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Serum iron, ferritin, transferrin and haptoglobin concentration variations during repeated show jumping competition in horse

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Abstract

Modifications of the iron profile in athlete horses during two international three star (***) show jumping competitions performed in two consecutive weekends were evaluated. Serum iron, ferritin, transferrin, and haptoglobin were assessed in 12 well-trained Italian Saddle horses. Blood samplings were performed before the first day of competition (R1), within 10 min from the end of each competition (J1, J2) and on the day after competition (R2). The same plan was followed during the second weekend (J3, J4 and R3). One-way repeated measures analysis of variance (ANOVA) was applied on obtained data, and a significant effect of exercise ($P < 0.05$) on all studied indices was found. These results suggest that serum iron, transferrin, ferritin and haptoglobin are responsive to intense exercise and could be considered important indicators that may give important information about the horse's performance.

Iron profile, athletic horse, exercise, blood

Similarly to other stressors, physical exercise has considerable effects on animal metabolism and adequate responses are needed to re-establish homeostatic equilibrium. The physiological processes induced by physical exercise result in changes of the concentrations of several blood variables including iron. Iron is a fundamental element in the metabolic systems involved in O_2 transport and, consequently, in the aerobic capacity of athletes (Walker et al. 2001). Iron in the body is always bound to specific storage proteins (ferritin and haemosiderin) or to carrier protein (transferrin, lactoferrin, and haptoglobin), and its metabolism involves intestinal absorption in the duodenum and delivery to several tissues. Evaluation of iron metabolism in athletic horses can be carried out through the measurement of the content of serum iron, of the iron storage ferritin, of blood transport performed by transferrin, and even taking into consideration the binding to the particular carrier protein haptoglobin.

Ferritin is a globular protein located in several tissues, in particular spleen, liver, and bone marrow. It has several isoforms and represents the main iron storage and detoxification protein in biological systems (Proulx-Curry and Chasteen 1995; Chasteen and Harrison 1999). Ferritin is also an acute phase protein (Cavill 1999) and so variations in its concentrations may signal inflammation.

Transferrin is a β-globulin and it is produced in the liver, lymphoid tissue, ovaries, testes and mammary gland. It is the main iron carrier to storage tissues and to the bone narrow.

Haptoglobin fixes iron protein binding including haemoglobin and myoglobin. Haptoglobin-haemoglobin/myoglobin complexes are removed in the liver determining a rapid decrease in plasma haptoglobin concentrations (Shaskey 2000; Schaer et al. 2013). Moreover, both haptoglobin and ferritin are moderate acute phase protein in horses (Crisman et al. 2008) and their blood concentration may vary due to inflammation and stress caused by e.g. exercise.

Several studies carried out in athletic horses showed that physical exercise of different intensities and durations influence iron homeostasis (Mills et al. 1996; Karamizrak et

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al. 1996; Inoue et al. 2005; Scoppetta et al. 2012). It is noteworthy that iron depletion could influence athletic performance through low haemoglobin concentration with a consequent reduction in the oxygen transport capacity, decreasing the maximal oxygen uptake (Walker et al. 2001).

On the basis of this knowledge, the aim of this study was to investigate the changes in the serum concentration of iron, ferritin, transferrin and haptoglobin of show jumping horses subjected to two sessions of show jumping competition.

Materials and Methods

Animals

The study was carried out on 12 regularly trained Italian Saddle horses (7 geldings and 5 females, 10–12 years old, mean body weight 490 ± 30 kg). The study involved a laboratory component and a veterinary clinic component, both conducted at the University of Messina's School of Veterinary Medicine. Horses took part in jumping competitions held on two consecutive weekends. The competition type, the course length, the obstacle height and environment recording are shown in Table 1. Protocols of animal husbandry and experimentation were reviewed and approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 86/609 CEE.

All horses were managed equally with individual boxes, the same natural photoperiod, indoor temperature (18–20 °C) and feeding schedule. The horses were fed standard rations, calculated to fulfill all the nutritional requirements according to INRA (Institut National de la Richerche Agronomique) specifications (Martin-Rosset 1990).

Each standard ration was composed of hay of approximately 6 ± 1 kg/horse/day (first cut meadow hay, sun cured, with 6.9% crude protein on average) and a mixture of cereals (oats and barley, 50% each, approximately 3.5 kg/horse/day). Hay contained 100–250 mg iron/kg, cereal grains contained 30–90 mg/kg.

