	78.6	99.0	150
13	1.5	Labrador-R	F	30.0	55.1	98.7	104.1	53.1	97.8	144.8	200
14	1.5	Labrador-R	F	31.0	43.2	78.1	104.2	44.6	91.3	74.1	200
15	1.5	Bull Terrier	M	29.0	35.8	71.4	77.7	36.4	84.8	76.9	200
16	1.5	Labrador-R	F	29.0	26.5	99.0	70.0	47.8	90.9	86.8	200
17	2.0	Labrador-R	M	33.0	51.2	129.4	133.8	47.4	121.2	116.9	200
18	1.0	Labrador-R	M	29.0	44.3	80.3	95.9	51.5	73.6	88.8	200

T = terrier, MD = mountain dog, R = retriever, F = female, FS = female spayed, M = male, MC = male castrated

collected 4 h and 6 h later. Serum was harvested and stored at −20°C until assayed. The serum TT_4 was measured by radioimmunoassay (RIA) (Canine T4 Double Antibody, Diagnostic Products Corporation, Los Angeles, California, USA). Radioactivity was measured with a gamma counter (Iso-Data Gamma Counter, Model 10/600, ICN Biomedicals Canada Inc., Mississauga, Ontario). Accuracy of the RIA method was monitored by including three commercial controls (Con6, Immunoassay Tri-Level Control, Diagnostic Products Corporation). To avoid interassay variation within samples from individual dogs, all serum samples from each dog were tested within the same run.

Our laboratory reference ranges for basal TT_4 concentration are from 19 to 45 nmol/L, and for post-TSH TT_4 concentration either ≥ 45 nmol/L or an increase by at least 24 nmol/L over basal TT_4.

Mean increase in serum TT_4 concentration after TSH administration was calculated for each group of dogs and for both doses of TSH. A paired *t* test was used to determine differences in the TT_4 response to TSH using the low dose of frozen TSH compared to the high dose of freshly reconstituted TSH at 4 h and 6 h, respectively, and this for each group. A p value < 0.01 was considered significant. Analyses were performed with a statistical analysis system (SAS/STAT version 6, 1987, SAS Institute Inc., Cary, North Carolina, USA).

Results

Concentrations of TT_4 4 h post-TSH were increased over basal concentrations both after administration of the high dose of freshly reconstituted TSH ($p < 0.001$; $n = 18$) and the low dose of TSH frozen for 100 d, 150 d, and 200 d, respectively ($p \leq 0.001$, $n = 6$; $p \leq 0.001$, $n = 6$; $p \leq 0.001$; $n = 6$). Table 1 shows the results of both TSH stimulation tests in the 18 healthy dogs.

All dogs were confirmed euthyroid on the basis of the TSH stimulation test performed with 0.1 IU/kg BW of freshly reconstituted TSH (based on previously cited reference ranges).

The mean increases in serum TT_4 concentrations (± SD) at 4 h were not statistically different when dogs received the high dose of freshly reconstituted TSH versus the low dose of TSH previously frozen for 100 d (38.8 ± 12.6 vs 35.2 ± 10.7 nmol/L; $p = 0.25$), 150 d (41.9 ± 8.1 vs 41.6 ± 5.6 nmol/L; $p = 0.95$), or 200 d (50.1 ± 19.9 vs 46.5 ± 16.5 ± nmol/L; $p = 0.60$).

Similarly, the mean increases in serum TT_4 concentrations at 6 h were not statistically different when dogs received the high dose of freshly reconstituted TSH or the low dose of TSH previously frozen for 100 d (49.2 ± 17.0 vs 43.4 ± 19.6 nmol/L; $p = 0.08$), 150 d (43.6 ± 7.3 vs 47.8 ± 7.6 nmol/L; $p = 0.33$), or 200 d (54.9 ± 15.2 vs 51.3 ± 24.1 nmol/L; $p = 0.73$) (Figure 1).

Mean serum TT_4 concentrations at 4 h were not statistically different from concentrations at 6 h in dogs ($n = 18$) receiving the high dose of fresh TSH (83.27 ±17.8 vs 88.88 ± 18.8 nmol/L; $p = 0.09$). Nor was there any statistical difference between

mean serum TT_4 concentrations at 4 h and 6 h in dogs receiving the low dose of TSH frozen for 100 d (68.88 ± 14.3 vs 77.10 ± 22.0 nmol/L; p = 0.09), 150 d (77.68 ± 7.2 vs 83.90 ± 11.6 nmol/L; p = 0.17), or 200 d (93.27 ± 15.9 vs 98.05 ± 27.5 nmol/L; p = 0.64).

Discussion

The present study achieved three goals. First, we showed that when performing a TSH stimulation test in normal dogs weighing between 29.0 and 41.6 kg, there were no differences between results obtained using 1 IU/dog and 0.1 IU of TSH/kg BW. Second, we showed that aliquots of reconstituted TSH can be frozen at −20°C for up to 200 d without losing potency. Third, we indicated that even with this reduced dose of previously frozen TSH, in normal dogs, a diagnostic blood sample can be collected for post-TSH TT_4 measurement any time between 4 h and 6 h after TSH administration.

It is known that anaphylactic reactions, although uncommon, may develop in dogs after repeated IV injections of bovine TSH (2). Thus, our study was designed so that the TSH stimulation test was performed only twice in each dog. In addition, it is suggested that important changes in environmental temperature (eg, winter vs summer) and sexual status (estrus, diestrus) can influence thyroid function test results in dogs (3,4), so our tests were performed at one week intervals and each dog was used as its own control.

The value of a diagnostic test for hypothyroidism can not be judged unless the results of the test are compared to the results of thyroid biopsy (eg, lymphocytic thyroiditis, thyroidal atrophy) or are based upon the success of predicting a response to thyroid hormone replacement therapy (1). Using the above criteria and a specific TSH stimulation test protocol (serum TT_4 concentration before and after administration of a minimum of 1 IU of TSH/dog IV), our laboratory had established that in euthyroid dogs, the post-TSH TT_4 concentration should either exceed 45 nmol/L or increase by at least 24 nmol/L over the basal serum TT_4 value to rule out a diagnosis of hypothyroidism (unpublished observations). Dogs that fail to meet one or the other of these criteria are considered hypothyroid, and their response to thyroid hormone replacement therapy is evaluated.

A popular misconception is that post-TSH serum TT_4 should double the baseline serum TT_4 concentration for adequate response to TSH (1,3,11). In the current study, when using 0.1 IU/kg BW of freshly reconstituted TSH, this criterion was not met in 7 of 18 and 4 of 18 dogs at 4 h and 6 h, respectively. When 1 IU/dog of previously frozen TSH was used, the above diagnostic criterion was not met in 7 of 18 and 5 of 18 dogs using 4 h and 6 h post-TSH samples, respectively. The results support other reports (6,11) that the previously cited "doubling" criterion should not be used for interpreting TSH stimulation tests results.

In conclusion, results of this study suggest that a reduced dose of TSH (1 IU/dog) can adequately stimulate the thyroid gland of euthyroid dogs, even if the TSH has been frozen and stored at −20°C for up to 200 d. In addition, under these conditions there were no statistical differences between mean TT_4 increases at 4 h and 6 h post-TSH. Further studies are needed to evaluate diagnostic efficacy of this TSH test protocol in hypothyroid dogs and in dogs with nonthyroidal systemic illnesses. CVJ

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