

Comparison of the biological activity of recombinant human thyroid-stimulating hormone with bovine thyroid-stimulating hormone and evaluation of recombinant human thyroid-stimulating hormone in healthy dogs of different breeds

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Objective—To evaluate whether use of recombinant human (rh) thyroid-stimulating hormone (TSH) induces equivalent stimulation, compared with bovine TSH (bTSH), and to evaluate activity of rhTSH in dogs of various large breeds.

Animals—18 healthy research Beagles and 20 healthy client-owned dogs of various breeds with body weight > 20 kg.

Procedures—The 18 Beagles were randomly assigned to 3 groups, and each dog received either 75 µg of rhTSH, IM or IV, or 1 unit of bTSH, IM, respectively, in a crossover design. The 20 client-owned dogs received 75 µg of rhTSH, IV. Blood samples were taken before and 6 hours after TSH administration for determination of total serum thyroxine (T₄) concentration. Additional blood samples were taken after 2 and 4 hours in Beagles that received rhTSH, IM.

Results—There was a significant increase in T₄ concentration in all dogs, but there were no differences between values obtained after administration of bTSH versus rhTSH or IV versus IM administration of rhTSH. Although there was a significant difference in age and body weight between Beagles and non-Beagles, there was no difference in post-TSH stimulation T₄ concentration between the 2 groups.

Conclusions and Clinical Relevance—Results indicated an equivalent biological activity of rhTSH, compared with bTSH. Use of 75 µg of rhTSH, IV, did not induce a different magnitude of stimulation in large-breed dogs, compared with Beagles. Euthyroidism was confirmed if post-TSH stimulation T₄ concentration was ≥ 2.5 µg/dL and at least 1.5 times basal T₄ concentration. (*Am J Vet Res* 2006; 67:1169–1172)

ABBREVIATIONS

TSH	Thyroid-stimulating hormone
T ₄	Thyroxine
bTSH	Bovine TSH
rhTSH	Recombinant human TSH
cTSH	Canine TSH
T ₃	Triiodothyronine

The TSH stimulation test has long been recognized as an accurate measure of thyroid function and still serves as the gold standard for diagnosis of hypothyroidism in dogs. The increase in T₄ concentration after administration of a supraphysiologic dose of exogenous TSH, traditionally of bovine origin, provides an assessment of the functional reserve capacity of the thyroid gland and helps to discriminate true hypothyroidism from other conditions with low T₄ secretion.¹

Because bTSH is no longer available as a pharmaceutical preparation, rhTSH has been proposed as an alternative. However, species-specific differences in the biological activity of protein hormones have been detected and have been attributed to differences in glycosylation patterns.² Differences in glycosylation patterns have also been found between recombinant and purified protein hormones.³⁻⁵ Although the canine thyroid gland can respond to rhTSH,^{6,7} it is unknown whether the biological activity of rhTSH in dogs is the same as that of the traditionally used bTSH. Consequently, the degree of effectiveness of rhTSH in dogs cannot be predicted, and criteria for test interpretation cannot simply be adapted from bTSH without critical evaluation. Moreover, rhTSH has so far only been evaluated in Beagles; to our knowledge, there are no published studies of the effectiveness of rhTSH in healthy dogs of other breeds.

Objectives of the present study were to compare the biological activity of rhTSH with bTSH in TSH stimulation tests in healthy Beagles, evaluate the influence of the route of administration (IM and IV) of rhTSH, determine the activity of rhTSH in healthy dogs of various large breeds, and determine criteria for test interpretation to confirm euthyroidism.

Materials and Methods

Dogs—Eighteen healthy Beagles (8 sexually intact females and 10 sexually intact males) with a

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median age of 2 years (range, 1.5 to 2 years) and a median body weight of 14.3 kg (range, 10.4 to 16.5 kg) were used. Body weight and hydration status were assessed as normal before thyroid gland stimulations were performed. All female dogs were determined to be in anestrus on the basis of results of physical examination and clinical history. Prior to the experiment, all dogs had been adapted to their environment and handling by the investigators. Dogs were kept in groups at the research unit of the Vetsuisse Faculty of the University of Zurich and fed a standard commercial maintenance pellet diet once daily, and water was available ad libitum. Animal care was in accordance with the guidelines and directives established by the Animal Welfare Act of Switzerland.

In addition, 20 healthy student- and staff-owned large-breed dogs, including 10 males (3 castrated) and 10 females (7 spayed), with a median body weight of 27 kg (range, 20.3 to 49.7 kg) and a median age of 4 years (range, 1 to 11 years) were used. Informed consent of the owners was obtained before the tests were performed.

All dogs were determined to be healthy and euthyroid on the basis of their history and results of a physical examination, CBC, serum biochemical profile, serum total T_4 concentration, and cTSH concentration. All dogs had negative results of tests for autoantibodies against T_3 , T_4 , and thyroglobulin. None of the dogs had received any medication for at least 8 weeks prior to TSH stimulation.

