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Thyroid hormone concentrations differ between donkeys and horses

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Summary

Reasons for performing study: Reference intervals for thyroid hormones (TH) concentrations have not been previously established for donkeys, leading to potential misdiagnosis of thyroid disease.
Objectives: To determine the normal values of TH in healthy adult donkeys and compare them to TH values from healthy adult horses.
Methods: Thirty-eight healthy Andalusian donkeys and 19 healthy Andalusian horses from 2 different farms were used. Donkeys were divided into 3 age groups: <5, 5–10 and >11 years and into 2 gender groups. Serum concentrations of fT3, tT3, rT3, fT4 and tT4 were quantified by radioimmunoassay. All blood samples were collected the same day in the morning. None of the animals had received any treatment for 30 days prior to sampling or had any history of disease. Both farms were in close proximity and under similar management. Differences between groups were determined using a one-way ANOVA analysis followed by Fisher's LSD test. $P<0.05$ was considered significant.
Results: Serum TH concentrations were higher in donkeys than in horses ($P<0.01$). Donkeys <5 years had higher serum rT3, fT4 and tT4 concentrations than donkeys >5 years ($P<0.05$). Furthermore, older donkeys (>11 years) had lower serum fT3 and tT3 concentrations than younger donkeys' groups (<5 and 5–10 years, $P<0.05$). TH concentrations were not different between genders (fT3: $P = 0.06$; tT3: $P = 0.08$; rT3: $P = 0.15$; fT4: $P = 0.89$; and tT4: $P = 0.19$).
Conclusions: Thyroid hormone concentrations are different between healthy adult donkeys and horses.
Potential relevance: Establishing species-specific TH reference ranges is important when evaluating clinicopathologic data in equids in order to avoid the misdiagnosis of thyroid gland dysfunction. Further studies to elucidate the physiological mechanisms leading to these differences are warranted.

Keywords: horse; donkey; thyroid hormones; thyroid gland dysfunction; hyperthyroidism

Introduction

Thyroid gland dysfunction in horses is often misdiagnosed due to a poor understanding of equine thyroid physiology, but also because reference intervals for thyroid hormones (TH) differ among laboratories. The same principle applies to donkeys, for which no TH reference intervals exist, and therefore clinicians must extrapolate the reference ranges from horses. This could potentially lead to the wrong diagnosis of thyroid disease.

Anatomical variations [1] and differences in haematological and biochemical parameters have been previously documented between donkeys and horses [2–5]. Although they are phylogenetically close species, important clinicopathological variations in physiological parameters, including TH concentrations, are expected and donkeys could be misdiagnosed with thyroid gland dysfunction. Since nonthyroidal diseases can alter blood TH concentrations, having donkey-specific values for these hormones is compelling.

Despite the fact that equine thyroid gland diseases are rare, the hypothalamus–pituitary–thyroid gland endocrine axis plays a pivotal role in many body functions. Disorders of the thyroid gland in horses include hyperthyroidism, euthyroid sick syndrome, hypothyroidism (in neonates), tumours and other poorly described disturbances (e.g. anhidrosis) [6,7]. In addition, low TH concentrations have been measured in premature and septic foals [8] and in horses with equine metabolic syndrome [9].

The goal of this study was to determine whether serum TH concentrations in healthy adult donkeys differ from those of horses kept under similar conditions. We hypothesised that serum TH concentrations would be different in healthy donkeys from those in healthy horses, as has been previously documented for other clinicopathological parameters [4,5].

Materials and methods

Animals

Blood samples were collected in the spring from healthy adult Andalusian donkeys and healthy adult Andalusian horses. Donkeys were housed outdoors in sand paddocks with no access to free pasture or toxic plants. Horses were also housed outdoors in sand paddocks and some were kept indoors at night. Both donkeys and horses were fed twice daily with a similar diet (alfalfa hay and oats) with free access to water. There was no history of disease for at least 2 months prior to blood sampling. Donkeys and horses were up to date with worming and vaccinations and had received no treatments for at least one month before blood sampling. Mares and jennets were not pregnant and all male donkeys and horses were castrated. Both farms were under a Mediterranean climate and were 190 km apart (donkeys' farm: 37°19′N, 4°22′W; horses' farm: 37°52′N, 5°37′W).

Animals were considered healthy based on a normal clinical history, physical examination, haematology and plasma fibrinogen concentration. All the animals received humane care in compliance with the Spanish Guide for the Care and Use of Laboratory Animals.

