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IRON METABOLISM MODIFICATION DURING REPEATED SHOW JUMPING EVENT IN EQUINE ATHLETES

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Abstract

In athletic horse the evaluation of iron status is of great importance to improve physical performance and health status of animal. The aim of this study was to evaluate the changes of iron indices following show jumping. Ten regularly trained Italian Saddlebred horses aged 7–8 years (mean body weight 467±12 kg) were subjected to three days jumping competition. Blood samples were collected at 5 time points: T0 (the day before competitions), T1 (immediately after exercise at day 1), T2 (immediately after exercise at day 2), T3 (immediately after exercise at day 3) and during the recovery period T4 (24 h after day 3). On each blood sample the values of red blood cell (RBC), hemoglobin concentration (Hb), hematocrit (Hct), serum iron, ferritin, transferrin, total iron-binding capacity (TIBC) and unsaturated iron-binding capacity (UIBC) were assessed. One-way repeated measure analysis of variance (ANOVA) showed a statistical significant effect of exercise ($P<0.05$) on all studied parameters. The application of Bonferroni's post-hoc comparison showed a statistical significant increase in all studied parameters after exercise. These results provide new information about the changes in iron profile of jumper horse following exercise allowing for better evaluation of the health status and physical performance of this athlete horse.

Key words: exercise, ferritin, horse, iron, transferrin, show jumping

Iron is a micronutrient essential for a number of synthetic and enzymatic processes, it is required for adequate erythropoietic function, oxidative metabolism and cellular immune response (Muñoz et al., 2009). The dominant site for the iron utilization is the bone marrow for developing red blood cell hemoglobinization (Johnson

and Wessling-Resnick, 2012). Storage iron concentration is the major factor affecting the relative distribution of this micronutrient between ferritin and hemosiderin in mammals; at low storage levels, more iron is stored as ferritin than as hemosiderin (Kaneko et al., 1997). Iron is delivered to tissues by circulating transferrin, a transporter that captures iron released into the plasma mainly from intestinal enterocytes or reticuloendothelial macrophages (Wang and Pantopoulos, 2011).

Normal iron status in athletes is particularly important because of the central role of this mineral in oxygen transport and the synthesis of hemoglobin, myoglobin, and certain enzymes essential to energy production (Ming et al., 2000). The formation of hemoglobin and the body's subsequent ability to transport oxygen from the lungs to the tissues will be impaired in the athlete who is iron deficient (Brumitt et al., 2009). The process by which iron stores are depleted depends on the balance between iron intake and iron requirements and may occur rapidly or very slowly (Beard and Tobin, 2000). The clear consequences of iron depletion are a reduction in oxygen transport capacity and a reduction in the cellular oxidative capacity.

It has been shown that exercise affects the hematological profile and iron status of the athlete with significant impact on the oxidative metabolism and aerobic performance (Schumacher et al., 2002). Iron profile changes associated with exercise have been widely analyzed to provide information about performance and health status in human (Kasugai et al., 1992; Camus et al., 1993; Hinton, 2014; Alaunyte et al., 2015; Habte et al., 2015) and equine athlete (Mills et al., 1996; Inoue et al., 2002; Hyyppä et al., 2002; Inoue et al., 2005; Assenza et al., 2016). However, only one study dealt with iron status in show jumpers during competition (Assenza et al., 2014). In view of this, the aim of this study was to evaluate how iron profile and some hematological parameters change following repeated physical exercise in jumper horses.