Experimental design—A 3 × 3 crossover study design was used to evaluate the differences among 3 TSH stimulation protocols: bTSH administered IM, rhTSH administered IM, and rhTSH administered IV. Each of the Beagles received each of the stimulation protocols with a 4-week washout period between treatments. Six dogs were randomly assigned to each of the 3 protocols.

TSH stimulation tests—In the Beagles, bTSH^a was administered IM (triceps brachii muscle of the right thoracic limb) at a dose of 1 unit (corresponding to 500 µg) in 0.5 mL of sterile injection water. Recombinant human TSH^b was injected at a dose of 75 µg in 0.5 mL of sterile injection water, IV (jugular vein) and IM (triceps muscle of the left thoracic limb), respectively. Blood samples (jugular venipuncture) were collected immediately before and 6 hours after administration of TSH. In dogs that received rhTSH IM, additional blood samples were taken after 2 and 4 hours. Thyroid-stimulating hormone-stimulation tests in the non-Beagles were performed with 75 µg of rhTSH, IV, with the second blood sample taken after 6 hours. After clot retraction at room temperature (21°C), serum was harvested via low-speed centrifugation and transferred to tubes for storage at -20°C for subsequent hormone assay.

Analytical procedures—Serum T_4 concentration was measured by use of a commercially available radioimmunoassay^c validated for use in dogs. Serum cTSH concentrations were determined by use of a homologous solid-part, 2-site chemiluminescent

enzyme immunometric assay^d validated for use in dogs. Assays for autoantibodies against T_3 , T_4 , and thyroglobulin were performed at the Endocrine Section of the Animal Health Diagnostic Laboratory, Michigan State University.^{8,9}

Statistical analysis—Data were analyzed via non-parametric statistical methods.^{c,f} Kruskal-Wallis 1-way ANOVA by ranks and the Dunn posttest for multiple comparisons were used to compare T_4 concentrations among the 3 groups (bTSH, rhTSH IM, and rhTSH IV). A Wilcoxon matched-pair test was used to compare the T_4 and post-TSH stimulation T_4 concentrations within the groups of animals receiving bTSH, rhTSH IM, and rhTSH IV, respectively. Thyroxine concentrations tested at several time points after rhTSH IM stimulation were tested for statistical differences by use of the Friedman repeated-measures test for paired samples and the Dunn posttest for multiple comparisons. Age, body weight, and post-TSH stimulation T_4 concentrations of Beagles and non-Beagles were compared by use of the Mann-Whitney *U* test. Values of *P* < 0.05 were considered significant.

Results

There were no adverse reactions after bTSH and rhTSH administration and no evidence of pain at the injection site after IM administration of TSH in any of the 38 dogs. Also, despite 3 repeated doses of TSH administered during a 12-week period in the Beagles, no anaphylactic reactions were observed.

Comparison of bTSH and rhTSH—After 6 hours, all dogs had a significant increase in serum T_4 concentration of at least 1.5 times basal T_4 concentration, independent of the stimulation protocol. Dogs with the lowest post-TSH T_4 concentration induced by use of bTSH also had the lowest values induced by use of the rhTSH IV and IM protocols, respectively.

Repeated stimulation neither led to a decrease nor to an increase in magnitude of stimulation in any of the dogs. Therefore, results were combined for further analysis according to the type of stimulation, yielding 18 T_4 and post-TSH T_4 values for each of the 3 treatments.

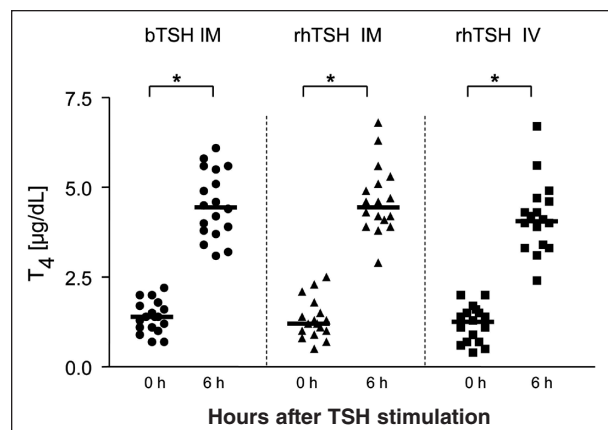


Figure 1—Scatter plot of serum T_4 concentrations before (time 0) and 6 hours after administration of bTSH IM, rhTSH IM, and rhTSH IV to 18 Beagles. *Significant (*P* < 0.05) difference between sampling times. Median values are indicated by horizontal lines.

