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Coefficient of variation of and effect of conditioning on concentration of plasma total and free Iodothyronines in Thoroughbred horses

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Summary

The coefficient of variation of T_4, fT_4,T_3, and fT_3, concentrations in blood plasma of horses before and after exercise was determined. In addition, the effect of six different conditioning programs on plasma T_4, fT_4, T_3, and fT_3, concentration of five Thoroughbred horses was studied in a latin square cross over study design. Each conditioning programme consisted of eleven repetitions of exercise of 5, 15 or 25 minutes' duration at a velocity at which, mathematically, horses had a blood lactate concentration of 2.5 or 4 mmol/l.
The mean coefficient of variation before exercise was 11.4%, 13.8%, 9.8%, and 20.0%, for T_4, fT_4, T_3, and fT_3, and 19.9%, 17.3%, 9.7% and 9.9% after exercise.
None of the conditioning programmes had an effect on plasma concentration of thyroid hormones.

Keywords: conditioning, exercise, coefficient of variation, thyroid hormones, blood plasma, horse

Variationskoeffizient der und Einfluß von Training auf die Konzentration von Schilddrüsenhormonen im Blutplasma von englischen Vollblütern

Der Variationskoeffzient der Konzentration von T_4, fT_4,T_3, und fT_3 im Plasma von fünf Pferden vor und nach körperlicher Belastung wurde gemessen. Außerdem wurde die Wirkung von sechs verschiedenen Trainingsprogrammen auf die Konzentration von T_4, fT_4,T_3, und fT_3 im Plasma derselben Pferde untersucht. Jedes Trainingsprogramm bestand aus elf Belastungen von 5, 15 oder 25 Minuten Dauer bei der Geschwindigkeit, die in einem standardisierten Belastungstest bei jedem Pferd eine Laktatkonzentration im Blut von 2,5 oder 4 mmol/l hervorrief.
Der mittlere Variationskoeffizient von T_4, fT_4,T_3, und fT_3 betrug vor Belastung 11,4%, 13,8%, 9,8%, und 20,0%, und nach Belastung 19,9%, 17,3%, 9,7% und 9,9% jeweils.
Keines der Trainingsprogramme verursachte signifikante Veränderungen der Konzentration von T_4, fT_4,T_3, und fT_3 im Plasma der Pferde.

Schlüsselwörter: Training, Belastung, Variationskoeffizient, Schilddrüsenhormone, Blutplasma, Pferd

Introduction

The involvement of the pituitary-thyroid axis in physical activity and performance is not fully understood. However, it could play an important role in supporting the energy requirements during exercise, improving both neuromuscular performance and thermal adaptation (*Irvine* 1983; *Lomax* and *Robertson* 1992).
Data obtained from man after exercise (*Terblanche* 1989) and conditioning (*Irvine* 1968; *Balsam* and *Leppo* 1975; *Boyden* et al. 1982; *Pakarinen* et al. 1988; *Alen* et al. 1993) is not very conclusive, probably due to the large differences in exercise and conditioning programmes studied.
In horses, the behaviour of plasma total and free iodothyronines after a prolonged riding-school session (*Ferlazzo* and *Piccione* 1991), after a light exercise session (*Fazio* et al. 1995), after a race (*Fazio* et al. 1994), and after a standardized exercise test (*Ferlazzo* et al. 1995) suggests a higher responsiveness of free hormones to exercise and an influence of competition experience on T_3 and fT_4 levels. Data on the effect of conditioning on thyroid hormone levels in horses is not available, and the data found in literature to estimate the value of measuring blood thyroid hormone concentrations for evaluation of performance capacity is not sufficient (*Thornton* 1985).

In this study the following questions were addressed:
1. What is the coefficient of variation of T_4, fT_4, T_3, and fT_3 concentration in plasma of horses before and after exercise?
2. What effect do different conditioning programmes have on T_4, fT_4, T_3, and fT_3 concentration in plasma of horses?

Materials and methods

Horses
Five Thoroughbred horses were included in this study (two were 3 years old and three were 2 years old at the beginning of the study; Mean body weight ± standard deviation of 448 ± 19 kg; One gelding and four mares). They were trained for two months to run on a motorized treadmill (6% incline). All standardized exercise workouts and tests were done on a treadmill.

