



Original Research

Influence of Marcha Exercise on the Serum Concentrations of Acute-Phase Proteins in Mangalarga Marchador Horses

Paula Alessandra Di Filippo ^{a, *}, Laura Pereira Martins ^a, Marcos Aurélio Dias Meireles ^a, Célia Raquel Quirino ^a, Andressa Francisca da Silva Nogueira ^b, Paulo Moreira Bogossian ^c

^a Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, Rio de Janeiro, Brazil

^b Universidade Federal do Piauí, UFP, Bom Jesus, Piauí, Brazil

^c Universidade de São Paulo, USP, Butantã, São Paulo, Brazil

ARTICLE INFO

Article history:

Received 6 October 2017

Received in revised form

16 January 2018

Accepted 23 January 2018

Available online 13 February 2018

Keywords:

Biomarker

Exercise physiology

Inflammation

SDS-polyacrylamide gel electrophoresis

Stress

ABSTRACT

The acute-phase response (APR) is a rapid, nonspecific, systemic response occurring secondary to skeletal muscle damage and might be a protective physiological mechanism. The response has a number of components including increases in serum concentrations of the acute-phase proteins (APPs), which are primarily produced in the liver. This study was designed to evaluate the influence of marcha gait exercise on serum concentrations of the immunoglobulin A (IgA), immunoglobulin G (IgG), ceruloplasmin, transferrin, albumin (Alb), α_1 -antitrypsin, haptoglobin (Hp), and α_1 -acid glycoprotein in 35 Mangalarga Marchador horses (18 geldings and 17 mares). The study also aimed to evaluate the differences between genders of the response to marcha gait exercise. Blood samples were assessed before and after official marcha contest. Acute-phase proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The results were submitted to analysis of variance using the SAS statistical program, and means were compared by Student-Newman-Keuls test ($P < .05$). In both geldings and mares, APP concentrations did not increase in response to exercise. Significant differences of total serum protein concentration, Alb, Hp, IgA, and IgG between geldings and mares were noticed. In conclusion, this study provides evidence that marcha gait exercise performed during official contest is not intense enough to stimulate an APR, demonstrated by no change in serum concentrations of APPs in Mangalarga Marchador horses. The differences between genders in the present study warrant further investigation.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

The acute-phase response (APR) is a common reaction to a range of threats to homeostasis, including bacterial infection, surgery, immunological disorders, stress, neoplasia, tissue damage, and inflammatory diseases [1–4]. The APR is characterized by high temperature, endocrine changes, increased protein catabolism, and

negative nitrogen balance. Moreover, APR is related to changes of lipid metabolism, serum concentrations of cations, iron metabolism, and increased leukocytes release from bone marrow and lymphokine-mediated function, complement activation, and increases in plasma levels of acute-phase proteins (APPs) [5–7]. All these reactions appear to be beneficial with regard to host survival by increasing resistance to infection, altering metabolism to promote wound repair, and activating both cellular and immune reactivity [8].

Several studies have provided further evidence that the APR to exercise is analogous to that which occurs in general medical and surgical conditions [8–12], but this response should not be confused with indicators of disease [13]. Furthermore, others studies have reported that moderate physical exercise is beneficial to health, whereas strenuous exercise can induce an inflammation-like state [14,15].

Understanding the effects of physical exercise on inflammatory response is important for the development of strategies to

Animal welfare/ethical statement: This study was approved by the Animal Ethics Committee of Darcy Ribeiro State University of Northern Rio de Janeiro (protocol number 2014/250).

Conflict of interest statement: The authors declare no competing interests. None of the authors has any financial or personal relationships with other people or organizations that could inappropriately influence or be perceived to influence the content of the paper.

* Corresponding author at: Paula Alessandra Di Filippo, Avenida Alberto Lamego nº 2000, 28013-602, Campos dos Goytacazes, Rio de Janeiro, Brazil.

E-mail address: pdf@uenf.br (P.A. Di Filippo).

modulate inflammation and can prove increasingly useful for the future health and welfare of animals [1,2,16]. In addition, the knowledge of the extent and nature of the APR to various types of exercise is important, as changes related to the response may need to be taken into account for interpretation of hematological and biochemical measurements made during and after exercise or competition [9]. To the best of our knowledge, there are no published data concerning APR during marcha competition in Mangalarga Marchador horses.