Donkeys were grouped based on age frequency distribution (range: 2–19 years) into the following 3 groups: *Group 1*: <5 years old (n = 13), *Group 2*: 5–10 years old (n = 12) and *Group 3*: >10 years old (n = 13). In addition, the age-effect was also studied as a continuous variable.

Sample handling and measurement

Blood samples were collected by jugular venipuncture into serum separator tubes to determine serum TH concentrations and into heparin tubes for complete blood cell count, total protein and fibrinogen concentration. After clotting, samples were centrifuged at 1000 ***g*** for 15 min at the farm, chilled on ice and frozen at -20°C until measured (less than 2 months from collection). Samples for haematology and fibrinogen concentrations were kept cold and analysed the same day. Plasma fibrinogen concentrations were determined estimating the difference between refractometric total protein readings in control and heat-treated samples (heat-denaturation method) [10].

Thyroid hormones measured included: free triiodothyronine (fT3), total triiodothyronine (tT3), reverse triiodothyronine (rT3), free thyroxine (fT4) and total thyroxine (tT4). Hormone concentrations were determined by radioimmunoassay using commercially available kits recently validated for horses [8]. Donkey and horse samples were analysed using the same assay. Technical parameters for each kits were: fT3 (Coat-a-Count Free T3)[a] analytical sensitivity: 0.2 pg/ml; tT3 (Coat-a-Count Total T3)[a] analytical

TABLE 1: Thyroid hormone concentrations in healthy donkeys and horses. Data are expressed as mean ± s.e., range and below the median and 25th–75th percentiles and number of animals. fT3: free triiodothyronine; tT3: total triiodothyronine; rT3: reverse triiodothyronine; fT4: free thyroxine; tT4: total thyroxine

	Donkeys Mean (range) Median (25th–75th)	Horses Mean (range) Median (25th–75th)	P values
fT3 (pg/ml)	1.81 ± 0.10 (0.67–3.25) 1.70 (1.40–2.06) (n = 38)	1.01 ± 0.10 (0.28–1.87) 0.94 (0.65–1.13) (n = 19)	<0.01
tT3 (ng/dl)	67.1 ± 2.93 (40.4–130.4) 65.1 (51.9–77.7) (n = 38)	47.7 ± 1.79 (32.7–62.8) 45.8 (43.3–50.0) (n = 19)	<0.01
rT3 (ng/ml)	0.63 ± 0.03 (0.18–0.99) 0.63 (0.48–0.77) (n = 38)	0.41 ± 0.04 (0.15–0.73) 0.37 (0.30–0.53) (n = 15)	= 0.01
fT4 (ng/dl)	0.44 ± 0.02 (0.14–0.85) 0.44 (0.39–0.48) (n = 38)	0.23 ± 0.02 (0.12–0.34) 0.24 (0.19–0.29) (n = 17)	<0.01
tT4 (µg/dl)	3.53 ± 0.25 (0.57–8.14) 3.12 (2.59–4.10) (n = 38)	1.64 ± 0.15 (0.37–3.20) 1.74 (1.39–2.15) (n = 19)	<0.01

sensitivity: 7 ng/dl; rT3 (rT3 RIA)[b] analytical sensitivity: 0.009 ng/ml; fT4 (Coat-a-Count Free T4)[a] analytical sensitivity: 0.01 ng/dl and tT4 (Coat-a-Count Total T4)[a] lowest detection limits: 0.25 µg/dl.

Data analysis

Results are expressed as mean ± standard error of the mean (s.e.). Normality was assessed by the Kolmogorov–Smirnov test. Differences between 2 groups were determined by unpaired *t* tests and for 3 or more groups by means of a one-way ANOVA followed by Fisher's LSD test. The median and 25th and 75th percentiles were calculated using Turkey's Hinges test. Outlier values (lower or upper quartile ± 1.5 times the interquartile range) were determined by Huber's test. Since the mean 5% trimmed did not change significantly the final results, the outlier values were not excluded from statistical analysis. Correlations between donkey TH concentrations and between donkey age and TH concentrations were determined using the Pearson's correlation coefficient. P<0.05 was considered significant. Statistical analysis was performed using commercial statistical software[c].

Results

Thyroid hormones

Thirty-eight donkeys (18 jacks, 20 jennets; 8.4 ± 0.8 years old, range: 2–19 years old) and 19 horses (15 geldings and 4 mares; 6.5 ± 0.5, range: 4–9 years old) were used in this study.

