Serum Triiodothyronine (T_3) and Thyroxin (T_4) Concentrations in Lipizzan Horses

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Abstract

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The Lipizzan horses are a breed with a long breeding history that may have developed differences to other breeds in thyroid hormone concentrations. The objectives of this study were to determine the influences of age, sex and pregnancy on total serum triiodothyronine (T_3) and thyroxin (T_4) levels in Lipizzans and to compare the results with reference values of other horse breeds. T_3 and T_4 concentrations were determined in blood serum of 131 stallions, 118 mares and 25 foals of the Lipizzan breed by using a radioimmunoassay (RIA), validated for horse serum. The highest thyroid hormone levels were found in foals. The lowest serum T_4 values were obtained in animals aged 16 years and more, the lowest T_3 values in the range of 1 to 3 years. At 4 years, T_3 concentrations rose and then steadily declined. In all categories of horses, significantly higher serum T_4 was found in males, but the sex dependent differences of T_3 were less evident. T_3 and T_4 values in pregnant mares were significantly lower ($P < 0.01$) than in non-pregnant ones and in stallions. In contrast to the data in the literature, a significant decrease of T_4 was observed between the 7th and 10th month of pregnancy, while T_3 remained almost unchanged. The mean total serum T_3 and T_4 values in Lipizzans were in the normal range for warm-blooded horses and the age and sex-related fluctuations were in agreement with those for other horse breeds.

Horse, lipizzan, age, sex, pregnancy, RIA, thyroxin; triiodothyronine.

In horses, as in other animal species, serum thyroid hormone concentrations vary over a wide range due to internal or external factors (Duckett et al. 1989). A variety of factors, including age, sex, environmental temperature, seasonal and daily rhythms, feeding and training stages influence the physiological values in euthyroid horses. During foetal development, circulating concentrations of T_3 and T_4 increase, reaching a peak immediately *post partum*, and then decline to their lowest values at 16 to 22 years (Malinowski et al. 1996; Chen and Riley 1981). Different data have been presented on the influence of sex on T_3 and T_4 concentrations. Thyroid hormone levels have been reported to be lower in mares than in stallions (Reap et al. 1978), lower in stallions and geldings than in mares (Motley 1972) or the same in the two sexes (Johnson 1986). There are no significant fluctuations in T_3 and T_4 at any stage of the oestrous cycle (Johnson 1986) but, as in other animals (Reimers et al. 1984), concentrations of both hormones are elevated in pregnant mares (Katowich et al. 1974; De Martin 1977; Slebodzinski 1994). Seasonal increases in thyroid activity are recognised during acclimation to cold temperatures (Nachreiner 1981) and during acute cold exposure (Katowich et al. 1974; Johnson 1986). Daily rhythms also contribute significantly to variations in T_3 and T_4 concentrations (Duckett et al. 1989). In horses, food deprivation causes a decrease in concentration of total and free thyroid hormones (Messer et al. 1995a). The T_3 and T_4 levels can also be affected by iodine-containing substances (Duckett et al. 1989) and glucocorticoid administration (Messer et al. 1995b). In the middle stages of training, T_4 concentration

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decreases, whereas in the later stages it is increased (Takagi 1974). After racing, only T_3 is significantly elevated (De Martin 1977).

In Lipizzans, the thyroid hormone concentrations and their changes under physiological conditions have not been investigated previously. The breed has a long breeding history and may have developed differences in thyroid hormone values compared to other breeds, as reported for steroid hormones (Palme et al. 2001). The aim of this work was to determine the influence of age, sex and pregnancy on serum T_3 and T_4 levels in this breed and to compare the obtained values with reference values for other horse breeds.

Materials and Methods

Total serum T_3 and T_4 concentrations were determined in 131 stallions, 118 mares and 25 foals of the Lipizzan breed. The stallions aged from 1 to 27 years (average 7.4 years), the mares from 1 to 23 years (average 6.6 years) and the foals from 17 to 131 days (average 81 days). The blood sampling in stallions and mares was performed in February and in foals in June. The study was carried out at the Lipica stud farm in Slovenia.