Material and methods

Animals and housing

Ten regularly trained Italian Saddlebred horses (7–8 years; 6 geldings, 4 females, mean body weight 467±12 kg) were enrolled in the present study. All animals were housed in individual boxes (3.5×3.5 m) at the same horse training centre in Sicily (latitude 36°54'52"N; longitude 14°51'12"E). Horses were fed standard rations twice a day (07.00 AM; 06.00 PM). The concentrate ratio was formulated to meet the requirements of horses based on the recommendations of the Institut National de la Recherche Agronomique (Martin-Rosset, 1990). The daily feed allowance consisted of hay (first-cut meadow hay, sun-cured, late-cut, 8 kg/horse/day; 6.9% crude protein on average) and a mixture of cereals (oats and barley, 50% each; 3.5 kg/horse/day). Hay typically contains 100–250 mg iron/kg and cereal grains have 30–90 mg/kg. The average dry matter content of the daily ration was 87%. 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% Unitè Fourragère Cheval/kg. Water was available *ad*

libitum. All animals were clinically healthy and free from internal and external parasites. Their health status was evaluated through a clinical exam. All horses competed in a three days jumping competition, according to the "Federazione Italiana Sport Equestri" (FISE) rules, in the afternoon (12.00 AM – 06.00 PM). The maximum height and the required speed (135 cm and 350 m/min, respectively) was the same for all course. The length of the course was 400 m with 12 efforts (7 verticals, 5 oxers, 2 double combinations) on day 1; 450 m and 14 efforts (7 verticals, 7 oxers, 2 double combinations) on day 2; and 500 m and 15 efforts (9 verticals, 6 oxers, 1 double and 1 triple combinations) on day 3. On the days of the jumping course the mean environmental temperature and relative humidity were 13.4±1.7°C and 68±4%, respectively.

All treatments, housing and animal care were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments.

Blood sampling and analysis

Blood samples were collected from each animal by jugular vein puncture into two vacutainer tubes (Becton, Dickinson and Company): one tube contained EDTA for hematological analyses and the other tube (Terumo Corporation Japan) without anticoagulant agent for biochemical analyses. Blood was sampled at 5 time points: T0 (the day before competitions in their training centre), T1 (immediately after exercise at day 1), T2 (immediately after exercise at day 2), T3 (immediately after exercise at day 3) and during the recovery period T4 (24 h after day 3, the day before competitions in their training centre). EDTA blood samples were refrigerated and analyzed within 2 h from the collection by means of an automated blood cell counter (HeCo Vet C, Naples, Italy). All samples were tested for red blood cell (RBC), hemoglobin concentration (Hb) and hematocrit (Hct). Blood samples without anticoagulant agent were refrigerated and centrifuged within 2 h from the collection at 1300 g for 10 min, at room temperature, and obtained sera were stored at –20°C until analysis. Serum iron concentration was determined by a colorimetric method (Wako Pure Chemical Industries, USA) with a Hitachi Model 7070 automated analyzer. Ferritin levels were measured using Quantimmune Ferritin IRMA kit (Bio-Rad Laboratories, USA), whereas transferrin concentrations were obtained by an immunoturbidimetric assay (Biosystems S.A., Spain). Total iron-binding capacity (TIBC) was measured by multiplying the values of transferrin by the constant factor 1.27. Unsaturated iron-binding capacity (UIBC) was calculated as the subtraction of TIBC and the serum iron (Gottschalk et al., 2000).

Statistical analysis

One-way measures analysis of variance (ANOVA) was applied to determine significant effect of exercise on studied parameters. P-value <0.05 was considered statistically significant. Bonferroni's multiple comparison test was applied for post hoc comparison. Correlation analysis was applied to investigate the relationship between studied parameters. Data were analyzed using statistical software Prism v. 4.00 (Graphpad Software Ltd., USA, 2003).

Results

One-way ANOVA showed a statistical significant effect of exercise on red blood cell (P<0.01), hemoglobin concentration (P<0.01), hematocrit (P<0.01), iron (P<0.05), ferritin (P<0.05), transferrin (P<0.05), TIBC (P<0.05) and UIBC (P<0.05). The application of Bonferroni's post-hoc comparison showed statistical significant increase of all studied parameters after exercise (Table 1). Every studied parameter was significantly positive correlated to each other, with the exception of ferritin that did not show any significant correlation (Table 2).