Study design
Study to determine coefficient of variation: Blood was taken from the horses before exercise at 7:00 a.m. and immediately after the end of exercise. This was repeated four times for each horse on days 1, 7, 13 and 19 of a single conditioning programme. Dura-

Tab. 1: Coefficient of variance (%) of plasma thyroid hormone concentrations in horses before and after exercise (exercise repeated four times/horse)

Horse	Variable							
	T_4		fT_4		T_3		fT_3	
	before	after	before	after	before	after	before	after
1	7.27	14.05	9.56	13.42	10.30	11.02	16.67	28.57
2	13.48	5.16	12.27	9.53	5.06	60.75	16.67	12.50
3	19.72	11.68	13.27	8.77	17.32	5.84	16.67	16.67
4	10.64	9.72	12.49	9.00	9.49	11.39	16.67	14.29
5	5.80	7.78	21.26	8.84	6.75	10.30	33.33	14.29

tion and intensity of the exercise were the same for a single horse, but differed between horses. The duration of exercise was 5, 15 or 25 minutes, and horses were run at $v_{2.5}$ or v_4 ($v_{2.5}$ or v_4: velocity at which, mathematically, a lactate concentration of 2.5 or 4 mmol/l blood is determined, when it is run under defined conditions). The $v_{2.5}$ and v_4 of each horse was determined with a standardized exercise test two days before the beginning of this study. The mean $v_{2.5}$ and v_4 were 6.50 ± 0.28 m/s (two horses) and 8.00 ± 0.48 m/s (three horses) respectively.

Conditioning study: In a randomized 5×6 latin square cross over study design (five horses × 6 conditioning programmes), horses were exercised at $v_{2.5}$ or v_4 during 5, 15 or 25 minutes for 11 times with one day of rest between two consecutive workouts. Before each conditioning programme horses performed a standardized exercise test to determine their individual $v_{2.5}$ and v_4. To examine the effect of the conditioning programme on T_3, fT_3, T_4 and fT_4 concentration in plasma, blood samples were always taken from the horses at 7:00 a.m., before and after each conditioning programme. Horses had about one week without standardized exercise between conditioning programmes. During this period horses were walked and trotted on the treadmill every second day and had access to paddocks on days in between.

Standardized exercise test

The standardized exercise test consisted of several gallop workouts of five minutes' duration each. Between two consecutive steps there was a resting period of 60 s. The velocity in the first step was 6.0 m/s. Each consecutive step was increased by 0.5 m/s. The test was finished when the horses' blood lactate concentration was above 4 mmol/l. $v_{2.5}$ and v_4 were determined from the individual blood lactate concentration-running speed relationship by exponential regression equation (*Galloux* 1991).

Blood sample handling and T_4, fT_4, T_3, fT_3 analysis

Blood was collected from a jugular vein into evacuated tubes containing Na-heparinate. Within 30 minutes samples were centrifuged at 6,000g for 10 minutes, and the plasma was transferred to plastic vials and kept stored at –20°C until analysis, which was normally done within two months. Plasma concentrations of T_4, fT_4, T_3 and fT_3 were analyzed by enzyme-linked immunoassay methods (Boehringer Biochemica Robin).

Statistics

Data are presented as mean ± standard deviation. The coefficient of variation of the variables before and after exercise was calculated dividing the standard deviation through the mean and multiplying by 100. Effects of conditioning programmes on variables was examined with analysis of variance for repeated measures. $p<0.05$ was used as level to denote significant differences.