According to the breeding programme of the farm, each category of horses has specific housing conditions. Brood mares are kept together and have an outdoor release, while mares with foals are placed in box stalls. Colts and fillies (aged 1 to 3 years) are kept in separate groups and they are also given outdoor release. The stallions are kept in separate box stalls. Working mares and riding horses are tethered in separate standing stalls. Animals are fed twice a day with hay and oats. Drinking water is given *ad libitum*.

On the day of blood sampling all the usually free running animals were tethered. Because the horses are familiar with humans and used to various handling procedures, no restraint was necessary. Blood was collected from the jugular vein 5 to 7 h after the morning feed by double-ended needles and evacuated tubes without anticoagulant (Vaccuette® Greiner Labortechnik). During the sampling and transport, samples were kept at an environmental temperature (about 12 ºC for 6 h) until centrifuged in the laboratory. The serum was stored at -20 ºC until assayed.

Total serum T_3 and T_4 concentrations were measured by radio-immunoassay (RIA), using commercial kits (RIA-mat T_3® and RIA-mat T_4®, Byk-Sangtec Diagnostica, Germany) validated for horse serum. The intra-assay coefficient of variation (CV) was 5.4% and 2.71%, while inter-assay CV was 5.61% and 7.66%, for T_3 and T_4, respectively.

Statistical calculations were performed by using the Statistical Package for Social Sciences (SPSS for Windows. Release 8.0.0.), with subprogrammes Paired *t* -test and analysis of variance. Results are considered significant at the level $P < 0.05$ and are presented in the text as means ± standard error of the mean (S.E.M.).

Results

Mean serum T_3 and T_4 values in all examined horses, foals, young (1-to-3 years) and adult horses are listed in Table 1. The concentrations of both hormones were higher in males than in females in all categories of horses. Significant differences in T_4 were observed between all males and females ($P < 0.001$), male and female foals ($P < 0.01$), fillies and colts ($P < 0.01$) and adult mares and stallions ($P < 0.001$). On the other hand, T_3 concentrations differed significantly only between adult mares and stallions ($P < 0.001$). Differences between

Table 1
Serum T_3 and T_4 concentrations (mean ± S.E.M) in horses of different categories

Category of horses	Number (n)	T_3 (nmol/L)	T_4 (nmol/L)
Female (total)	118	0.97 ± 0.03	22.78 ± 0.75
Male (total)	131	1.01 ± 0.03	25.96 ± 0.72
Foals (female)	18	1.44 ± 0.09	31.53 ± 1.67
Foals (male)	7	1.54 ± 0.07	42.53 ± 2.39
Fillies (1 to 3 years)	39	0.74 ± 0.02	25.75 ± 0.97
Colts (1 to 3 years)	36	0.75 ± 0.03	29.99 ± 1.17
Mares (adult)	61	0.93 ± 0.04	18.18 ± 0.83
Stallions (adult)	88	1.09 ± 0.04	22.82 ± 0.65

female foals, fillies and mares as well as between male foals, colts and stallions were significant ($P < 0.001$) for T_3 and for T_4.

Serum T_3 and T_4 concentrations in pregnant mares are shown in Table 2. Mean T_3 (0.82 ± 0.04 nmol/L) and T_4 (12.99 ± 1.00 nmol/L) concentrations in pregnant mares were significantly lower than in non-pregnant ones (1.02 ± 0.06 nmol/L, $P < 0.01$ and 21.88 ± 0.76 nmol/L, $P < 0.001$, respectively) and stallions ($P < 0.001$). Statistically significant differences between various stages of pregnancy were observed only for T_4 ($P < 0.01$).