Thyroid hormone concentrations are shown in Table 1 and were normally distributed. Serum fT3 (1.81 ± 0.10 vs. 1.01 ± 0.10 pg/ml, P<0.01), tT3 (67.10 ± 2.93 vs. 47.7 ± 1.79 pg/ml, P<0.01) and rT3 (0.63 ± 0.03 vs. 0.41 ± 0.04 ng/ml, P = 0.01) were significantly higher in donkeys than in horses (Figs 1a–c). Both serum fT4 (Fig 2a) and tT4 (Fig 2b) concentrations were significantly higher in donkeys (0.44 ± 0.02 ng/dl and 3.53 ± 0.25 µg/dl, respectively, P<0.01) than in horses (0.23 ± 0.02 ng/dl and 1.64 ± 0.15 µg/dl, respectively; P<0.01).

Serum fT3 was correlated with tT3 (r = 0.829, P<0.01), fT4 (r = 0.399, P<0.02) and tT4 (r = 0.423, P<0.01) concentrations. Serum fT4 was correlated with rT3 (r = 0.688, P<0.01) and tT4 concentrations (r = 0.738, P<0.01). Serum tT4 concentration was correlated with rT3 (r = 0.664, P<0.01).

Furthermore, in the groups established in this study (Table 2), donkeys <5 years had higher serum fT3 (1.99 ± 0.17 pg/ml), tT3 (70.59 ± 4.17 ng/dl), rT3 (0.78 ± 0.05 ng/ml), fT4 (0.50 ± 0.03 ng/dl) and tT4 (4.99 ± 0.44 µg/dl) concentrations than donkeys >5 years (P<0.05). Moreover, when donkey age was correlated with TH concentrations, correlations for rT3 (r = -0.415, P<0.01) and tT4 (r = -0.573, P<0.01) were observed and a trend was found for fT3 (r = -0.292, P = 0.07) and fT4 (r = -0.293, P = 0.07). However, no correlations between TH concentrations and age were observed in horses. When both species were combined, correlations were found for rT3 (r = -0.274, P<0.05) and tT4 (r = –0.287, P<0.05). Furthermore, when donkeys and horses aged between 4 and 9 years old were compared, donkeys also had higher (P<0.01) serum TH concentrations than horses (Table 3).

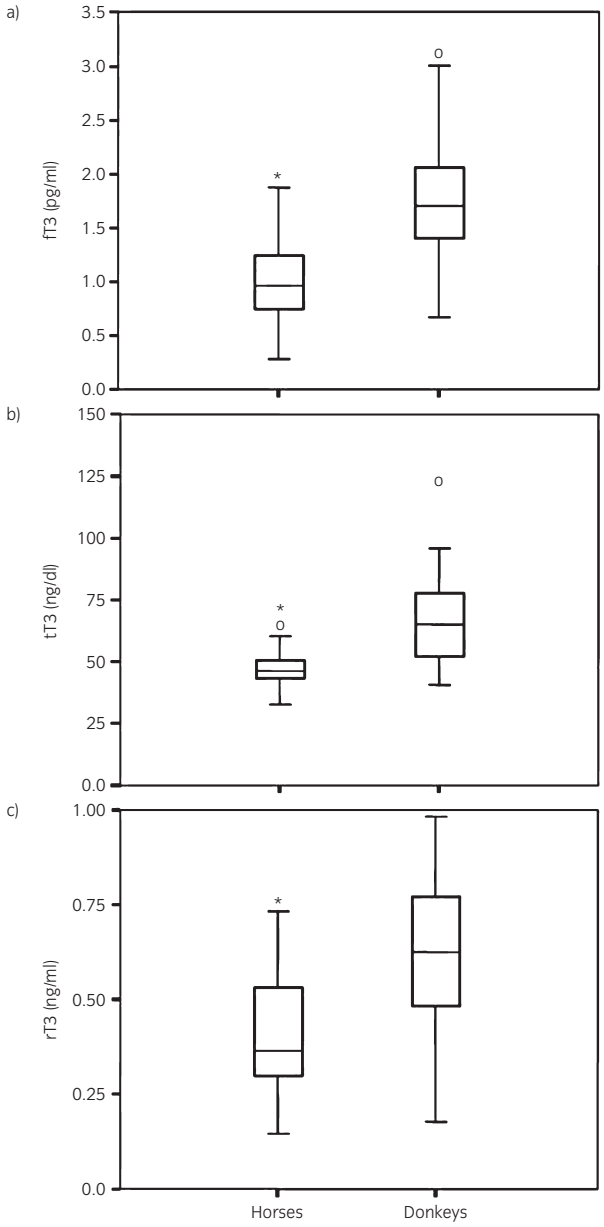

Fig 1: a) Free triiodothyronine (fT3) values in healthy donkeys and horses. b) Total triiodothyronine (tT3) concentrations in healthy donkeys compared to healthy horses. c) Reverse triiodothyronine (rT3) concentrations in donkeys and horses. In all graphs the central box represents the values from the lower to the upper quartile (25th–75th percentiles). The middle line represents the median. The vertical line extends from the minimum to the maximum value, excluding outliers (lower or upper quartile ± 1.5 times the interquartile range), displayed as separate points (o). *Statistically significant (P<0.01) when donkeys were compared with horses.

