



Original Research

Serum Thyroid Hormone and Thyrotropin Concentrations in Adult Horses as They Age



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ABSTRACT

With more horses remaining active longer in life, it is important to characterize changes that occur normally with aging, so that these can be differentiated from development of disease. The objective of the study was to test the hypotheses that geriatric horses have lower circulating concentrations of thyroid hormones and/or higher serum thyrotropin (TSH) concentrations compared to younger horses. Serum thyroid hormone and TSH concentrations from 71 normal, healthy horses that had participated in prior research projects were analyzed for effects of age, sex, and season when samples were obtained. All samples had been assayed in the same previously validated radioimmunoassays. There were no differences in serum concentrations of thyroid hormones or TSH by sex or season. Serum total thyroxine (T4) was greater in 3- to 6-year-old horses compared to all other age groups and was negatively correlated with age. There were no differences among age groups for free T4 and total and free tri-iodothyronine (T3). Serum TSH concentration was significantly greater in old horses (≥ 15 or ≥ 20 years) compared to young (3–10 years) and intermediate (11–14 years) age groups. Serum TSH was positively correlated with age. There were no significant differences in thyroid hormone responses to thyrotropin releasing hormone (TRH) among young, intermediate, or old horses. However, the TSH response to TRH was significantly different in both groups of older horses compared to intermediate and young horses. Serum total thyroxine concentrations decrease and serum TSH concentrations increase in horses as they age, with no changes in free T4 or T3.

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1. Introduction

Over the past 20 years or so, there has been increased interest in the health and well-being of geriatric horses. Horses are staying active longer, and the proportion of veterinary caseload attributed to older horses has increased [1,2]. It is therefore important to characterize changes that occur normally with aging, so that these can be differentiated from development of disease.

Animal welfare/ethical statement: Horses were client owned or belonged to research or teaching herds at North Carolina State University. All university-owned horses were cared for according to principles outlined in the NIH Guide for the Care and Use of Laboratory Animals, and all studies had been approved by the North Carolina State University Institutional Animal Care and Use Committee.

Conflict of interest statement: The author has no conflicting interests to report.

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Changes in circulating thyroid hormone and thyrotropin (TSH) concentrations with aging have been noted in other species. In general, thyroid hormones decrease with age in healthy dogs and cats, although they tend to remain within established reference ranges [3–5]. Multiple studies in elderly people have shown that TSH increases, especially after the age of 70 years, and is variably associated with either no change or a slight increase in free thyroxine (fT4) and/or decreased tri-iodothyronine (T3) concentrations [6–13]. However, TSH has also been shown to decrease with age in people living in iodine-deficient environments [14–16].

Very little attention has been given to serum thyroid hormone or TSH concentrations in adult horses as they age. Neonatal horses are born with very high circulating concentrations of thyroid hormones that gradually decrease to the adult reference range over the first few months of life [17–20]. One study measured serum thyroid hormone concentrations in Thoroughbred foals over the first 13 months of life and reported some changes with aging and with gender [21]. Mendoza et al reported

that thyroid hormone concentrations (total thyroxine [TT4], fT4, total T3 [TT3], free T3 [fT3], and reverse T3) were lower in donkeys that were aged >10 years compared to donkeys aged <5 years [22]. These authors also found that serum thyroid hormones were higher in donkeys (aged 2–19 years) compared to horses (aged 4–9 years). However, in that study, there were no horses >10 years, and TSH was not measured. To test the hypotheses that thyroid hormones decrease with age and that TSH is increased in geriatric horses, serum thyroid hormone and TSH concentrations and their responses to thyrotropin releasing hormone (TRH) were examined in a group of horses that had served as normal controls for previous thyroid hormone studies in horses [23–27].

