

Physiological variables of horses after road transport

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(Received 11 October 2008; Accepted 22 April 2009; First published online 22 May 2009)

In order to investigate the effects of short road transport stress on total and free iodothyronines, body weight (BW), rectal temperature and heart rate (HR) changes, 126 healthy stallions were studied in basal conditions, before and after transport. A total of 60 Thoroughbred and 66 crossbred stallions aged 4 to 15 years with previous travelling experience were transported by road in a commercial trailer for a period of about 3 to 4 h (distance under 300 km). Blood samples and functional variables were collected in each horse box, one week before loading and transport in basal conditions (control samples), one week later immediately before loading (pre-samples) and again after transport and unloading (about 3 to 4 h) in each new horse box, within 30 min of their arrival at the breeding stations (post-samples). Compared to the before-transport values, increases in circulating T_3 , T_4 and fT_4 levels ($P < 0.01$) were observed after transport, irrespective of breed, but not for fT_3 levels. Lower T_4 and fT_4 levels were observed in basal II (at 1100 h) ($P < 0.01$) than in basal I (at 0800 h) conditions and before transport. Thoroughbreds showed higher fT_3 ($P < 0.05$) and fT_4 ($P < 0.01$) levels after transport than crossbred stallions. No significant differences were observed for T_3 and T_4 . Compared to the before-transport values, significant increases in rectal temperature ($P < 0.01$) and HR ($P < 0.05$) were observed after transport. No differences were observed between basal I, II and before values for functional variables. Significant correlations between T_3 and rectal temperature, BW and HR were found. The results indicate that short road transport induces a preferential release of T_3 , T_4 and fT_4 hormones from the thyroid gland in relation to different breed, and an increase in rectal temperature and HR. No significant changes in BW were observed. No differences were observed in relation to different ages. The data obtained suggest that the stallion's thyroid hormones and functional variables may play an important role in assessing the effects of transport stress and a horse's coping strategy.

Keywords: horse, iodothyronines, rectal temperature, heart rate, transport

Implications

The results of the current study suggest that the stallion's thyroid hormones and functional responses may play an important role in providing complementary information for the assessment of transport stress and animal coping strategies. In addition, the existence of a significant correlation between T_3 and rectal temperature, body weight and heart rate confirms that T_3 is the most metabolically active iodothyronine. Moreover, the importance of the thyroid hormone, namely triiodothyronine (T_3) generated locally by 5'-monodeiodinase (5'-MD) in the muscle tissue for metabolic needs during transport is still under investigation. In addition, when using circulating iodothyronine changes for the evaluation of transport stress the presence of peripheral 5'-MD should be further investigated. This may indicate that T_3 generation in peripheral tissues possibly plays a

paracrine role supporting transport stress and metabolic activity of skeletal muscle.

Introduction

Physiologic thyroid hormone concentrations reported for horses have a wide range of values because many intrinsic and extrinsic variables can influence their levels. These include: age (Irvine and Evans, 1975; Chen and Riley, 1981), breed (Malinowski *et al.*, 1996), gender (Fazio *et al.*, 2007), seasonal period (Flisińska-Bojanowska *et al.*, 1991), altitude (Greene *et al.*, 2002), changes in fertility (Gutierrez *et al.*, 2002; Meredith and Dobrinski, 2004), daily rhythms (Morris and Garcia, 1983; Duckett *et al.*, 1989), diet (Messer *et al.*, 1995; Powell *et al.*, 2000), training and exercise (González *et al.*, 1998; Suwannachot *et al.*, 2000) and disease, both severity and duration (Peeters *et al.*, 2006).

Thyroid hormones are important physiologic indices which are commonly used in metabolic rate and animal

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thermogenesis studies (McGuire *et al.*, 1991; Bird *et al.*, 1998) and their supplementation may increase metabolic rate and promote significant weight loss with dietary restriction. In addition, hormonally mediated events play an important role in central nervous system development and function, and a number of researchers have speculated that changes in cognitive function are also mediated by thyroid hormones in humans and animals (Schantz and Widholm, 2001; Helmreich *et al.*, 2006).