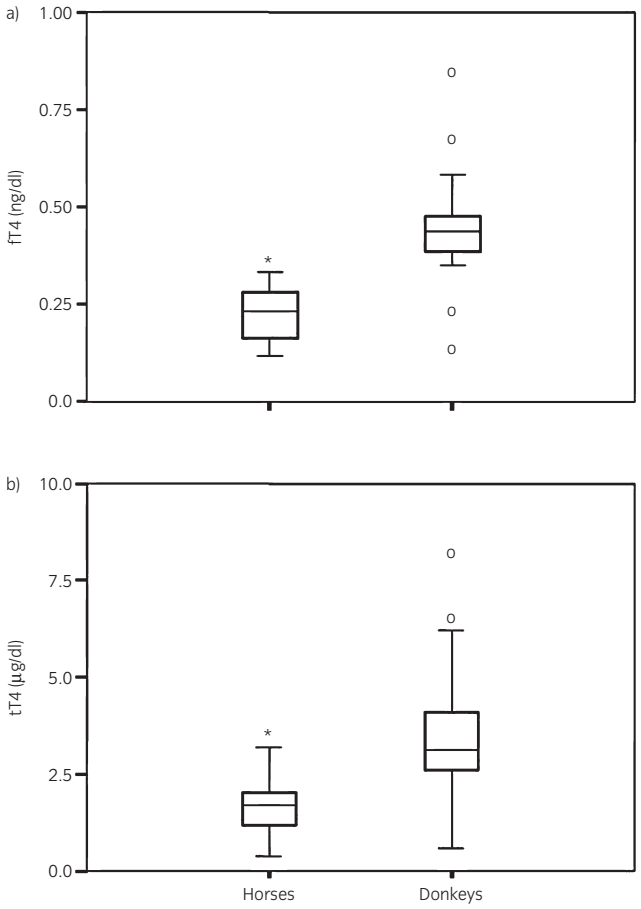

Fig 2: a) Free thyroxine (fT4) values in healthy donkeys and horses. b) Total thyroxine (tT4) concentrations in donkeys compared to horses. In both median and 25th–75th percentiles are represented. The vertical line extends from the minimum to the maximum value, excluding outliers (lower or upper quartile ± 1.5 times the interquartile range), displayed as separate points (o).*Statistically significant (P<0.01) when donkeys were compared with horses.

No differences between jacks and jennets were found for any of the TH (Table 4). There was a trend for male donkeys to have higher concentrations of fT3 (2.01 ± 0.16 pg/ml) and tT3 (72.55 ± 4.60 ng/dl) than females (1.63 ± 0.12 pg/ml and 62.19 ± 3.49 ng/dl; P = 0.059 and 0.078, respectively).

TABLE 3: Thyroid hormone concentrations in healthy donkeys and horses grouped between 4 and 9 years old. Data are expressed as mean ± s.e. fT3: free triiodothyronine; tT3: total triiodothyronine; rT3: reverse triiodothyronine; fT4: free thyroxine; tT4: total thyroxine

	Donkeys	Horses	P values
fT3 (pg/ml)	2.12 ± 0.15 (n = 14)	1.01 ± 0.10 (n = 19)	<0.01
tT3 (ng/dl)	76.94 ± 5.95 (n = 14)	47.55 ± 1.88 (n = 19)	<0.01
rT3 (ng/ml)	0.60 ± 0.05 (n = 14)	0.40 ± 0.05 (n = 15)	<0.01
fT4 (ng/dl)	0.45 ± 0.04 (n = 14)	0.22 ± 0.02 (n = 17)	<0.01
tT4 (µg/dl)	3.41 ± 0.39 (n = 14)	1.61 ± 0.15 (n = 19)	<0.01

In order to compare TH concentrations in donkeys to those in different horse populations and breeds, results from previous published studies are shown in Table S1.