2. Materials and Methods

Thyroid hormone and TSH data from normal horses that had participated in several previously reported studies [23–27] were chosen for analysis because the same assays had been used to measure thyroid hormones and TSH in these studies. Horses from the various studies were client owned or belonged to research or teaching herds at North Carolina State University. All university-owned horses were cared for according to principles outlined in the NIH Guide for the Care and Use of Laboratory Animals, and all studies had been approved by the North Carolina State University Institutional Animal Care and Use Committee. Horses had received no medications for ≥ 4 weeks before sampling and were determined to be systemically healthy based on history and physical examination (including evaluation of body temperature, heart rate, respiratory rate, mucous membrane color, capillary refill time, pulse quality, digital pulse strength, heart and lung auscultation, gastrointestinal motility, manure quality, and manual palpation of the neck to detect possible thyroid gland enlargement). Blood sampling was performed with each horse in its home environment. Baseline samples were obtained in the mornings between 8:00 AM and 12:00 PM, by jugular venipuncture. In addition, TRH stimulation tests were performed in some of the horses, as described previously [23–25]. Briefly, a control blood sample was obtained, 1 mg TRH was given IV, and then, additional blood samples were drawn at 60, 120, and 240 minutes after TRH administration for thyroid hormone measurements and at 15, 30, 45, 60, 120, and 240 minutes for TSH measurements. After sampling, blood was allowed to clot at room temperature for 30 minutes, and then, samples were centrifuged at 4°C, and serum was removed and stored at –70°C until assayed.

Serum TT4, free T4 by equilibrium dialysis (fT4D) and fT3 concentrations were measured using commercially available

radioimmunoassay (RIA) kits (Magic T4; Ciba Corning Diagnostics, East Walpole, MA; Free T4 by equilibrium dialysis; Nichols Institute Diagnostics, San Clemente, CA; Magic fT3; Ciba Corning Diagnostics, East Walpole, MA). Total T3 and TSH were measured by RIA using described procedures and modifications [23,28–30]. All assays had been previously validated for use in the horse [23,26,31]. Total T4 assay sensitivity was 3 nmol/L; fT4D assay sensitivity was 1.8 pmol/L; fT3 assay sensitivity was 0.1 pmol/L; TT3 assay sensitivity was 0.3 nmol/L; and TSH assay sensitivity was 0.02 ng/mL.

Commercial software was used for statistical analyses and graph generation (SigmaPlot Software, Inc, Chicago, IL). Parametric tests were used for normally distributed data (Shapiro–Wilk); nonparametric tests were used when data were not normally distributed. For analysis of baseline thyroid hormone and TSH concentrations, horses were initially divided into five groups based on their age: 3 to 6 years ($n = 15$), 7 to 10 years ($n = 27$), 11 to 14 years ($n = 13$), ≥ 15 years ($n = 16$), and ≥ 20 years ($n = 10$) and compared by a one-way analysis of variance (ANOVA) with multiple comparisons by the Fisher least significant difference method or by Kruskal–Wallis ANOVA on ranks. Pearson product moment and Spearman rank order correlations between baseline thyroid hormones and TSH and age were also performed. Any differences associated with sex (mare, gelding) were analyzed by *t* test or Mann–Whitney Rank Sum test. Season that blood was drawn (spring, summer, fall, winter) was analyzed by one-way ANOVA or Kruskal–Wallis one-way ANOVA on ranks. Because a TRH stimulation test was not performed in every horse, the two younger groups were combined into one group (3–10 years, $n = 19$) for analysis. The 11- to 14-year-old group ($n = 5$) was kept as a separate group because those horses were considered to be intermediate between young and old horses. These two groups were compared to horses that were ≥ 15 years ($n = 11$) or ≥ 20 years ($n = 7$) by two-way repeated measures ANOVA on raw data or on ranks, as appropriate. Peak serum concentrations as well as maximum increases and percentage increases in thyroid hormones or TSH in response to TRH were also compared by one-way ANOVA or by Kruskal–Wallis ANOVA on ranks, as appropriate. Significance was set at $P < .05$.