Table 2
Serum T_3 and T_4 concentrations (mean ± S.E.M.) in non-pregnant and pregnant mares

Month of gestation	Number (n)	T_3 (nmol/L)	T_4 (nmol/L)
7	4	0.86 ± 0.05	17.59 ± 0.22
8	4	0.95 ± 0.08	14.85 ± 0.68
9	6	0.65 ± 0.05	10.13 ± 1.14
10	12	0.84 ± 0.06	11.30 ± 1.15
Total	26	0.82 ± 0.04	12.99 ± 1.00
Non-pregnant	92	1.02 ± 0.06	21.88 ± 0.76

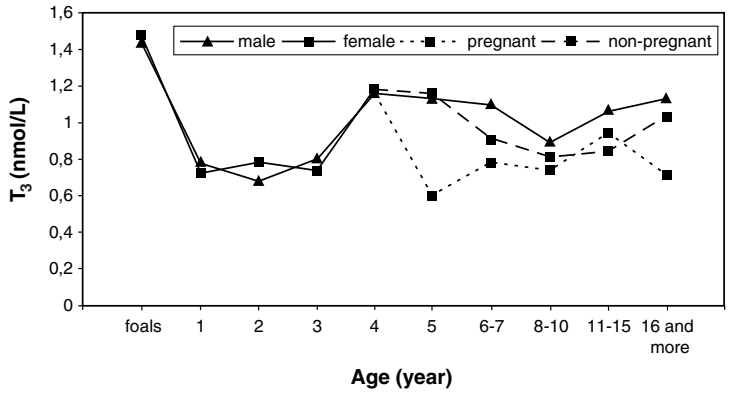

Fig. 1. Mean serum T_3 concentrations in male and female horses of various age groups

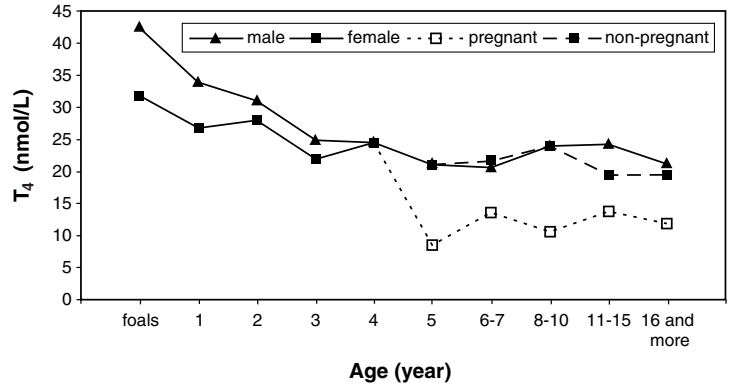

Fig. 2. Mean serum T_4 concentrations in male and female horses of various age groups

Serum T_3 and T_4 concentrations in male and female horses of different ages are presented in Figures 1 and 2. Within the groups of stallions and mares, the differences were significant ($P < 0.001$) for both hormones. Negative significant correlation between age and hormone concentration was observed only for T_4 (in females r = - 0.7986; P < 0.01 in males r = - 0.6379, $P < 0.05$).

T_3 concentrations were insignificantly higher in male yearlings and in 3 years old horses than in female, but lower in male foals and male animals aged 2 and 4 years. In pregnant mares of all ages, serum T_3 values were lower than in stallions, but significant differences were detected only in the age group of 6 - 7 years. T_3 values were lower in non-pregnant mares than in stallions (5 year old animals were the exception), but higher than in pregnant mares of all age groups (exception those aged 11 – 15 years).

In male foals and young horses (aged 1-to-3 years), T_4 concentrations were not significantly higher than in females. In pregnant mares of all age groups, serum T_4 values were lower than in non-pregnant ones and in stallions. Significant differences between the non-pregnant mares and stallions were determined in 6-to-7 ($P < 0.05$), 8-10 ($P < 0.01$) and 1-to-15-year-old ($P < 0.01$) animals.

Discussion

Serum T_3 and T_4 concentrations in Lipizzans are in the usual range for warm-blooded horses, the highest values being in foals (Chen et al. 1981; Duckett et al. 1989; Sojka et al. 1993; Malinowski et al. 1996). The highest thyroid hormone values in various species are measured in autumn or winter and the lowest in spring or summer (Flisinska-Boyanowska et al. 1991; Slebodzinski 1994; Johnson 1986). Therefore, high T_3 and T_4 values in Lipizzan foals could not be contributed to seasonal influences. High serum values of the hormones are essential for normal muscular and nervous function and for growth in foals (Chen et al. 1981). As in Standardbred horses (Malinowski et al. 1996), T_4 values were the lowest in horses aged 16 years and more. T_3 values were the lowest at the age of 1 to 3 years, followed by rise at 4 years. In older animals, T_3 declined steadily from then on. A strong decrease in T_3 values in growing Lipizzans, without changes in T_4, could be connected with rapid growth and increased need of T_3 for general metabolism (Malinowski et al. 1996). A decrease of T_3 values can also be connected with the composition of food, lacking glucose and fatty acids (Slebodzinski 1994).