Numerous studies to determine the amount of transport stress in horses have been carried out (Leadon, 1994; Fazio and Ferlazzo, 2003; Stull *et al.*, 2004) and thus it is well known that long-term stress (24 to 48 h) can influence a number of systems in the horse, including the immune, digestive and reproductive systems (Baucus *et al.*, 1990a and 1990b). The extent to which short transport stress influences the horse's thyroid function has not been sufficiently studied and only limited scientific data are available that demonstrate its effect on body weight (BW), rectal temperature and heart rate (HR) changes (Stull, 1999).

Given the above, the aim of the present research was to evaluate stallion thyroid function before and after road transport over short distances (under 300 km), taking into account the effects of different breed and age. Rectal temperature, BW and HR changes were also considered.

Material and methods

Animals

The study was carried out on a total of 126 healthy Thoroughbreds ($n = 60$) and crossbred ($n = 66$) stallions, aged 4 to 15 years and weighing 492 ± 15 kg. The horses were transported from the same stud farm (Istituto di Incremento Ippico of Sicily, Italy) to various other breeding stations in Sicily. All stallions had lived together and were a reproductive group of great value; they were therefore familiarized with the other animals in the group. All subjects were used exclusively for reproduction and all had previous transport experience and were familiar with the trailer as well as loading and unloading procedures. To ensure the homogeneity of the groups of horses per load and to exclude any chance of social competition stress, the same subjects were grouped together on all journeys over the three years of the study.

Each stallion was transported on one outbound journey (March to April) and one return journey (July to August) in every year, in accordance with the breeding season for three consecutive years (2004–06). The subjects were transported over exactly the same distance and route. However, this study examined only the subjects submitted to outbound journeys in order to minimize the effects of reproductive activity stress in stallions.

Samples

Two blood samples (10 ml) were collected by jugular venipuncture using evacuated tubes (Venoject; Terumo[®], Terumo Europa N.V., Leuven, Belgium), one at 0800 h (basal I) and the

other at 1100 h (basal II), in each horse box, one week prior to the loading and transport in basal conditions (control samples), then one week later, at 0800 h (before) in each horse box immediately before loading and about 3 to 4 h after transport and unloading (after), within 30 min of their arrival at the breeding stations (post-samples), in each new horse box. Serum samples were harvested after centrifugation at $1500 \times g$ for 15 min at 4°C and stored at -20°C .

The recording of rectal temperature and HR was started before blood sampling to exclude a possible influence on HR changes. These measurements were recorded in an individual box for each horse to exclude the effect of loading procedure. Rectal temperature was measured using an electronic digital thermometer (Artsana, Grandate-Como, Italy) and HR using a Polar Sport Tester (Polar Electro Europe BV, Fleurier, Switzerland), which consisted of an electrode belt with built-in transmitter and a wristwatch receiver. The receiver had a memory function and stored data from the transmitter, averaging HR over 5 s intervals.

Individual live BWs were recorded in basal conditions, before and after transport using large animal scales.

Study design

The commercial trailer used was 9.515 m long and 2.5 m wide, with a ceiling height of 2.5 m. Six compartments with swinging gates were available to each group of stallions transported (i.e. six horses per load). Stocking density was about $2 \text{ m}^2/\text{horse}$. Rubber padding lined the sides of the trailers from the floor to an approximate height of 1.2 m. The number of horses per load, the available floor area, distance travelled and time between loading and unloading were recorded. No feed or water was available during transportation, but they were made available before loading. The subjects were transported on main roads in a commercial trailer and were driven by the same professional driver. Driving speeds ranged from 0 to 80 km/h and the route took approximately 3 to 4 h to complete, and exactly the same route was followed on all journeys. Temperatures were recorded outside and inside the trailer before each journey commenced. Temperature and relative humidity inside the trailer at start and after transport ranged from 19.5°C to 22.4°C and from 62.4% to 65.1%, respectively. These were monitored continually using a Hygrothermograph ST-50 (Sekonic Corporation, Tokyo, Japan) placed near the centre of the trailer.

All methods and the procedures used in this study were reviewed and approved by the Messina University Institutional Board for the Care and Use of Animals.

Hormone assays

Serum total and free iodothyronine concentrations were determined in duplicate using a commercially available immunoenzymatic kits (Roche Diagnostics GmbH, Mannheim, Germany). The respective intra- and inter-assay coefficients of variation were as follows: 7.3% and 11.4% for T_3 , 2.3% and 5.7% for T_4 , 4.2% and 11.9% for fT_3 , 6.6% and 9.6% for fT_4 .