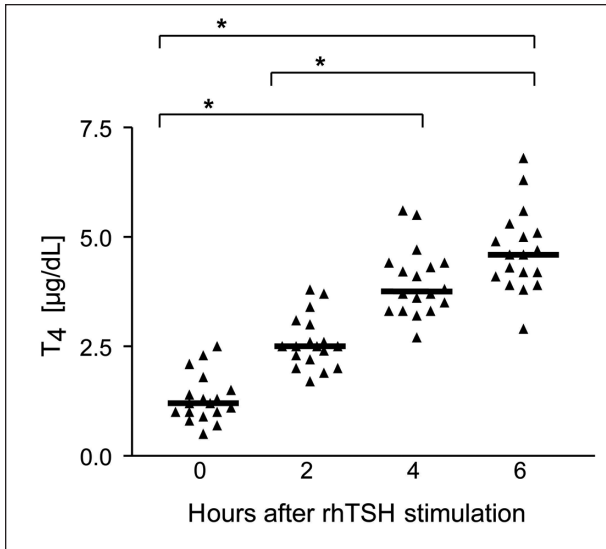


Figure 2—Scatter plot of serum T₄ concentrations before (time 0) and 2, 4, and 6 hours after IM administration of rhTSH to 18 Beagles. See Figure 1 for key.

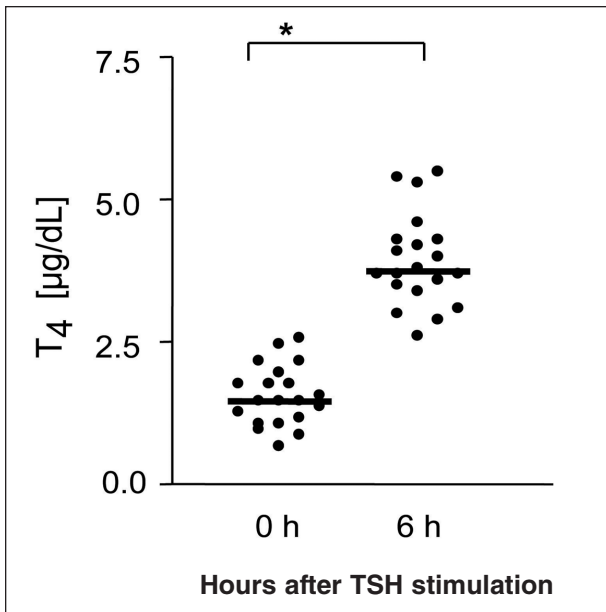


Figure 3—Scatter plot of serum T₄ concentrations before (time 0) and 6 hours after administration of rhTSH IV to 20 non-Beagles. See Figure 1 for key.

Median (range) post-TSH stimulation T₄ concentrations were 4.4 µg/dL (3.1 to 6.1 µg/dL), 4.6 µg/dL (2.9 to 6.8 µg/dL), and 4.0 µg/dL (2.3 to 6.6 µg/dL) after bTSH, IM; rhTSH, IM; and rhTSH, IV; respectively, and there were no significant differences among the 3 groups (Figure 1). After IM administration of rhTSH, T₄ concentration was significantly increased after 4 hours; there was a further nonsignificant increase in T₄ after 6 hours, compared with the 4-hour value (Figure 2).

Non-Beagles—There was a significant increase in serum T₄ concentration of at least 1.5 times basal T₄ concentration 6 hours after rhTSH administration (Figure 3). Median post-TSH stimulation T₄ concentra-

tion after rhTSH administration was 3.5 µg/dL (range, 2.5 to 5.2 µg/dL). Although age and weight were significantly ($P < 0.001$) greater, compared with body weight in Beagles, post-TSH stimulation T₄ concentration did not differ between the 2 groups.

Criteria to confirm euthyroidism—Because there was no difference in post-TSH stimulation T₄ concentration between the 18 Beagles and the 20 non-Beagles, values were combined to calculate reference values. Median and 5th- and 95th-percentile values of post-TSH stimulation T₄ concentration were 3.8, 2.5, and 5.5 µg/dL, respectively. According to the results, criteria to confirm euthyroidism were established as an increase in T₄ concentration after 6 hours of at least 1.5 times basal T₄ concentration and a post-TSH stimulation T₄ concentration of at least 2.5 µg/dL.

Discussion

Although species-related differences in the biological activity of protein hormones are known, this study did not detect a difference in magnitude of stimulation associated with administration of either rhTSH or bTSH in the same dogs. Stimulation of healthy beagles with 75 µg of rhTSH resulted in post-TSH stimulation serum T₄ concentrations equivalent to those after administration of 1 unit of purified bTSH. On the basis of these results, it seems that rhTSH is a valuable substitute for the bovine preparation.

