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Original Research

Different Training Schedules Influence Serum Electrophoretic Protein Profile in the Athletic Horse

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ABSTRACT

Physical exercise induces various physiological responses and metabolic adaptations that have not been completely elucidated. The monitoring of alterations in blood parameters related to exercise is required to detect subclinical conditions. In this study, changes in serum total proteins and globulin fractions (albumin, α1, α2, β1, β2, and γ-globulins) were investigated in 15 clinically healthy horses subjected to different physical exercise for 5 weeks. Animals were divided in three equal groups: group A performed an intense training schedule, group B performed a light training schedule, and group C included sedentary subjects. After 5 weeks, group B was subjected to the same training schedule used for group A and it was indicated as group B1. Blood samples were collected from all animals at rest conditions. During the first week, three blood samples were collected at first, third, and fifth day and afterward, once a week for additional 4 weeks. Two-way repeated measures analysis of variance showed a statistical effect of sampling time and of different training schedules on total proteins and albumin levels in groups A and B as compared with groups C and B1. Based on these results, it is evident that the interpretation of changes in electrophoretic parameters in athletic horses cannot be limited to the comparison with a static normal range, but must consider their dynamic evolution with the progression of training.

1. Introduction

In equine sport medicine, the effects of stress related to exercise are generally associated with poorly understood disorders such as the poor performance syndrome, the overtraining syndrome, the exertional rhabdomyolysis, and the exercise-induced pulmonary hemorrhage [1–7]. Depending on the intensity, duration, and different type of physical exercise, equine metabolism has to adapt to nervous, cardiovascular, endocrine, and respiratory system requirements [1–8]. It was found that the horse has a regulatory system that effectively reacts to stressful events such as exercise, to restore homeostatic equilibrium [1,9,10] demonstrating that horses have a great capability for physical work.

Many studies have been performed to evaluate changes in physiological and biochemical parameters in horse during physical exercise [9,11–18].

In particular, the evaluation of hemogram and plasma or serum biochemistry has been used to assess the health status and fitness level, as well as to investigate poor performance cases in athletic horses [19]. Serum electrophoresis represents an effective diagnostic tool in equine laboratory medicine, and it is frequently used in horse with specific or unspecific clinical signs [20]. This screening test

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is based on the knowledge of the chemical composition of proteins, which determines their electrophoretic migration, and can inform about changes in albumin, α-, β-, and γ-globulins concentrations.

Studies carried out on athletic horse reported changes in serum protein profile as a response to exercise [9,20,21].

On the basis of this knowledge, the aim of this study was to investigate the serum total proteins levels and electrophoretic profile in horses subjected to different training schedules, to evaluate how these parameters change in relation to the time and intensity of exercise.

2. Materials and Methods

The study was carried out on 15 Italian saddle horses (nine males and six females, 7 to 10 year old) from the same training center, located in Sicily (latitude 38°14′27″96 N, longitude15°26′17″52 E), and with an average body weight of 420 ± 40 kg. Each animal, subjected to routine hematology and plasma biochemistry testing, was considered healthy and included in the study. The horses were fed standard rations, calculated to fulfill all the nutritional requirements according to the Institut National de la Richerche Agronomique specifications [19].

Each standard ration was composed 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, about 3.5 kg/horse/day). The latter ration was provided three times a day, 7 AM, 1 PM, and 7 PM. The percent composition of the mixture was dry matter 86.36% and moisture 13.63%. The dry matter contained 9.11% horse digestible protein, 13.05% crude protein, 20.7% crude fiber, and 3.42 crude lipids, as well as 0.80 Unité Fouragire Cheval 7 kg. Water was available ad libitum.

Horses were divided into three equal groups (A, B, and C) according to the training schedules. Group A included horses that performed the following daily training schedule for 5 weeks: warm-up (10-minutes walk, 30-minutes trot, and 10-minutes gallop) and show jumping course with 10 fences of 100 ± 10 cm average height. Group B was trained twice a week during the first 5 weeks, warm-up (10-minutes walk, 20-minutes trot, and 10-minutes gallop) and show jumping course with seven fences of 80 ± 10 cm average height.

Afterward, horses from group B were daily lounged (10-minutes trotting and 10-minutes cantering) for 2 weeks. Then, they were subjected to a higher-level training schedule for additional 5 weeks (group B1), warm-up (10-minutes walk, 30-minutes trot, and 10-minutes gallop), and show jumping course with 10 fences of 100 ± 10 cm average height. Group C (control group) included sedentary subjects that performed no training during the study.

From animals of each group, blood samples were collected by jugular venipuncture into Vacutainer tubes (Terumo Corporation, Tokyo, Japan) without anticoagulant agent, at rest conditions before feeding (at 6 AM).

During the first week, three blood samples were collected at first, third, and fifth day (T1.1, T1.2, and T1.3). Afterward, blood samples were collected once a week (first day of week) every seven days for additional 4 weeks (T2, T3, T4, and T5). Following standing at room temperature for 20 minutes, the tubes were centrifuged at 3,000 rpm for 10 minutes, and the obtained sera were stored at −20°C until analyzed.

The concentration of serum total proteins was determined by the biuret method using an automated analyzer UV Spectrophotometer (SEAC, Slim, Florence, Italy). The serum protein electrophoresis was performed using an automated system (Sel Vet 24; SELEO Engineering, Naples, Italy) according to the procedures described by the manufacturer and previously used in horse [22]. The major protein fractions revealed were albumin, α1, α2, β1, β2, and γ-globulins.

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 2010/63/EU for animal experiments.

2.1. Statistical Analysis

Data were normally distributed ($P > .05$, Kolmogorov-Smirnov test). Two-way repeated measures analysis of variance was used to determine a statistically significant effect of sampling time and different training schedules on total proteins and protein fractions values. Bonferroni test was also applied as post hoc test comparison ($P < .05$).

All calculations were done using the PRISM package version 4.00 (GraphPad Software Ltd, 2003).

3. Results

All the results are expressed as mean values ± standard deviation.

Analysis of variance showed a statistical effect of sampling time on total proteins, albumin, and albumin/globulins ratio (A/G ratio) levels in groups A and B ($P < .001$), whereas no difference was observed on globulin fractions ($P > .05$). In particular, a significant gradual increase of total proteins and albumin levels was found in groups A and B from T1.1 until T2 followed by a slight decrease until T4 and by a subsequent increase at T5.

The statistically significant effect of training schedules was observed on total proteins ($P < .05$) and albumin ($P < .01$). Higher total proteins values were found in groups A compared with groups C and B1 at T2 and T5, and in group B as compared with groups C and B1 at T5. Albumin concentration was higher in group A as compared with groups C and B1 at T1.3 and T2, and in group B as compared with groups C and B1 at T2. No statistical modifications were found on globulin levels and A/G ratio ($P > .05$) among groups (Fig. 1).

4. Discussion

Serum total proteins and their fractions in all the data points tested in the present study were within the physiological range suggested for horses [23].

According to other authors [22,24,25], all serum protein electrophoretograms were characterized by the absence of a prealbumin region and by six different bands, albumin, α1, α2, β1, β2, and γ-globulins.