Statistical analysis

Data are presented as mean values ± standard deviation (s.d.) of duplicate measurements. One-way repeated measures analysis of variance (RM-ANOVA) was applied to: (i) determine whether transport stress had any effect, (ii) compare changes in BW, rectal temperature and HR values before loading and after unloading and transport and between basal I and basal II conditions, (iii) compare different stallion breeds and ages. The level of significance was set at <math>P < 0.05</math>. All calculations were performed using the PRISM package (GraphPad Software Inc., San Diego, CA).

The correlations between rectal temperature, BW, HR measurements and thyroid hormone concentrations were evaluated by correlation and linear regression.

Results

Effects of transport

The results obtained showed significant ($P < 0.01$) increases in levels of circulating T_3 (Figure 1), T_4 (Figure 2) and ft_4 (Figure 4) after transport under 300 km, compared to the

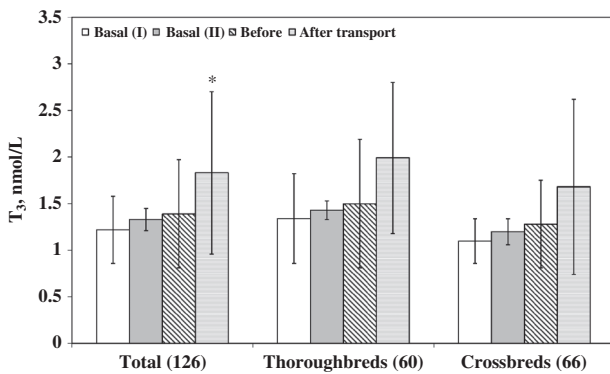


Figure 1 Circulating triiodothyronine levels (mean ± s.d.) in basal conditions, before and after short road transport of stallions. Label on x-axis: groups and number of subjects. Asterisk indicates significant ($*P < 0.01$) differences in average hormone concentrations v. before.

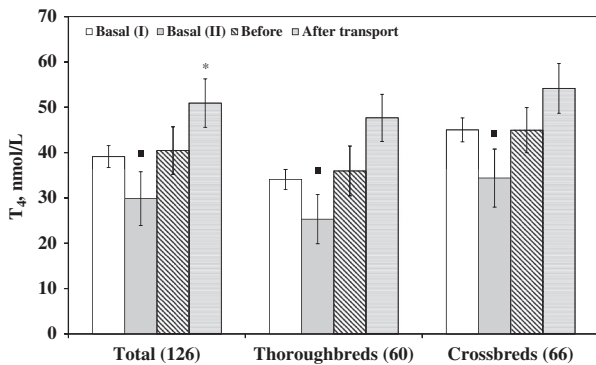


Figure 2 Circulating thyroxine levels (mean ± s.d.) in basal conditions, before and after short road transport of stallions. Label on x-axis: groups and number of subjects. Asterisk indicates significant ($*P < 0.01$) differences in average hormone concentrations v. before. Symbol indicates significant ($\blacksquare P < 0.01$) differences in average hormone concentrations v. basal I.

before values. Transport effects were therefore observed for T_3 , T_4 and ft_4 hormones ($P < 0.01$), but not for ft_3 levels (Figure 3). T_4 and ft_4 showed significantly lower levels ($P < 0.01$) in basal II than in basal I conditions. No significant differences were observed between the before and basal values for T_3 and ft_3 .

Rectal temperature ($P < 0.01$) and HR ($P < 0.05$) showed significant increases (Table 1) after transport, compared to before values. A positive correlation between T_3 and rectal temperature ($r = 1.000$; $P = 0.001$) was recorded. In addition, HR was positively correlated with T_3 ($r = 0.996$; $P = 0.01$), T_4 ($r = 1.000$; $P = 0.001$), ft_3 ($r = 0.998$; $P = 0.05$) and ft_4 ($r = 0.996$; $P = 0.01$) (Table 2)

No significant decrease in BW after transport was observed (Table 1). No significant differences were observed for these parameters between basal and before values. However, a negative correlation between T_3 and BW ($r = -0.996$; $P = 0.05$) was observed (Table 2).

Effects of age

There was no effect of age on total and free iodothyronines, and no significant differences were observed between stallions aged 4 to 9 years and stallions aged 10 to 15 years in basal conditions, and both before and after transport.