Influences of dose and route of administration (IV versus IM) of bTSH have been discussed controversially for decades and finally resulted in different test protocols. The same situation seems to be developing for rhTSH. In the first study that evaluated rhTSH in Beagles, Sauve and Paradis⁶ concluded that IV administration of 50 µg was clinically useful. Daminet et al⁷ administered a dose of 75 µg of rhTSH to lean and obese Beagles that weighed from 10.8 to 25.9 kg, which resulted in adequate stimulation of the thyroid gland. Because dogs with hypothyroidism are often of large-breed origin (> 20 kg), it is important to evaluate rhTSH in breeds other than Beagles. Although the influence of TSH dose on magnitude of stimulation was not systematically evaluated in the present study, the post-TSH stimulation T₄ concentration of 20 dogs with body weights significantly greater than those of Beagles did not differ from that of those Beagles. Therefore, results suggest that administration of 75 µg induces adequate thyroid gland stimulation in healthy dogs independent of their body weight. Consequently, this dose might be recommended as low-dose protocol because the cost of rhTSH is a major limiting factor in a clinical setting.

In the present study, bTSH was administered IM. Considerable disagreement exists regarding the optimal route of TSH administration. Allergic and anaphylactoid reactions have been reported, most notably after repeated IV usage.^{10,11} We have traditionally administered 1 unit of bTSH, IM, and used modified criteria for an adequate response as proposed by Beale et al¹² and have not observed any adverse reactions. Interestingly and in contrast to the study by Sauve and Paradis,⁶ route of administration (IV or IM) did not result in a significant difference in post-TSH stimula-

tion T_4 concentration 6 hours after stimulation in Beagles. There are several possible explanations for the observed discrepancy including differences in the muscle selected for IM administration, differences in hydration status of the dogs, and body fat content. Furthermore, use of needles with different gauges could also have influenced results. In view of these findings, it is recommended that IM administration should be used cautiously, particularly in obese dogs, which are common among dogs with hypothyroidism.

The optimal time point of the second blood sampling has already been debated regarding bTSH and has resulted in a recommendation of sampling after 4 hours versus after 6 hours.^{11,13} Of course, earlier blood sampling would be more advantageous because the test is already associated with considerable inconvenience and costs associated with the required hospitalization of the animal. A time-dependent increase in serum T_4 concentration was observed in the present study in that T_4 values at 6 hours were greater than those at 2 hours, suggesting that with 75 μg of rhTSH administered IM, sampling 6 hours after the injection of rhTSH may be preferable. Therefore, it is proposed that performing the TSH stimulation test with 75 μg of rhTSH and taking the second blood sample after 6 hours is indicated. However, this issue deserves further study before general recommendations can be made.

By use of the proposed protocol, hypothyroidism can be excluded if post-TSH stimulation T_4 concentration is at least 2.5 $\mu\text{g}/\text{dL}$ and 1.5 times basal T_4 concentration. Interestingly, the same criteria were found in an earlier study¹⁴ of bTSH. This additionally confirms an equivalent biological activity of bTSH, compared with rhTSH.

In the first study⁶ using rhTSH, criteria for test interpretation were based on published reference ranges for bTSH.¹⁵ The authors considered dogs euthyroid if the post-TSH stimulation T_4 value was $> 45 \text{ nmol/L}$ (3.6 $\mu\text{g}/\text{dL}$) or increased by at least 24 nmol/L (1.87 $\mu\text{g}/\text{dL}$). These criteria were also used by Daminet et al⁷ and are commonly recommended. However, applying the suggested criteria to the results of the present study reveals that 1 of the 18 Beagles and 8 of the 20 healthy non-Beagles did not have the criteria for euthyroidism. This discrepancy could be explained by the fact that the reference values for T_4 concentration applied in the studies mentioned were greater than the reference range used in the present study. It is therefore highly recommended that criteria for test interpretation should not merely be adopted from published literature regarding rhTSH. As for any endocrine or analytical test, it is important for each laboratory to establish reference ranges rather than to use values extracted from the literature.

Results of the present study cannot be regarded as conclusive. Before any recommendations for general use in veterinary practice can be made, the diagnostic efficacy of the proposed rhTSH test protocol and the

criteria for test interpretation must be evaluated in dogs with clinically suspected hypothyroidism and preferably in dogs in which diagnosis has been confirmed by other means.

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- a. Thyrotropic hormone, Sigma Chemical Co, St Louis, Mo.
 - b. Thyrogen, Genzyme Corp, Suffolk, UK.
 - c. Canine DPC radioimmunoassay, Coat-A-Count, Diagnostic Products Corp, Los Angeles, Calif.
 - d. Immulite canine TSH, Diagnostic Products Corp, Los Angeles, Calif.
 - e. SPSS, Statistical Package for the Social Sciences, Software Packets for Windows, version 11, SPSS Inc, Chicago, Ill.
 - f. GraphPad PRISM for Windows, version 3.0, GraphPad Software Inc, San Diego, Calif.
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