Table 1. Effect of show jumping on some iron status and related blood parameters (Mean±Root-Mean-Square Error)

Parameters	Experimental Period				
	T0	T1	T2	T3	T4
RBC (10^6/µL)	7.86±0.91	9.87±0.47 ab	9.88±0.38 ab	9.37±0.24 sb	7.32±0.54
Hct (%)	34.94±3.61	43.13±1.77 ab	43.12±1.63 ab	41.33±1.34 ab	32.66±2.03
Hb (g/dL)	13.36±1.65	16.92±0.86 ab	16.88±0.79 ab	16.14±0.47 ab	12.40±0.92
Iron (µg/dL)	150.13±13.12	172.14±18.14 a	173.15±19.05 a	173.20±18.92 a	159.08±14.68
Ferritin (µg/dL)	21.31±6.04	24.75±7.01	24.65±3.66	27.99±5.14 ab	21.03±6.79
Transferrin (mg/dL)	260.14±32.71	282.14±36.17	294.75±40.49	306.82±23.41	270.17±26.89
TIBC (µg/dL)	325.18±40.88	352.68±45.21	368.44±41.07	383.52±29.61 a	337.71±51.82
UIBC (µg/dL)	152.03±45.21	179.48±54.21	196.30±45.35	224.44±32.27 a	187.58±52.16

Observations significance: a *vs* T0 and b *vs* T4; T0: the day before competitions; T1: immediately after exercise at day 1; T2: immediately after exercise at day 2; T3: immediately after exercise at day 3; T4: 24 h after T3 during the recovery period.

Table 2. Coefficients of correlation between iron profile and some hematological parameters calculated for horses during and after a three days jumping competition. Significant correlations (P<0.05) are indicated in bold letters

	Ferritin (µg/dL)	Transferrin (mg/dL)	TIBC (µg/dL)	UIBC (µg/dL)	RBC (106/ µL)	Hb (g/dL)	Hct (%)
Iron (µg/dL)	0.15 P=0.22	0.24 P<0.05	0.40 P<0.01	0.34 P<0.01	0.43 P<0.01	0.44 P<0.01	0.43 P<0.01
Ferritin (µg/dL)		0.05 P=0.69	0.04 P=0.76	0.04 P=0.72	0.17 P=0.16	0.17 P=0.16	0.17 P=0.16
Transferrin (mg/dL)			0.56 P<0.01	0.51 P<0.01	0.34 P<0.01	0.31 P<0.01	0.29 P<0.01
TIBC (µg/dL)				0.94 P<0.01	0.35 P<0.01	0.34 P<0.01	0.32 P<0.01
UIBC (µg/dL)					0.24 P<0.05	0.23 P<0.05	0.22 P=0.07
RBC (10^6/µL)						0.98 P<0.01	0.96 P<0.01
Hb (g/dL)							0.98 P<0.01

Abbreviations: total iron-binding capacity (TIBC), unsaturated iron-binding capacity (UIBC), red blood cell (RBC), hemoglobin concentration (Hb), hematocrit (Hct).

Discussion

Physical exercise is one of the most physiologically stressful stimuli an animal can undergo; during physical exertion, the animal experiences reversible alterations in various homeostatic variables that are detectable by the quantification of laboratory variables. The scientific community is currently interested in studying the hematochemical and hematological changes that result from physical exercise (Gondin et al., 2013). 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. Most of the investigations carried out on the athlete horse showed an engagement of RBC, Hb, Hct and serum iron immediately after exercise, with increases interpreted as the result of splenic contraction (Piccione et al., 2007) and adjustments functional to increase the oxiphoric power of blood (Kearns et al., 2002). In the present study significant changes occurred in RBC, Hb and Htc values after jumping exercise in all horses. The RBC count showed a significant increase with concomitant significant changes in Hb and Hct after each day of jumping competition (T1–T3). The Hct and/or Hb are widely used to evaluate iron status, but each is relatively insensitive because of splenic contraction that may make it difficult to determine accurately the true value (Smith et al., 1984). In response to exercise, splenic erythrocytes are released due to splenic contraction under the influence of catecholamines (Householder and Douglas, 2005). The significant increase in Hct, RBC and Hb values recorded in horses after each jumping course was likely due to the splenic contraction (Piccione et al., 2007), as it is well known that the equine spleen functions as reservoir of 4-12 liters of red blood cells that can be released into circulation at the beginning of exercise (Hinchcliff et al., 2004).