Effects of breed

Thoroughbreds showed higher ft_3 ($P < 0.05$) and ft_4 ($P < 0.01$) levels than crossbred stallions only after transport (Figures 3 and 4). No differences between Thoroughbreds and crossbreds were observed in basal and before conditions for total and free iodothyronine levels.

Thoroughbreds and crossbreds showed lower T_4 and ft_4 ($P < 0.05$) levels in basal II than in basal I conditions.

Discussion

The results observed document how the thyroid function (evaluated by means of the modifications in circulating total and free iodothyronine levels) actively modulates the response to short road transport stress in stallions with a

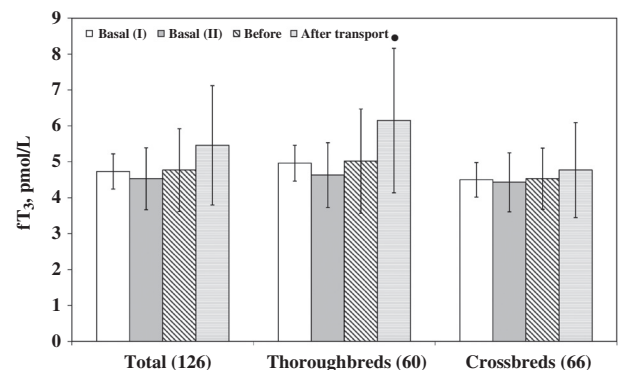


Figure 3 Circulating free triiodothyronine levels (mean ± s.d.) in basal conditions, before and after short road transport of stallions. Label on x-axis: groups and number of subjects. Symbol indicates significant ($\bullet P < 0.01$) differences in average hormone concentrations v. crossbreds.

Table 1 Rectal temperature, body weight and heart rate measurements in basal conditions, before and after short road transport of stallions

Parameters	Sampling time			
	Basal I	Basal II	Before	After
Rectal temperature (°C)	37.22 ± 0.32	37.33 ± 0.20	37.32 ± 0.10	37.58 ± 0.10 ^b
Body weight (kg)	493.00 ± 20.3	493.00 ± 20.3	492.5 ± 20.7	491.7 ± 22.10
Heart rate (beats/min)	39.40 ± 3.00	39.20 ± 2.05	40.33 ± 3.67	46.67 ± 6.02 ^a

^{a,b}Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 2 Correlations and linear regression (r) between thyroid hormone concentrations, rectal temperature, body weight and heart rate measurements of stallions

Hormones	Measurements					
	Rectal temperature (°C)		Body weight (kg)		Heart rate (beats/min)	
	r	P	r	P	r	P
T ₃ (nmol/l)	1.000	0.001 ^c	-0.996	0.05 ^a	0.996	0.01 ^b
T ₄ (nmol/l)	0.986	0.107	-0.959	0.183	1.000	0.001 ^c
fT ₃ (pmol/l)	0.975	0.142	-0.942	0.218	0.998	0.05 ^a
fT ₄ (pmol/l)	0.954	0.195	-0.911	0.270	0.996	0.01 ^b

^{a,b,c}Within a row, means without a common superscript letter differ ($P < 0.05$).

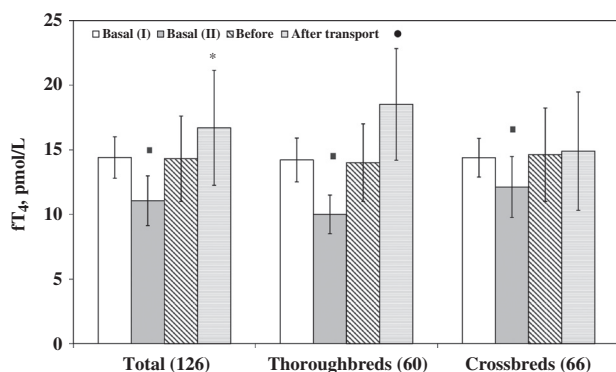


Figure 4 Circulating free thyroxine levels (mean ± s.d.) in basal conditions, before and after short road transport of stallions. Label on x-axis: groups and number of subjects. Asterisk indicates significant ($*P < 0.05$) differences in average hormone concentrations *v.* before. Symbol indicates significant ($\bullet P < 0.01$) differences in average hormone concentrations *v.* crossbreds. Symbol indicates significant ($\blacksquare P < 0.05$) differences in average hormone concentrations *v.* basal I.

greater release of T₃, T₄ and fT₄. The significant response of T₃ to short transport stress could be either the result of the preferential release of T₃ from the thyroid gland or of an increased peripheral monodeiodination of T₄, although a concomitant increase of T₄ levels were observed. This data confirms that T₃ is a metabolically active hormone (Larsen *et al.*, 1981).