3. Results

Thyroid hormone and TSH data from 71 normal horses were examined and found to include 15 horses ranging in age from 3 to 6 years, 27 horses ranging in age from 7 to 10 years, 13 horses ranging in age from 11 to 14 years, 16 horses that were aged ≥ 15 years, and 10 horses that were aged ≥ 20 years. They represented a mixture of breeds including primarily Quarter Horse and Quarter Horse–related breeds, Thoroughbreds, warmbloods,

Table 1
Serum concentrations of TT4, fT4D, TT3, fT3, and TSH in young horses (aged 3–6 years and 7–10 years), intermediate horses (aged 11–14 years), and old horses (≥ 15 years and ≥ 20 years).

Age group	TT4, nmol/L	fT4D, pmol/L	TT3, nmol/L	fT3, pmol/L	TSH, ng/mL
3–6 y, $n = 15$	24 \pm 5.80 ^{b,c,d,e} (8–44)	23 \pm 4.45 (9–40)	1.20 \pm 0.22 (0.60–2.00)	1.70 \pm 0.53 (1.20–4.20)	0.31 \pm 0.08 (0.03–0.42)
7–10 y, $n = 27$	18 \pm 3.56 ^a (6–46)	19 \pm 3.71 (9–47)	0.80 \pm 0.22 (0.40–2.90)	1.50 \pm 0.56 (0.50–5.7)	0.37 \pm 0.08 (0.06–0.80)
11–14 y, $n = 13$	19 \pm 4.30 ^a (8–32)	16 \pm 7.01 (7–40)	0.90 \pm 0.23 (0.40 + 1.60)	1.30 \pm 0.78 (0.80–4.50)	0.34 \pm 0.09 (0.03–0.50)
≥ 15 y, $n = 16$	15 \pm 4.53 ^a (6–32)	24 \pm 3.17 (11–33)	0.90 \pm 0.19 (0.30–1.80)	2.05 \pm 0.81 (0.10–5.90)	0.44 \pm 0.13 ^{b,c} (0.14–0.97)
≥ 20 y, $n = 10$	14.5 \pm 6.85 ^a (6–32)	23.5 \pm 4.45 (11–32)	0.95 \pm 0.20 (0.40–1.40)	2.05 \pm 1.15 (0.10–5.90)	0.52 \pm 0.20 ^{a,b,c} (0.17–0.97)

Abbreviations: TT4, total T4; TT3, total T3; fT3, free T3; fT4D, free T4 by dialysis.

Data are expressed as median \pm 95% confidence interval (range).

^a Significant difference compared to horses 3 to 6 years old ($P < .05$).

^b Significant difference compared to horses 7 to 10 years old ($P < .05$).

^c Significant difference compared to horses 11 to 14 years old ($P < .05$).

^d Significant difference compared to horses aged ≥ 15 years ($P < .05$).

^e Significant difference compared to horses aged ≥ 20 years ($P < .05$).

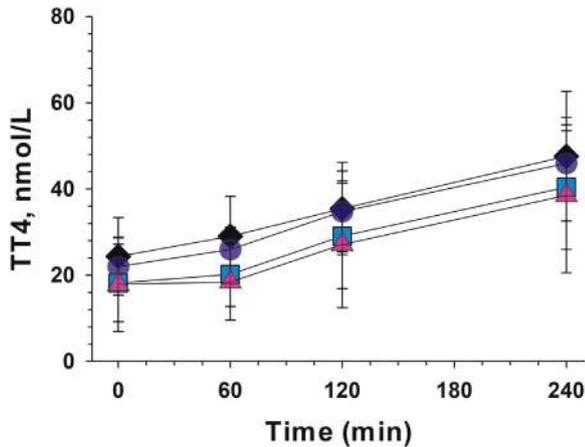


Fig. 1. TT4 responses to TRH in 3- to 10-year-old horses ($n = 19$, diamonds), 11- to 14-year-old horses ($n = 5$, open circles), ≥ 15 -year-old horses ($n = 11$, triangles), and ≥ 20 -year-old horses ($n = 7$, squares). Responses are not significantly different from each other. TT4, total T4; TRH, thyrotropin releasing hormone.

