

# Effect of Thyrotropin Storage on Thyroid-Stimulating Hormone Response Testing in Normal Dogs

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The stability of reconstituted, refrigerated thyrotropin was evaluated. Thyrotropin (TSH) was reconstituted at the start of the study and stored at 4°C. A TSH stimulation test was performed in eight healthy, euthyroid dogs at weekly intervals for 1 month. In seven of eight dogs, there was no significant difference ( $P < 0.05$ ) between the post-TSH  $T_3$  concentrations and the post-TSH  $T_4$  concentrations for the duration of the study. For one dog, the post-TSH  $T_4$  concentration was below the normal post-TSH  $T_4$  range following the administration of reconstituted TSH that had been stored 4 weeks. The  $T_3$  response to the TSH, however, was normal. This dog responded normally to freshly reconstituted TSH. The results of this study suggest that reconstituted bovine TSH can be stored at 4°C for at least 3 weeks without loss of biologic activity in the dog. (Journal of Veterinary Internal Medicine 1987; 1:91-94)

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CANINE hypothyroidism is a common metabolic disorder resulting from decreased circulating thyroid hormone.<sup>1-3</sup> The deficiency in circulating thyroid hormone affects the metabolic function of almost all organ systems. As a result, clinical signs are diffuse in nature, variable, often vague, and rarely pathognomonic for hypothyroidism.<sup>1,3</sup> The determination of basal 3,3',5-triiodothyronine ( $T_3$ ) and thyroxine ( $T_4$ ) concentrations has been recommended to help establish a diagnosis of hypothyroidism.<sup>4</sup> Unfortunately, variables such as concurrent drug therapy, concomitant disease, nutritional status, plasma concentrations of certain endogenous hormones, estrus, and normal daily fluctuations can all influence plasma thyroid hormone concentrations.<sup>2,5-11</sup> Most of these variables can result in falsely low basal thyroid hormone concentrations and a misdiagnosis of hypothyroidism in a euthyroid dog.

The thyrotropin (TSH) stimulation test is designed to evaluate the responsiveness of the patient's thyroid gland to exogenously administered TSH. This function test is not affected by many of the variables that can alter

basal thyroid hormone concentrations and, as such, is considered to be a more accurate diagnostic test in the evaluation of the thyroid gland.<sup>12-14</sup> Unfortunately, the TSH stimulation test is not commonly performed in private practice because of the perceived ease of establishing a diagnosis by trial therapy, the unreliable availability of TSH, the time required to complete the test, and the additional expense compared to the measurement of basal thyroid hormone concentrations.

A number of different protocols for the TSH stimulation test have been advocated.<sup>4,12,14-17</sup> Most of these recommend the injection of 5 IU (1 vial) of TSH (Dermathycin, Wellcome Animal Health Division, Burroughs Wellcome Co., Kansas City, MO) per test. However, administration of TSH based on body weight (eg, 0.1 IU TSH/kg body weight) has also been utilized to perform the stimulation test.<sup>12,17,18</sup> Utilizing these protocols, only a portion of the TSH in the vial would be used. Unfortunately, because the manufacturer states that the shelf life of reconstituted TSH is only 48 hours (Dermathycin, Wellcome Animal Health Division, Burroughs Wellcome Co., Kansas City, MO), the unused TSH is frequently discarded. If the shelf life of the reconstituted TSH were longer than 48 hours, multiple TSH stimulation tests could potentially be performed following the reconstitution of one vial of TSH, thereby helping to minimize the cost of the test. The purpose of this study was to determine the stability of reconstituted TSH when stored at 4°C.

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This study was supported, in part, by the Canine Disease Research Fund, Purdue University, West Lafayette, Indiana.