The ration was administered × 3 a day: at 8:00 h, 12:00 h, and 17:00 h. The percentage composition of the mixture was dry matter 87% and moisture 13%. The dry matter contained 9.11% digestible protein, 13.05% crude protein, 20.7 % crude fibre and 3.42% crude lipid, as well as 0.80 Unité Fourragère Cheval/kg. Water was available *ad libitum*.

Blood sampling and analysis

Blood samples were collected by jugular venipuncture in vacutainer tubes with clot activator for serum analyses (Terumo Co., Tokyo, Japan). Blood samplings were performed before the first day of competition (R1), within 10 min from the end of each competition (J1, J2) and on the day after competition (R2); the same plan was followed during the second weekend (J3, J4, and R3).

Immediately after collection, blood samples were placed in refrigerated bags and transported to the laboratory for the analysis. Tubes were centrifuged at 1308 *g* for 10 min. Serum iron, transferrin, ferritin and haptoglobin were determined using commercially available kits – iron with colorimetric method (Wako Pure Chemical Industries, USA) with an automated analyser, Model 7070 (Hitachi LTD, Tokyo, Japan); ferritin with the Quantimmune ferritin IRMA kit (Bio-Rad Laboratories, USA); transferrin with the immunoturbidimetric assay (Boehringer-Mannheim, Germany); haptoglobin with the haptoglobin kit-second generation Phase Range (Tridelta Development LTD, Ireland).

The same operator assayed all samples in duplicate each time. Samples exhibited parallel displacement to the standard curve; the intra-assay coefficient of variation was < 7% for all indicators measured and the inter-assay coefficient of variation was < 9% for all indicators measured.

Statistical analysis

Bartlett's test was applied to verify the normal distribution of data and all passed the test. $P < 0.05$ was considered significant, with an alpha level of 95%. One-way repeated measures analysis of variance (ANOVA) was applied to determine significant effects of exercise on serum iron, ferritin, transferrin and haptoglobin in the horses involved in this study. $P < 0.05$ was considered

Table 1. Competition type, course length, obstacle height and environmental conditions recording during the two weekends of competition.

	Competition 1			Competition 2		
	Competition type	Course and obstacle	Environmental conditions	Competition type	Course and obstacle	Environmental conditions
Day 1	Two phases	550 ± 50 m; 140 cm	25 ± 6 °C; 65 ± 5% HR	Two phases	550 ± 50 m; 140 cm	26 ± 5; 68 ± 6% HR
Day 2	Mixed competition	550 ± 50 m; 145 cm	27 ± 4 °C; 66 ± 3% HR	Mixed competition	550 ± 50 m; 145 cm	28 ± 3; 70 ± 5% HR

significant. Bonferroni's multiple comparison test was applied for *post hoc* comparison. Statistical analysis was performed using Stats package of R Core Team (2013).

Results

Mean values ± standard error of the mean (SEM) of serum iron, ferritin, transferrin, and haptoglobin are shown in Table 2.

The results of ANOVA showed a significant effect of the time in serum iron, ferritin, transferrin, and haptoglobin in all experimental conditions ($P < 0.05$).

ANOVA showed a significant effect of the exercise on serum iron ($P < 0.05$), ferritin ($P < 0.05$), transferrin ($P < 0.05$), and haptoglobin ($P < 0.05$).

In particular, *post hoc* multiple comparisons showed a significant decrease of serum iron values in J1, J2, and R2 in respect to R1, J3, J4, and R3. ANOVA showed a significant increase of serum ferritin concentrations in J2 and J4 in respect to R1, J1, R2, J3, and R3. A significant increase of transferrin values was found in J2 and J4 in respect to R1, R2 and J3, and of haptoglobin concentrations in J2 and J4 in respect to R1, J1, R2, and R3.

Table 2. Mean values (± SEM) with significances of indices obtained in 12 Italian Saddle horses before the first competition day (R1), within 10 min from the end of each exercise (J1, J2) and on the day after competition (R2). The same plan was followed during second weekend (J3, J4 and R3).