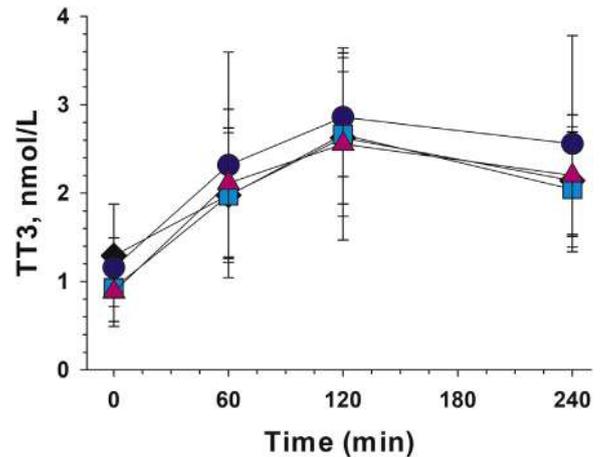


Fig. 3. TT3 responses to TRH in 3- to 10-year-old horses ($n = 19$, diamonds), 11- to 14-year-old horses ($n = 5$, open circles), ≥ 15 -year-old horses ($n = 11$, triangles), and ≥ 20 -year-old horses ($n = 7$, squares). Responses are not significantly different from each other. TT3, total T3; TRH, thyrotropin releasing hormone.

saddlebreds, and Arabians. There were no significant differences in serum thyroid hormone or TSH concentrations when compared by sex (mares vs. geldings) or by season of blood sampling (spring, summer, fall, winter).

Table 1 shows resting serum concentrations of thyroid hormones and TSH in the five groups of horses (3–6 years, 7–10 years, 11–14 years, ≥ 15 , and ≥ 20). For the most part, data were not normally distributed and therefore are expressed as median \pm 95% confidence interval and range. Resting serum TT4 concentrations were significantly greater in the 3- to 6-year-old age group compared to each of the other age groups. There was also a significant negative correlation between age and resting serum TT4 concentration (Pearson $P = .033$, Spearman $P = .022$). Horses that were aged ≥ 20 years had significantly greater resting serum TSH concentrations than 3- to 6-year-old, 7- to 10-year-old, and 11- to 14-year-old horses. Horses that were aged ≥ 15 years also had greater baseline serum TSH concentrations compared to 3- to 6-year-old and 11- to 14-year-old horses. There was a significant positive correlation between age and resting serum TSH concentration (Pearson $P = .001$, Spearman $P = .006$). There were no significant differences in resting serum concentrations of ft4D, TT3, or

ft3 among the age groups nor were any of these three thyroid hormone concentrations correlated to age.

In horses in which a TRH stimulation test was performed, there were no significant differences in thyroid hormone responses to TRH among young horses (3–10 years), intermediate age horses (11–14 years), and old horses (≥ 15 years or ≥ 20 years) when compared by repeated measures ANOVA on ranks (Figs. 1–4). However, TSH responses to TRH were significantly different for both groups of older horses compared to younger horses and intermediate age horses (Fig. 5). Peak serum TSH concentrations after TRH were not different among the four age groups (Table 2). Because resting or baseline TSH is higher in old horses compared to young or intermediate age horses, the actual increase in TSH or percent increase in TSH appears to be lower in the older horses. However, this was not statistically significant, perhaps due to the smaller number of horses in which a TRH stimulation test was performed.

4. Discussion

To the author's knowledge, this is the first study to measure serum TSH concentrations in adult horses as they age. The present

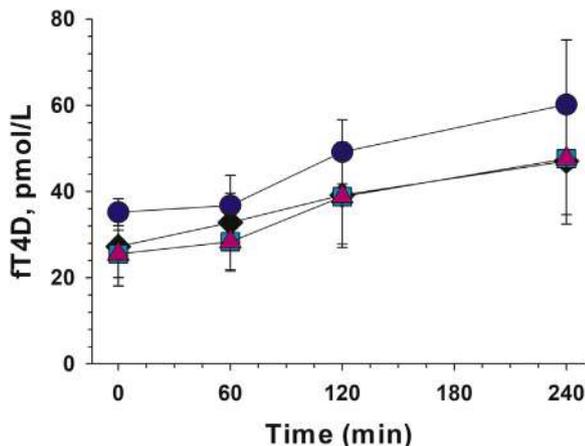


Fig. 2. ft4D responses to TRH in 3- to 10-year-old horses ($n = 19$, diamonds), 11- to 14-year-old horses ($n = 5$, open circles), ≥ 15 -year-old horses ($n = 11$, triangles), and ≥ 20 -year-old horses ($n = 7$, squares). Responses are not significantly different from each other. ft4D, free T4 by dialysis; TRH, thyrotropin releasing hormone.