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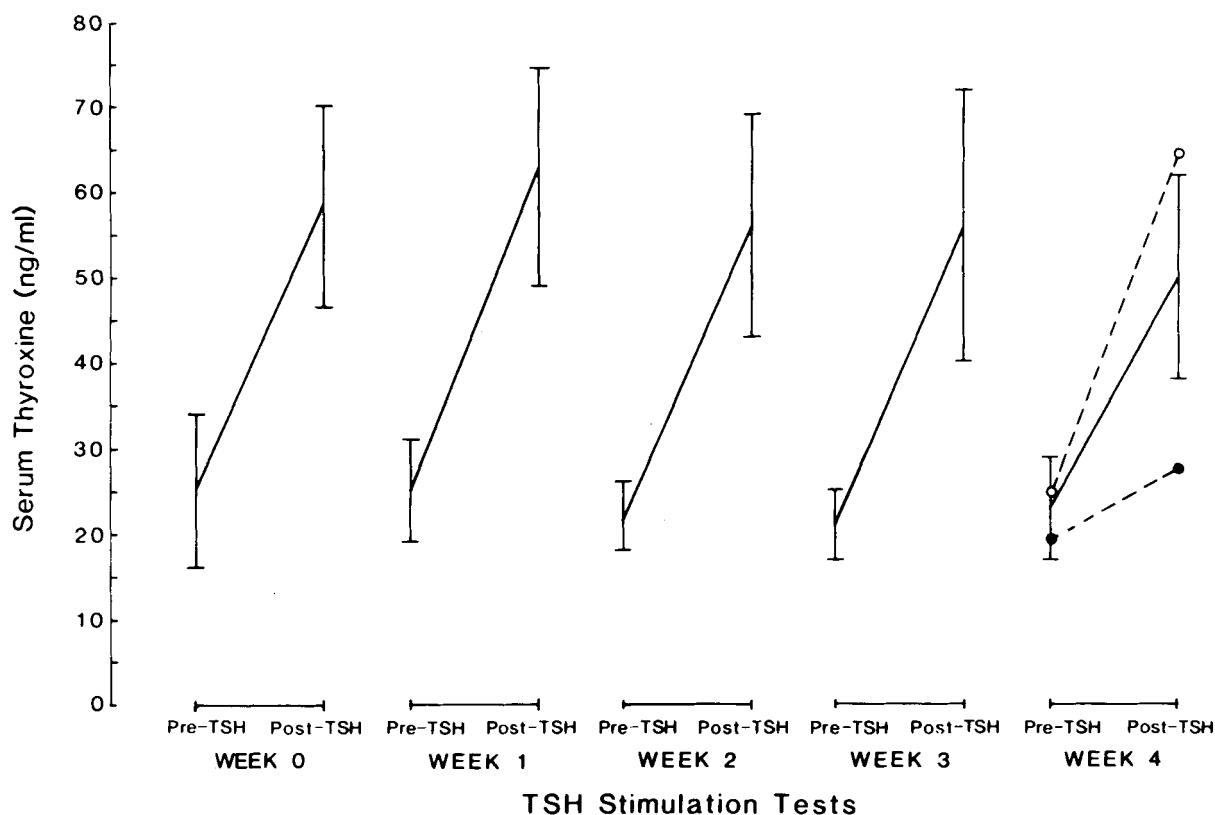


FIG. 1. Mean serum T<sub>4</sub> concentration for the eight dogs before and after the IV administration of TSH (0.1 u/kg). The vertical lines represent the standard deviation. The solid circles represent the response of one dog during week 4. The open circles represent the response of the same dog to freshly reconstituted TSH (normal pre-TSH T<sub>4</sub>: 20 ± 7 ng/ml; normal post-TSH T<sub>4</sub>: 52 ± 11 ng/ml).

### Materials and Methods

Eight dogs, three males and five females, were used in this study. All dogs were house pets, were alert and active in the home environment, and were not receiving any medications at the time of the study. None of the dogs had any previous history of illness. The dogs' ages ranged from 2–10 years, and their weights varied between 20 and 32 kg. The establishment of normal thyroid function in each dog was based on the lack of symptoms associated with hypothyroidism, in conjunction with results of basal serum T<sub>3</sub> and T<sub>4</sub> concentrations and response to TSH stimulation testing.