Indices	Experimental period						
	R1	J1	J2	R2	J3	J4	R3
Serum iron (µg/dl)	173.2 ± 8.4	137.9 ± 1.7^{a,e,f,g}	138.2 ± 2.8^{a,e,f,g}	119.0 ± 2.7^{a,e,f,g}	173.9 ± 8.6	159.1 ± 6.3	150.1 ± 4.3
sFerritin (µg/dl)	21.3 ± 1.8	19.9 ± 1.5	24.7 ± 1.9^{a,c,d,e,g}	21.0 ± 1.9	19.7 ± 0.7	26.7 ± 1.3^{a,c,d,e,g}	20.9 ± 1.5
Transferrin (mg/dl)	260.1 ± 9.0	282.2 ± 10.0^{a,d,e}	306.8 ± 9.1	270.2 ± 11.5	272.3 ± 4.4	309.8 ± 6.4^{a,d,e}	299.9 ± 7.6
Haptoglobin (mg/dl)	1.8 ± 0.02	1.8 ± 0.08	2.0 ± 0.1^{a,b,d,g}	1.6 ± 0.1	1.9 ± 0.05	2.0 ± 0.1^{a,b,d,g}	1.6 ± 0.1

Significances: [a] vs R1, [b] vs J1, [c] vs J2, [d] vs R2, [e] vs J3, [f] vs J4, [g] vs R3

Discussion

The type, intensity and duration of physical exercise (Rivero et al. 2007) together with athletic fitness and training level (Couroucè 1999) have a great influence on the changes occurring in the athlete's body. Normal iron status in athletes is particularly important because of the central role of this mineral in oxygen transport and the synthesis of haemoglobin, myoglobin, and certain enzymes essential to energy production. Due to the significant role of iron in optimal physical performance and health, the evaluation of iron status in athletes is of great importance in order to prevent iron deficiency. Similar to the results of previous studies (Haskviz et al. 1992; Eaton et al. 1999), our findings showed a modification in serum iron concentrations following exercise. In particular, higher iron values were found at J3 and J4 compared to J1, J2, and R2.

The rise in iron values could be due to intravascular haemolysis that is likely to occur during maximal exercise (Schott et al. 1995; Pellegrini Masini et al. 2003; Inoue et al. 2005) as well as due to the release of iron stocked in liver, spleen, and reticuloendothelial cells (Kimber et al. 1983; Gimenez et al. 1988). Moreover, the results obtained in the present study showed increased values of serum ferritin and transferrin in response to physical exercise. Ferritin is highly correlated to the intracellular iron storage both in human (Newhouse and Clement 1988) and horse (Smith 1984), and its synthesis is iron-dependent and regulated by a post-transcriptional gene regulation model which increases the production with

a rise of iron availability (Newhouse and Clement 1988). Although ferritin concentration is high in the liver and spleen, during exercise ferritin may leak from these tissues (Hyyppä et al. 2002) reaching blood. In horses, the increase in plasma ferritin was higher when the intensity and duration of exercise increased (Hyyppä et al. 2002).

The higher serum ferritin and transferrin concentrations found after physical activity could be due to haemoconcentration occurring during and immediately after exercise (Schumacher et al. 2002). In addition, the hyperferritinaemia found at J2 and J4 could be due to a stress response to exercise considering the well-known role of ferritin as an acute phase protein. The exercise-induced ferritin increase could be due to a tissue leakage of ferritin and in particular to splenic contraction but also to the increase of serum iron caused by intravascular haemolysis (Hyyppä et al. 2002; Friedrichs et al. 2010). The iron binding capacity of ferritin and its serum concentrations may be the first indicators of early iron deficiency (Ostojic and Ahmatovic 2008) which seems to be one of the most frequently encountered nutritional and/or exercise-related deficiencies in athletes.

In contrast to previous studies (Inoue et al. 2005), we observed a significant increase of haptoglobin during the second day of jumping competition (J2, J4). However, subsequent studies (Cywinska et al. 2011) showed a significant decrease in serum haptoglobin only during the first and second week of repeated exercises and a return to baseline levels after second week. The trend we found could be due to the proximity of sampling and the different exertion of exercise. For these reasons, the trend of haptoglobin in this study could be due only to its action as an acute phase protein.

In conclusion, it is possible to underline the importance of relating the evaluation and interpretation of iron indices to the evolution of the values with the progression of exercise without limiting it to a comparison with the static normal range.

Although the results of the present study provide insight into the jumper horse's physiological response to physical effort, further research is needed in order to better evaluate the mechanisms by which exercise influences the iron metabolism in the athletic horse.

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