Increases in T₃ and T₄ levels after transport confirm that thyroid hormone modifications are an indicator of stress in domestic animals (Fazio and Ferlazzo, 2003; Fazio *et al.*, 2005 and 2007) and also show that horse breed, but not age, appears to influence thyroid responsiveness. In fact, the value of thyroid hormone concentrations cannot be

used as an absolute value, as it is only useful in terms of its variation when relative basal values are known. The higher free iodothyronines observed in Thoroughbreds compared to crossbreds after transport confirm data previously obtained in horses (Malinowski *et al.*, 1996) and suggests that partial thyroid responses to short transport stress are influenced by breed.

The influence of circadian rhythms on thyroid hormones was not investigated because single measurements of total and free iodothyronines are insufficient and thus inadequate to interpret this factor. In addition, the thyroid function can be affected by many endogenous and exogenous factors, such as loading, unloading, environmental changes and diurnal rhythms. How these factors could influence hormonal rhythms remains to be proven. Moreover, T₄ and fT₄ hormones showed lower levels in basal II conditions than basal I conditions. This suggests a significant effect of transport on these hormones, irrespective of the existence of periodicity demonstrated for thyroid hormones in adult geldings (Duckett *et al.*, 1989).

However, the higher T₄ and fT₄ levels observed at 1100 h after transport showed that transport stress may mask the physiological pattern of those hormones, which one week earlier had been lower. The data obtained showed that the environmental conditions and the physiological state of an organism might modify the course and amplitude of rhythms.

Transport is inevitably associated with thermal stress, as confirmed by the positive correlation between rectal temperature measurements and T₃ concentrations. However, the stallions had not been previously acclimatized in our study design. It has been shown that acclimatization can increase the horse's ability to tolerate thermal load reflected by a decrease in respiration rate and rectal temperature

(Rammerstorfer *et al.*, 2001). Extremely warm temperatures of 24°C to 32°C are dangerous, as horses cannot dissipate body heat quickly enough to maintain homeothermy.

In addition, the energy expended for standing during transport may be considerable and posture movements may vary depending on the quality and duration of transport. Short road transport may mask the changes in metabolic reactions as a response to stress. In this sense, the increase in total iodothyronine and fT_4 levels could be a contribution to the energy requirements for muscles, including the reactions related to intermediary metabolism such as protein turnover, ion transport and substrate cycles.

These results indicate that short road transport can more severely affect thyroid patterns and related hormone secretion, but that effective compensatory mechanisms restore normal physiological adaptation to stress thus protecting the body against exaggerated catabolism. Thyroid hormone evaluation after short transport has been shown to be effective in evaluating short-term stress in horses and to provide an additional tool for distinguishing between total and free iodothyronine changes.

The physiologic responses of horses to short road transport show a decrease in BW (although not significant), as confirmed by a negative correlation only between BW and T_3 , as well as an increase in rectal temperature, in accordance with that reported after long, medium and short trips (Waran *et al.*, 1996; Stull, 1999; Stull and Rodiek, 2000). However, no differences were observed relating to different ages. The significant increase in HR observed after transport showed that HR remained higher, even up to 30 min after unloading. Though, previous results reported an increase in HR observed at loading and unloading (Waran and Cuddeford, 1995) and also during transport (Smith *et al.*, 1994) in horses. These data are confirmed by the positive correlations observed between HR and total and free iodothyronines.

The data obtained could suggest that novel environments may reinforce the total cumulative effect on an individual horse's heart rate, although no significant differences between Thoroughbreds and crossbreds were observed. Basal, pre- and post-transport values for rectal temperature and HR values of stallions fell within the physiological range reported for equine species (Rammerstorfer *et al.*, 2001).

These findings suggest that the stallion's thyroid hormones and physiological responses may play an important role in providing complementary information for the assessment of transport stress and animal coping strategies. In addition, the existence of a significant correlation between T_3 and rectal temperature, BW and HR confirms that T_3 is the most metabolically active iodothyronine, also during transport in stallions.

Acknowledgements

The authors express their gratitude to the management and staff of Istituto di Incremento Ippico of Sicily, Italy, for their practical and technical assistance.

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