As previously observed in human athletes (Kasugai et al., 1992; Pratt and Chrisley, 1996) and in sport horses (Mills et al., 1996; Inoue et al., 2002, 2005), our results showed a significant increase in the iron concentration after each day of competition (T1–T3), and a subsequent slight decrease, although not statistically significant, after recovery period (T4). This might be due to exercise-induced hemoconcentration. Hovewer, other authors, studying iron metabolism in human athletes, indicated that the serum iron concentration was increased 30 min after exercise, and, consequently, it was not related to hemoconcentration (Ohira et al., 1995). They suggested that the increase was due to serum Fe originating from ruptured erythrocytes. Hanzawa et al. (1998) suggested that the increase of the iron levels was due to serum iron released from ruptured erythrocytes induced by exercise in horses.

Therefore, we consider that the slow recovery of serum iron is, at least partly, induced by hemolysis during exercise. Suzuki et al. (1999) reported that serum creatine kinase activity and the myoglobin level rose after exercise, which resulted from muscle damage in humans. Snow et al. (1982) also indicated that exercise increased serum creatine kinase activity in horses. Myoglobin is probably released from muscle after exercise in horses because of muscle damage. The increase in serum myoglobin concentration may contribute to the slow recovery of serum Fe concentration after exercise in horses.

Our results showed a significant increase of the ferritin, transferrin, TIBC and UIBC concentration after competition at T3 compared to T0, and a subsequent decrease at T4. Ferritin is highly correlated to the intracellular iron stores 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 rise of iron availability (Newhouse and Clement, 1988). In horses, the increase in plasma ferritin was greater when the intensity and duration of exercise increased (Hyyppä et al., 2002). Although ferritin concentration is high in liver and spleen, during exercise ferritin may leak from these tissues (Franken et al., 1981; Suryakala and Deshpande, 1999; Hyyppä et al., 2002). Transferrin showed the response to exercise usually reported as a mild increase (Gimenez et al., 1988; Schumacher et al., 2002). As for ferritin, the increase of transferrin could be attributed to hemoconcentration, which occurs during and immediately after exercise. This hyphotesis seems to be confirmed by the results obtained from the correlation analysis that showed a positive correlation among studied parameters.

The increased TIBC concentration after exercise, compared with the resting state, seems to suggest an increased generation of transferrin carrying iron throughout the body (Kobayashi et al., 2014). However, the TIBC was measured by multiplying the values of transferrin by the constant factor 1.27 and UIBC was calculated as the subtraction of TIBC and the serum iron, therefore, the trend of these parameters was the same throughout the monitoring period.

Conclusions

The influence of physical exercise on iron metabolism was well documented, however, the nature and the extent of the changes in iron status depend on the degree of training, as well as exercise type and intensity. The results obtained in the present study showed an increase in the values of erythrocytes and iron indices in jumper horses during the three days competition that could be related to exercise-induced hemoconcentration. Moreover, the failure of the iron profile values to lower up to baseline levels during the recovery period, probably resulted from iron influx from ruptured erythrocytes and/or damaged muscular tissue. Although the results of the present study provide insight into the jumper horse's physiological response to exercise, further studies are needed to investigate the mechanisms by which exercise affects iron homeostases in order to better evaluate the health status and the physical performance of jumper horse.

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