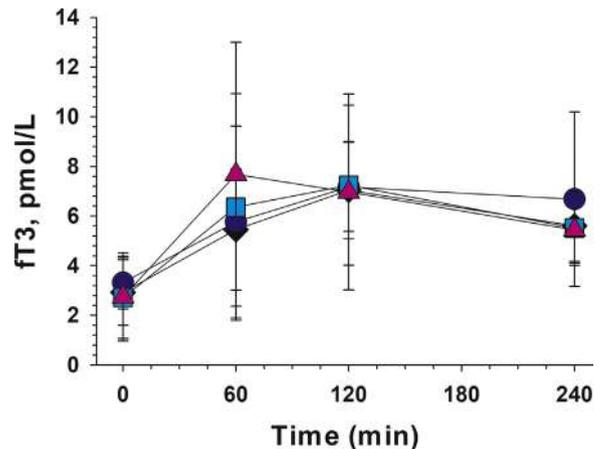


Fig. 4. ft3 responses to TRH in 3- to 10-year-old horses ($n = 19$, diamonds), 11- to 14-year-old horses ($n = 5$, open circles), ≥ 15 -year-old horses ($n = 11$, triangles), and ≥ 20 -year-old horses ($n = 7$, squares). Responses are not significantly different from each other. ft3, free T3; TRH, thyrotropin releasing hormone.

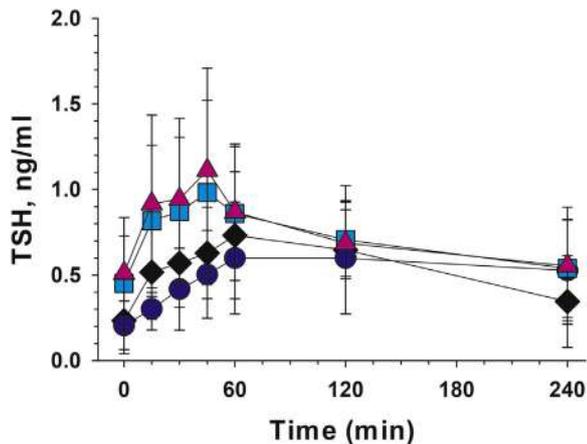


Fig. 5. TSH responses to TRH in 3- to 10-year-old horses ($n = 19$, diamonds), 11- to 14-year-old horses ($n = 5$, open circles), ≥ 15 -year-old horses ($n = 11$, triangles), and ≥ 20 -year-old horses ($n = 7$, squares). Responses for both groups of older horses (≥ 15 and ≥ 20 years) are significantly different from the younger (3–10 years) and intermediate (11–14 years) horses. TRH, thyrotropin releasing hormone.

study found TSH to be significantly greater in older horses compared to younger and intermediate age horses, no matter whether older horses were defined as ≥ 15 years or as ≥ 20 years. The increase in serum TSH concentration in old horses was associated with lower serum TT4 concentrations but no changes in any of the other thyroid hormones measured, including fT4D. Thus, the decrease in TT4 may be a result of a decrease in binding proteins or altered binding affinity rather than decreased production by the thyroid gland. The TSH findings of this study are similar to those reported in aging people living in iodine-sufficient environments [6–13].