In all foals, young and adult Lipizzan horses, significantly higher serum T_4 levels were found in males, as reported for warm-blooded horses (Reap et al. 1978; Chen et al. 1981). The sex dependent differences for serum T_3 values were not significant. In contrast to our findings, thyroid hormone levels have been reported to be higher in mares than in stallions and geldings (Motley 1972) or, again, the same in both sexes (Duckett 1989). This inconsistency in results may be associated with regional, laboratory or assay technique effects (Duckett 1989).

There are no significant fluctuations in thyroid hormone levels at any stage of the oestrous cycle (Johnson 1986), but pregnancy importantly elevates the thyroid hormone concentrations in mares (De Martin 1977; Slebodzinski 1994) and in other species (Reimers et al. 1984). Total and free T_3 and T_4 are increased up to 3-fold in mid-pregnancy in horses and sheep. The same increases are observed in non-pregnant animals during the winter when seasonal breeders are in mid-pregnancy (Slebodzinski 1994). Contrary to these reports, a steady decline of T_3 values for all 11 months of pregnancy was observed in Standardbred mares, while T_4 values remained almost unchanged (Flisinska-Boyanowska et al. 1991). In Lipizzan mares, T_4 values were significantly different between the 7^{th} and 10^{th} month of pregnancy, while T_3 values changed only slightly. In contrast to other experiments, the blood sampling in Lipizzan mares was performed on the

same day, excluding the possibility of seasonal influences (Flisinska-Boyanowska et al. 1991; Slebodzinski 1994). On the other hand, however, the numbers of animals in different groups of pregnancy were too small for accurate estimation of the whole gestation period. The influence of pregnancy on thyroid hormone levels in Lipizzan mares needs further study.

Significantly lower T_3 and T_4 values were observed in pregnant than in non-pregnant Lipizzan mares, as also reported for Standardbred mares (Flisinska-Boyanowska et al. 1991). The reason for this phenomenon is probably the influence of oestrogens on thyroid function, which is inhibited by high oestrogen levels in pregnant mares (Slebodzinski 1994).

Koncentrace trijodthyroninu (T_3) a thyroxinu (T_4) v krevní plazmě lipicánů

Lipicáni jsou plemenem s dlouhou historií chovu a je možné, že se u nich vyvinuly oproti jiným plemenům rozdíly v koncentracích hormonů štítné žlázy. Cílem této studie bylo stanovit vliv věku, pohlaví a březosti na koncentraci celkového trijodthyroninu (T_3) a thyroxinu (T_4) lipicánů a výsledky porovnat s referenčními hodnotami pro jiná plemena koní. Koncentrace T_3 a T_4 byly stanoveny v krevní séru 131 hřebců, 118 klisen a 25 hříbat tohoto plemene s použitím radioimunoassay (RIA) pro koňské sérum. Nejvyšší koncentrace hormonů štítné žlázy byly nalezeny u hříbat, nejnižší koncentrace T_4 byly u zvířat starších 16 let, a nejnižší T_3 koncentrace byly u zvířat ve věku 1-3 let. U 4letých jedinců se koncentrace T_3 zvýšila a poté postupně klesala. Ve všech věkových kategoriích byla signifikantně nižší koncentrace T_4 u hřebců, ale sexuální rozdíly v T_3 byly méně výrazné. Koncentrace obou hormonů byly u březích klisen nižší než u klisen nebřezích a hřebců. Na rozdíl od údajů v literatuře, zaznamenali jsem signifikantní pokles koncentrace T_4 mezi 7. a 10. měsícem březosti, zatímco koncentrace T_3 téměř neměnila. Průměrná koncentrace T_3 a T_4 byla u lipicánů v rozmezí hodnot nalézaných u teplokrevných koní a věkové a sexuální diference byly podobné jako u jiných plemen koní.

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