Results

The coefficient of variation before exercise of single horses ranged from 5.8% to 19.7%, 9.6% to 21.3%, 5.1% to 13.4%, and 16.7%

Tab. 2: Effect of different conditioning programmes on blood plasma thyroid hormones of horses (n = 5; mean ± standard deviation)

Hormone	Time of blood sampling	Conditioning programme					
		$v_{2.5}$ 5 minutes	$v_{2.5}$ 15 minutes	$v_{2.5}$ 25 minutes	v_4 5 minutes	v_4 15 minutes	v_4 25 minutes
T_4 (nmol/l)	before	25.3 ± 6.3	25.9 ± 7.1	26.4 ± 3.3	24.1 ± 5.7	22.9 ± 6.5	26.2 ± 5.5
	after	25.4 ± 9.5	26.8 ± 3.8	28.5 ± 3.8	27.6 ± 5.0	22.0 ± 5.5	28.8 ± 4.4
fT_4 (pmol/l)	before	8.5 ± 2.4	8.2 ± 2.0	8.7 ± 1.9	7.3 ± 1.5	8.7 ± 2.3	8.5 ± 1.2
	after	8.6 ± 2.6	8.2 ± 2.5	9.0 ± 2.1	8.2 ± 1.2	7.2 ± 0.8	9.6 ± 0.7
T_3 (nmol/l)	before	2.12 ± 0.54	1.87 ± 0.39	1.97 ± 0.12	2.01 ± 0.40	1.96 ± 0.31	2.10 ± 0.40
	after	1.80 ± 0.16	1.84 ± 0.22	1.98 ± 0.64	1.98 ± 0.45	2.09 ± 0.38	2.06 ± 0.40
fT_3 (pmol/l)	before	0.07 ± 0.03	0.06 ± 0.02	0.06 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	0.07 ± 0.02
	after	0.06 ± 0.01	0.06 ± 0.00	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.02	0.07 ± 0.01

to 33.3% for T_4, fT_4, T_3 and fT_3. After exercise the values were between 5.2% and 14.1%, 8.8% and 13.4%, 5.8% and 60.8%, and 12.5% and 28.6% for T_4, fT_4, T_3 and fT_3 respectively (Tab. 1). None of the conditioning programmes had an effect on plasma concentration of thyroid hormones (Tab. 2).

Discussion

The variability of the plasma Thyroid hormones within a horse before and after exercise can be remarkably high and may be a reason for the lack of congruent results in horses on the practical value of blood thyroid hormone evaluation for performance diagnosis (*Thornton* 1985).

There was no effect of conditioning on plasma thyroid hormone levels in resting horses (7:00 a.m. samples). This may be because intensities and the durations of exercise chosen were not large enough to induce adaptations of the organism sufficiently large to overcome the great variability encountered for these variables in plasma. It also may be possible that the adaptation of the pituitary-thyroid axis to conditioning was only at the level of the receptors or in total hormone production and therefore was not reflected in the plasma hormone concentrations (*Katzeff* et al. 1988). The lack of measurable effects of the conditioning programmes used may be also because our horses went through a thorough preparation period before starting the experiment. During the pretrial period heart rate during exercise and blood lactate concentration after exercise decreased, and v_4 increased indicating the adaptation of the horses to the training workloads imposed. Therefore, it may be that the lack of measurable changes of thyroid hormone concentrations in blood plasma was due to the rather high endurance capacity of the horses which could not be further improved by the conditioning programmes examined (*Werkmann* et al. 1996).

Studies in horses on the effect of conditioning on thyroid hormone concentrations in blood were not found. In man, the interpretation of the results on the effect of conditioning on thyroid hormone levels is conflicting. The results of *Irvine* (1968) showed that conditioning increases the degradation rate of T_4 whilst the study of *Balsam* and *Leppo* (1975) did not support this hypothesis. *Boyden* et al. (1982) have suggested that conditioning may impair thyroid function, and the data of *Pakarinen* et al. (1991) demonstrated that very intense exercise during one week inhibits the secretion of TSH and TRH.

The results of this study demonstrated that exercise of up to 25 minutes' duration and at $v_{2.5}$ and v_4 during a period of three weeks does not affect basal plasma thyroid hormone levels. The sometimes very high individual variability of plasma thyroid hormone concentrations before and after exercise may hamper its value for assessing conditioning effects and performance diagnosis of horses. Nevertheless, further studies on the effects of conditioning with longer and with shorter but more intensive exercise on thyroid hormone levels in blood and their interaction with the different tissues involved in exercise should be done to find out whether it is worthwhile to measure these endocrinological variables in healthy horses.

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