Increased TSH in older populations could indicate the development of subclinical hypothyroidism with aging, or it could represent a normal change with aging, potentially representing a change in the TSH set point, decreased TSH bioactivity, or altered TSH receptor sensitivity at the thyroid gland [10,12]. In people, there is evidence to suggest that increasing TSH is a normal aging change. Using data from the National Health and Nutrition Examination Survey, Surks and Hollowell showed that TSH frequency distribution curves were similar in shape in young versus old people, but the TSH distribution curve for older people was shifted to the right (i.e., to higher TSH) [7]. The authors argued that if the increase in TSH had been caused by development of subclinical hypothyroidism, the peak frequency in old people would have occurred at the same TSH concentration as in young individuals, but there would be a larger skew toward higher values in the older population. Based on their analysis, Surks and Hollowell recommended adoption of age-based reference ranges to prevent erroneous diagnosis of subclinical hypothyroidism in older people. In a longitudinal study in which TSH was measured in the same people 13 years

apart, Bremnar et al also recommended adoption of age-based reference ranges for TSH [10]. The authors suggested that the increase in TSH with age resulted from an age-related change in TSH set point or reduced TSH bioactivity with aging rather than occult thyroid disease.

It seems unlikely that all or most of the older horses in the present study had subclinical hypothyroidism, partly because primary hypothyroidism is rare in horses and partly because the serum concentrations of TSH in the older horses in this study, although greater than younger horses, were still within what is considered to be the normal range of TSH for this assay [23,32–37]. Also, in a previous study that documented the development of hypothyroidism in horses that were administered propylthiouracil, serum TT3 and fT3 concentrations decreased right away, before TSH started to increase, and serum TT4 concentrations were not significantly decreased until serum TSH concentrations were greater than 1 ng/mL [23]. In the present study, none of the horses had serum TSH concentrations as high as 1 ng/mL, and there were no differences in serum concentrations of TT3, fT3, or fT4D.

In this study, we were primarily interested in whether serum concentrations of thyroid hormones or TSH would be different in horses considered to be geriatric compared to younger adults, similarly to what has been observed in other species. Most previous studies of aging in horses have used chronologic age, more specifically from 15 to 20 years or older, to define the geriatric population [38]. For this reason, we initially chose to classify horses aged ≥ 20 years as “old.” However, due to the limited number of horses in that age group, we chose to also examine horses aged ≥ 15 years. Because aging is most likely a continuum, horses that might be at different stages of aging (i.e., 11–14 years) were included in a separate group in the present analysis. The smaller number of old horses compared to young horses is a limitation of this study, and results perhaps should be considered to be preliminary. That being said, the proportion of horses aged ≥ 15 or ≥ 20 years in the group of horses described in the present study was 23% and 14% respectively, which is similar to the proportions of older horses that have been reported in previous larger studies [39–42].

In conclusion, this study suggests that serum concentrations of TSH increase and TT4 decrease as horses age. It is important to keep this in mind when interpreting results of thyroid hormone and TSH measurements in older horses. Also, when studying the effects of different treatments, diets, illnesses, and so forth on thyroid function in older horses, it is important to have age-matched controls. It would be interesting to measure thyroid hormones and TSH in a larger group of horses and to follow those horses over a 10- to 20-year period.

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Table 2

Peak serum concentrations of TSH, increases in TSH (peak–baseline), and percent increases in TSH in horses in which a TRH stimulation test was performed.

	Peak TSH, ng/mL	Increase TSH, ng/mL	% Increase TSH
Young horses (3–10 y, $n = 19$)	0.93 ± 0.18 (0.26–1.61)	0.66 ± 0.21 (0.05–1.42)	420 ± 497 (17.5–4067)
Intermediate age horses (11–14 y, $n = 5$)	0.76 ± 0.18 (0.50–0.85)	0.40 ± 0.25 (0.24–0.77)	144 ± 1130 (92–2200)
Old horses (≥ 15 y, $n = 11$)	0.89 ± 0.31 (0.78–0.97)	0.58 ± 0.25 (0.15–1.44)	108 ± 155 (37–800)
Old horses (≥ 20 y, $n = 7$)	0.89 ± 0.46 (0.14–2.02)	0.54 ± 0.38 (0.33–1.44)	108 ± 255 (59–800)

Abbreviation: TRH, thyrotropin releasing hormone.

Data are expressed as median \pm 95% confidence interval, (range).

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