Serum Triiodothyronine (T₃) and Thyroxin (T₄) Concentrations in Lipizzan Horses

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Abstract

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 (T₃) and thyroxin (T₄) levels in Lipizzans and to compare the results with reference values of other horse breeds. T₃ and T₄ 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 T₄ values were obtained in animals aged 16 years and more, the lowest T₃ values in the range of 1 to 3 years. At 4 years, T₃ concentrations rose and then steadily declined. In all categories of horses, significantly higher serum T₄ was found in males, but the sex dependent differences of T₃ were less evident. T₃ and T₄ 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 T₄ was observed between the 7th and 10th month of pregnancy, while T₃ remained almost unchanged. The mean total serum T₃ and T₄ 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.

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 T₃ and T₄ 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 T₃ and T₄ 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 T₃ and T₄ 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; Slebdzinski 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 T₃ and T₄ 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 T₃ and T₄ 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, T₄ concentration...
decreases, whereas in the later stages it is increased (Takagi 1974). After racing, only T3 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 T3 and T4 levels in this breed and to compare the obtained values with reference values for other horse breeds.

Materials and Methods

Total serum T3 and T4 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 (Vacuette® Greiner Labortechnik). During the sampling and transport, samples were kept at an environmental temperature (about 12 °C for 6 h) until centrifuged in the laboratory. The serum was stored at -20 °C until assayed.

Total serum T3 and T4 concentrations were measured by radio-immunoassay (RIA), using commercial kits (RIA-mat T3® and RIA-mat T4®, 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 T3 and T4, 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 T3 and T4 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 T4 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, T3 concentrations differed significantly only between adult mares and stallions (P < 0.001). Differences between

Table 1

<table>
<thead>
<tr>
<th>Category of horses</th>
<th>Number (n)</th>
<th>T3 (nmol/L)</th>
<th>T4 (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (total)</td>
<td>118</td>
<td>0.97 ± 0.03</td>
<td>22.78 ± 0.75</td>
</tr>
<tr>
<td>Male (total)</td>
<td>131</td>
<td>1.01 ± 0.03</td>
<td>25.96 ± 0.72</td>
</tr>
<tr>
<td>Foals (female)</td>
<td>18</td>
<td>1.44 ± 0.09</td>
<td>31.53 ± 1.67</td>
</tr>
<tr>
<td>Foals (male)</td>
<td>7</td>
<td>1.54 ± 0.07</td>
<td>42.53 ± 2.39</td>
</tr>
<tr>
<td>Fillies (1 to 3 years)</td>
<td>39</td>
<td>0.74 ± 0.02</td>
<td>25.75 ± 0.97</td>
</tr>
<tr>
<td>Colts (1 to 3 years)</td>
<td>36</td>
<td>0.75 ± 0.03</td>
<td>29.99 ± 1.17</td>
</tr>
<tr>
<td>Mares (adult)</td>
<td>61</td>
<td>0.93 ± 0.04</td>
<td>18.18 ± 0.83</td>
</tr>
<tr>
<td>Stallions (adult)</td>
<td>88</td>
<td>1.09 ± 0.04</td>
<td>22.82 ± 0.65</td>
</tr>
</tbody>
</table>
female foals, fillies and mares as well as between male foals, colts and stallions were significant \((P < 0.001)\) for T3 and for T4.

Serum T3 and T4 concentrations in pregnant mares are shown in Table 2. Mean T3 (0.82 \(\pm\) 0.04 nmol/L) and T4 (12.99 \(\pm\) 1.00 nmol/L) concentrations in pregnant mares were significantly lower than in non-pregnant ones (1.02 \(\pm\) 0.06 nmol/L, \(P < 0.01\) and 21.88 \(\pm\) 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 T4 \((P < 0.01)\).

### Table 2

<table>
<thead>
<tr>
<th>Month of gestation</th>
<th>Number (n)</th>
<th>T3 (\pm) S.E.M. (nmol/L)</th>
<th>T4 (\pm) S.E.M. (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>4</td>
<td>0.86 (\pm) 0.05</td>
<td>17.59 (\pm) 0.22</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>0.95 (\pm) 0.08</td>
<td>14.85 (\pm) 0.68</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>0.65 (\pm) 0.05</td>
<td>10.13 (\pm) 1.14</td>
</tr>
<tr>
<td>10</td>
<td>12</td>
<td>0.84 (\pm) 0.06</td>
<td>11.30 (\pm) 1.15</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>0.82 (\pm) 0.04</td>
<td>12.99 (\pm) 1.00</td>
</tr>
<tr>
<td>Non-pregnant</td>
<td>92</td>
<td>1.02 (\pm) 0.06</td>
<td>21.88 (\pm) 0.76</td>
</tr>
</tbody>
</table>

Fig. 1. Mean serum T3 concentrations in male and female horses of various age groups

Fig. 2. Mean serum T4 concentrations in male and female horses of various age groups
Serum T₃ and T₄ 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 T₄ in females \(r = -0.7986; P < 0.01\) in males \(r = -0.6379, P < 0.05\).

T₃ 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 T₃ values were lower than in stallions, but significant differences were detected only in the age group of 6-7 years. T₃ 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), T₄ concentrations were not significantly higher than in females. In pregnant mares of all age groups, serum T₄ 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 T₃ and T₄ concentrations in Lipizzan 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 T₃ and T₄ 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), T₃ values were the lowest in horses aged 16 years and more. T₄ values were the lowest at the age of 1 to 3 years, followed by rise at 4 years. In older animals, T₄ declined steadily from then on. A strong decrease in T₃ values in growing Lipizzans, without changes in T₄, could be connected with rapid growth and increased need of T₃ for general metabolism (Malinowski et al. 1996). A decrease of T₃ 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 T₄ 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 T₃ 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 T₃ and T₄ 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 T₃ values for all 11 months of pregnancy was observed in Standardbred mares, while T₄ values remained almost unchanged (Flisinska-Boyanowska et al. 1991). In Lipizzan mares, T₄ values were significantly different between the 7th and 10th month of pregnancy, while T₃ 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; Słebodzinski 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 (Słebodzinski 1994).

**Konzentrace trijodthyroninu ($T_3$) a thyroxinu ($T_4$) v krevní plazmě lipicánů**

Lipcání 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 koncentrace 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 klisenců a 25 hřebíků tohoto plemene s použitím radioimmunoassay (RIA) pro koňské sérum. Nejvyšší koncentrace hormonů štítné žlázy byly nalezeny u hřebců, nejnižší koncentrace $T_3$ 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 klisenců nižší než u klisenců a hřebců. Na rozdíl od údajů v literatuře, zaznamenali jsem signifikantní pokles koncentrace $T_3$ 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 věkově a sexuální diference byly podobné jako u jiných plemen koní.

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