Discussion

The main objective of this study was to determine whether TH concentrations were different between healthy adult donkeys and horses. The results demonstrate that donkeys had higher serum fT3, tT3, rT3, fT4 and tT4 concentrations than horses. Furthermore, it is of interest that in addition to higher TH concentrations, donkeys also had a broader range of fT3, tT3 and tT4 concentrations. When the donkeys were grouped according to age, young donkeys (<5 years old) had higher serum TH concentrations than older donkeys (>5 years old). A similar age-dependent difference has been demonstrated between foals and adult horses but not in donkeys. This information has clinical value, since extrapolating data between donkeys and horses is common, in particular when normal values are not available for donkeys.

In addition to comparing donkeys to horses from the same breed and region to reduce variability due to geography or management, we also found that TH concentrations in the donkeys of our study were different from ranges published for horses using the same analytical method [11–22]. Of interest, TH concentrations in the Andalusian horses of this study were in the reference range of previous published studies. Furthermore, no interbreed differences for TH concentrations have been demonstrated in horses [23,24], making it unlikely that the variation in TH between horses and donkeys was due to breed but rather to species.

As TH reference intervals for donkeys have not been established prior to this study, if clinicians extrapolate values from horses they could

TABLE 2: Thyroid hormone concentrations in donkeys arranged in 3 age groups: *Group 1*: <5 years old (n = 13), *Group 2*: 5–10 years old (n = 12) and *Group 3*: >10 years old (n = 13). Data are expressed as mean ± s.e., range and below the median and 25th–75th percentiles. fT3: free triiodothyronine; tT3: total triiodothyronine; rT3: reverse triiodothyronine; fT4: free thyroxine; tT4: total thyroxine. [a]P<0.05 vs. *Group 1*. [b]P<0.05 vs. *Group 2*

Donkeys	*Group 1*: <5 years old (n = 13)	*Group 2*: 5–10 years old (n = 12)	*Group 3*: >10 years old (n = 13)
fT3 (pg/ml)	1.99 ± 0.17 (1.07–3.25) 2.03 (1.48–2.51)	1.92 ± 0.17 (1.23–3.01) 1.89 (1.42–2.37)	1.52 ± 0.16 (0.67–2.98)[a] 1.49 (1.07–1.79)
tT3 (ng/dl)	70.59 ± 4.17 (47.77–96.04) 71.64 (56.24–85.34)	73.75 ± 6.65 (49.48–130.40) 71.60 (54.01–87.76)	57.46 ± 3.24 (40.39–79.98)[a,b] 53.47 (50.75–65.67)
rT3 (ng/ml)	0.78 ± 0.05 (0.49–0.98) 0.87 (0.61–0.93)	0.54 ± 0.05 (0.30–0.89)[a] 0.50 (0.41–0.70)	0.57 ± 0.05 (0.18–0.77)[a] 0.59 (0.47–0.71)
fT4 (ng/dl)	0.50 ± 0.03 (0.37–0.85) 0.48 (0.42–0.51)	0.42 ± 0.02 (0.24–0.58)[a] 0.44 (0.36–0.46)	0.41 ± 0.02 (0.14–0.50)[a] 0.41 (0.39–0.47)
tT4 (µg/dl)	4.99 ± 0.44 (2.26–8.14) 4.36 (3.84–6.18)	2.85 ± 0.22 (1.54–3.75)[a] 3.01 (2.18–3.56)	2.70 ± 0.23 (0.57–4.12)[a] 2.94 (2.39–3.08)

TABLE 4: Thyroid hormone concentrations in donkeys arranged by gender. Data are expressed as mean ± s.e., range and below the median and 25th–75th percentiles. fT3: free triiodothyronine; tT3: total triiodothyronine; rT3: reverse triiodothyronine; fT4: free thyroxine; tT4: total thyroxine

Donkeys	Jack (n = 18)	Jennet (n = 20)	P values
fT3 (pg/ml)	2.01 ± 0.16 (0.99–3.25)	1.63 ± 0.12 (0.67–2.72)	0.059
	1.99 (1.49–2.56)	1.51 (1.25–1.92)	
tT3 (ng/dl)	72.55 ± 4.60 (47.77–130.40)	62.19 ± 3.49 (40.40–96.04)	0.078
	71.69 (54.59–83.55)	57.70 (51.10–75.65)	
rT3 (ng/ml)	0.68 ± 0.05 (0.18–0.98)	0.59 ± 0.59 (0.34–0.93)	0.154
	0.69 (0.49–0.89)	0.56 (0.46–0.72)	
fT4 (ng/dl)	0.44 ± 0.02 (0.14–0.68)	0.44 ± 0.02 (0.24–0.85)	0.893
	0.45 (0.39–0.49)	0.41 (0.39–0.47)	
tT4 (μg/dl)	3.88 ± 0.45 (0.57–8.14)	3.22 ± 0.23 (1.71–6.44)	0.190
	3.67 (2.54–5.81)	3.08 (2.56–3.60)	