At the time of the initial TSH stimulation test, enough TSH was reconstituted to perform four additional TSH stimulation tests at weekly intervals on each dog. The reconstituted TSH was refrigerated at 4°C for the duration of the study. The TSH stimulation test was performed on the same day of each week, at approximately 8 AM, for each dog. For the TSH stimulation test, TSH (0.1 IU/kg body weight) was injected intravenously and blood was collected just prior to and 6 hours following TSH administration. Serum was stored at –60°C until thyroid hormone concentrations could be determined. The assay for determining T<sub>3</sub> and T<sub>4</sub> has been previously described.<sup>18</sup> Normal thyroid hormone concentrations

(mean ± SD) for our laboratory are basal T<sub>4</sub> 20 ± 7 ng/ml, post-TSH T<sub>4</sub> 52 ± 11 ng/ml, basal T<sub>3</sub> 1.0 ± 0.2 ng/ml, and post-TSH T<sub>3</sub> 1.6 ± 0.4 ng/ml.

The Newman-Kuels method of statistical analysis was used to evaluate the significance of changes in serum thyroid hormone concentrations following TSH administration. One-way analysis of variance was used to evaluate the significance of changes in the mean post-TSH T<sub>3</sub> and T<sub>4</sub> concentrations between weeks.

### Results

The initial basal serum thyroid hormone concentrations were within the normal range for all eight dogs. In addition, all eight dogs had a normal increase in the plasma T<sub>4</sub> and T<sub>3</sub> concentrations following the administration of TSH (Figs. 1, 2). These results established the presence of euthyroidism in the dogs used in this study.

In seven of the eight dogs, there was no significant difference ( $P < 0.05$ ) between the post-TSH T<sub>3</sub> concentrations or the post-TSH T<sub>4</sub> concentrations for the duration of the study (Figs. 1, 2). All seven dogs still responded normally to the TSH 1 month after its reconstitution and storage.

One dog responded normally to the reconstituted, stored TSH through week 3. At week 4, the post-TSH T<sub>4</sub>