misdiagnose thyroid gland dysfunction based upon clinicopathological testing. Under this premise, many of these donkeys could have been classified as having hyperthyroidism [25,26]. On the other hand, donkeys with hypothyroidism (rare adult disease) could be diagnosed as normothyroid.

Age-related differences in equine TH concentrations have been previously described [23]. For example, TH concentrations in newborn foals are almost 10 times higher than in adult horses, declining to a plateau at 9–10 months of age [24,27]. An age-related effect was also observed in the donkeys of this study, although younger animals (neonates and weanling) to support this assumption were not included. Correlations between age and TH concentrations have been previously demonstrated in horses [23], with younger animals having higher T3 and T4 concentrations, possibly because of their importance in growth, development and tissue differentiation. However, no correlations between age and TH concentrations were found in the horses of this study, possibly because this was a narrow age group.

At this time the potential influence of gender on TH concentration in horses remains unclear [23,28]. In this study, a slight trend in fT3 and tT3 concentrations was found between male and female donkeys. The lack of significance between genders in this study may be attributed to either a low number of animals or wide variability in the animals used, leading to a possible type II error. In order to establish differences between genders, further studies with larger sample sizes are necessary to support this statement.

In addition to genetics, other factors could have contributed to TH concentration differences. For example, sample type (serum vs. plasma), storage, haemolysis and lipaemia may affect TH determination [29–31]. In both donkeys and horses, all blood samples were collected using the same type of tube. In this study, samples were centrifuged on the farm immediately after collection and held on ice for up to 6 h then frozen at -20°C. Neither lipaemia nor haemolysis was evident in the samples.

There is evidence that season, circadian rhythm, feeding, physiological status, exercise, drug administration, plants and nitrates may affect TH concentrations in horses [12,13,15,32–40]. These effects were unlikely to affect TH in this study, as animals were housed in similar premises, fed the same diet, did not have access to goitrogenic plants, and samples were collected at the same time of the day.

Numerous techniques have been validated for the determination of thyroid hormones in animals, notably in horses [38,40–43]. However, although all the radioimmunoassay kits used in this study have been previously validated for horses [8], a species-dependent effect cannot be ruled out. To the authors' knowledge, kits validated in horses have previously been reliably and accurately used for donkeys [3–5].

Differences in TH concentrations between donkeys and horses could be explained by several mechanisms. One of them could be due to the Jod–Basedow phenomenon, but donkeys and horses were fed a similar diet, making this condition unlikely. Differences in metabolism have been described in donkeys, since higher therapeutic doses and/or shorter dosing intervals of several drugs are required compared to horses [44]. Therefore, possible metabolic mechanisms could be: 1) differences in thyroid-binding protein concentrations or affinity for TH, but unfortunately thyroid-binding protein assays have not been validated for horses; 2) a more prolonged half-life in comparison to horses; and 3) different metabolic rate, since TH concentrations are inversely proportional to the size of the animal, as is demonstrated in foals vs. adult horses [23]. Another possible theory could be that donkeys have a higher secretion of thyrotropin-releasing hormone or thyroid-stimulating hormone that could induce higher TH production, but TRH assays for equidae have not yet been validated. In a previous study, the authors attempted to measure equine and donkeys TSH concentrations using human-specific and canine-specific TSH RIAs but results were unreliable (data not shown). The mechanisms responsible for the differences between species remain unclear, and further studies are warranted to elucidate them.

In conclusion, our results show that serum TH concentrations in healthy donkeys are different from those reported in the literature for horses. In addition, this study is the first one reporting serum TH concentrations for healthy donkeys. Further studies are necessary to determine the physiological mechanism of these differences.

Authors' declaration of interests

No competing interests have been declared.

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Manufacturers' addresses

[a]Siemens Healthcare Diagnostic Ltd, Los Angeles, USA.
[b]Immunodiagnostic Systems Ltd (IDS), Paris, France.
[c]SPSS 15.0 software package. Chicago, Illinois, USA.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1: Previous published thyroid hormone concentrations in horses established by means of radioimmunoassay.