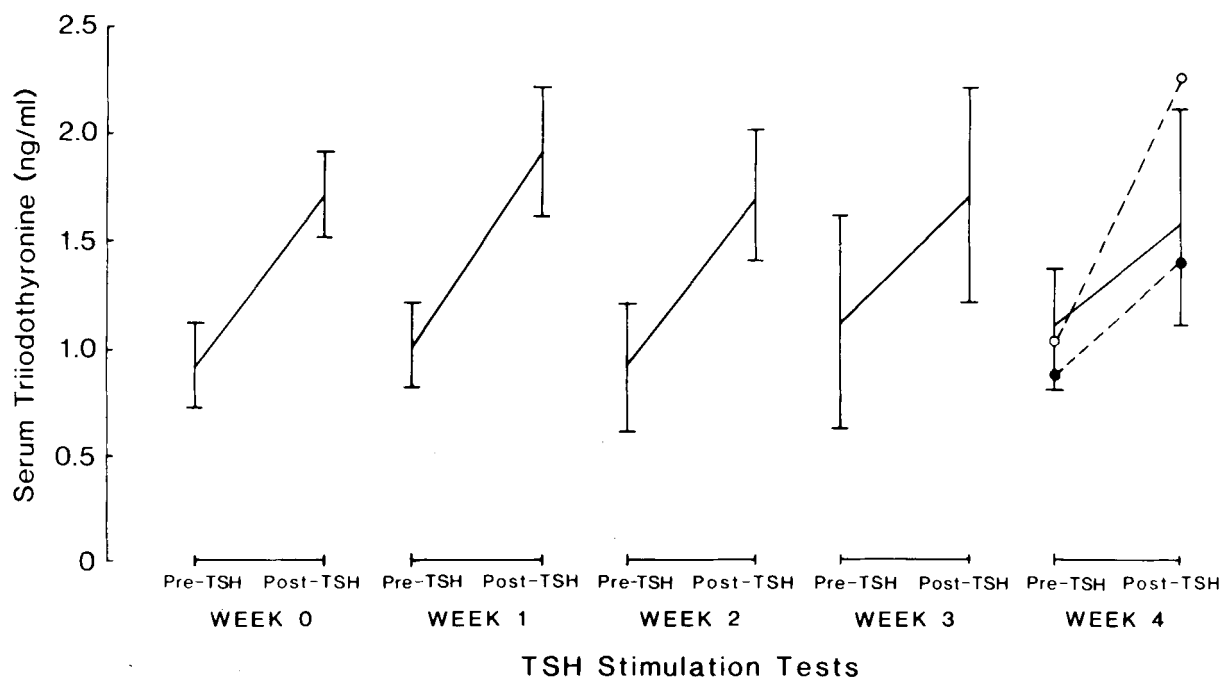


FIG. 2. Mean serum T<sub>3</sub> concentrations for the eight dogs before and after the IV administration of TSH (0.1 u/kg). The vertical lines represent the standard deviation. The solid circles represent the response of one dog during week 4. The open circles represent the response of the same dog to freshly reconstituted TSH (normal pre-TSH T<sub>3</sub>:  $1.0 \pm 0.2$  ng/ml; normal post-TSH T<sub>3</sub>:  $1.6 \pm 0.4$  ng/ml).

concentration was 28 ng/ml, which is more than two standard deviations below the normal post-TSH T<sub>4</sub> mean ( $52 \pm 11$  ng/ml) for the laboratory being used. The post-TSH T<sub>3</sub> concentration, however, was 1.4 ng/ml, which is within the normal post-TSH T<sub>3</sub> range ( $1.6 \pm 0.4$  ng/ml). In addition, this dog had a normal response to freshly reconstituted TSH, which was administered a few days after the end of the study (Figs. 1, 2).

### Discussion

The results of this study suggest that reconstituted bovine TSH can be stored at 4°C for at least 3 weeks without loss of biologic activity. Seven of eight dogs responded normally to TSH 1 month following its reconstitution, suggesting that the stability may, in fact, be longer than 4 weeks. One dog, however, had a subnormal post-TSH T<sub>4</sub> response 1 month after reconstitution of the TSH. The post-TSH T<sub>4</sub> concentration fell within three standard deviations of the normal mean and may have merely represented a normal response. Loss of activity of the reconstituted TSH seems unlikely because of the normal T<sub>3</sub> response to the TSH in this dog, and a normal response following administration of TSH from the same vial to another dog in the study. In the later dog, the T<sub>3</sub> concentration went from 1.7 to 2.4 ng/ml and the T<sub>4</sub> concentration from 16 to 47 ng/ml following the administration of the TSH in question. The development of hypothyroidism in this dog during the study also seems unlikely because of the normal basal T<sub>3</sub> and

T<sub>4</sub> concentrations and the normal response to freshly reconstituted TSH administered a few days later (Figs. 1, 2).

The development of anti-TSH antibodies in this dog following multiple injections of a foreign protein (ie, bovine TSH) may have interfered with the test by inactivating the injected TSH. The greater response following administration of freshly reconstituted TSH may represent a difference in antigenicity between lot numbers of TSH used for the two tests. Although not determined in this study, anti-TSH antibodies that inactivate TSH seem unlikely because of the normal post-TSH T<sub>3</sub> response in all the dogs throughout the study.

Clinical impressions with the use of reconstituted TSH in evaluating thyroid function would tend to support the findings in this study. During a 2-month period following completion of this study, reconstituted TSH that had been stored from 1–3 weeks was used for TSH stimulation tests in 28 euthyroid dogs. The mean pre and post-TSH T<sub>3</sub> and T<sub>4</sub> concentrations in these dogs was  $0.9 \pm 0.3$  ng/ml,  $1.9 \pm 0.5$  ng/ml,  $23 \pm 9$  ng/ml, and  $58 \pm 16$  ng/ml, respectively. These values fell within the normal range for the laboratory utilized. The use of reconstituted, stored TSH consistently allowed the accurate establishment of euthyroidism in these dogs. No euthyroid dog had a response suggestive of hypothyroidism. During the same period, unresponsiveness to reconstituted, stored TSH was documented in four dogs. Similar results were found following administration of

freshly reconstituted TSH. These four dogs subsequently responded to thyroid hormone supplementation.

The reasons for the discrepancy between the manufacturer's recommended shelf-life of reconstituted TSH and the results of this study are not readily apparent. Nevertheless, the results of this study suggest that reconstituted bovine TSH can be stored at 4°C for at least 3 weeks without loss of biologic activity in the dog.

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