The marcha contest is a functional evaluation of Mangalarga Marchador horses, unique in the world, which can be defined as a long-duration exercise with great energy expense, in which the animal develops, in circle, a long course without rest and in constant and excessive speed [17]. The marcha is a comfortable four-beat lateral and diagonal gait with moments of triple support and no suspension [18]. Therefore, we hypothesized that marcha competition would induce an APR in healthy Mangalarga Marchador horses and sought to determine whether the response differs between genders. To test this hypothesis, serum concentrations of APPs (immunoglobulin A [IgA], immunoglobulin G [IgG], ceruloplasmin [Cp], transferrin [Tf], albumin [Alb], α_1 -antitrypsin [Ant], haptoglobin [Hp], and glycoprotein [Gp]) were measured before and after official marcha contest in male and female Mangalarga Marchador horses.

2. Material and Methods

2.1. Horses and Competition

Thirty-five clinically healthy Mangalarga Marchador horses (18 geldings and 17 mares), with average age of 4 ± 2 years and weighing 399 ± 45 kg, were included in the study. Evaluations were carried out during an official marcha contest in a rodeo park in the city of Campos dos Goytacazes, Rio de Janeiro, Brazil ($20^\circ 48' 21''$ south latitude, $40^\circ 38' 52''$ west longitude, and altitude 13 m). The mean temperature during the competitions was 24°C , and relative humidity was 80%. Competitions were held in the evening and involved batida gait at 2.5–3.3 m/s during 45 minutes (22.5 minutes on reach rein), covering roughly 6–7 km. The animals were selected on the basis of clinical examination performed by qualified veterinarian to exclude pathological conditions. All animals were considered healthy in the clinical examination and had no history of previous diseases and/or application of medications. The animals were transported to the park on roads and covered a maximum distance of 100 km. Before the competitions, the animals remained in rest for 24 hours.

2.2. Blood Sampling

Five milliliters of blood were drawn from the jugular vein of each horse using evacuated collection tubes with no additive (Vacutainer; Becton Dickinson, Ribeirão Preto, SP). Blood samples were collected under informed consent of the owners at rest (24 hours), 2 hours before competition (T0) and 2 hours after completion of the marcha contest (T1). Within 2 hours of collection, blood samples were centrifuged at 4,380 g for 5 minutes, and the serum was aspirated, immediately frozen, and stored at -20°C until being analyzed.

2.3. Total Serum Protein and Acute-Phase Protein Assays

Total protein levels were determined by the Biuret method (Labquest; CELM-E-225-D, BR), and the proteinogram were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The electrophoretic fractionation was performed by a

unidimensional electrophoresis system on 4%–10% gradient acrylamide gels. The separating gel consisted of 15 mL of distilled water, 3 mL of 2M Tris 1M glycine (pH 9.0), 7.5 mL of 30% acrylamide, 1.5 mL of 2% bis acrylamide, 1.5 mL of glycerol, 0.6 mL of 0.5 M ethylenediaminetetraacetic acid (pH 8.3), and 0.6 mL of 20% SDS. It was polymerized by adding 15 mL of tetramethylethylenediamine and 0.3 mL of 10% ammonium persulfate. The stacking gel was polymerized by adding 20 mL of tetramethylethylenediamine and 0.1 mL of 10% ammonium persulfate. The upper electrode buffer contained 30 mM Tris base, 150 mM glycine, 0.1% SDS, and sufficient water to make 1 L. The lower buffer contained 75 mM glycine, 0.1 SDS, and sufficient water to make 1 L. Both buffers were adjusted to pH 8.5. The serum samples (5 mL) were prepared in 40 mL of Dulbecco phosphate-buffered saline solution and 10 mL of gel mix (10% water, 2% SDS, 5% 2-mercaptoethanol, 10 mM ethylenediaminetetraacetic acid, 20 mM Tris phosphate [pH 7.4], 5% glycerol, and 0.001% bromophenol blue as dye). The electric current for the 8×8 -inch vertical gel electrophoresis system was programmed at 35 and 50 mA, while samples were in the stacking and running gel, respectively. After fractionation, the gel was stained in 0.2% Coomassie brilliant blue solution for 10 minutes. Next, the gel was destained in a solution containing 250 mL of methanol, 100 mL of acetic acid, and 650 mL of water until protein fractions appeared clear. The concentrations of these protein fractions were determined using a digital densitometer (Shimadzu 9301PC, Tokyo, Japan). Proteins were identified through reference markers (Sigma Chemical Co, St Louis, MO) with molecular weights of 29, 45, 66, 97.4, 116, and 205 kDa. Also, electrophoretic migration of proteins was compared with that of pure proteins (Sigma Chemical Co, St Louis, MO) including Alb, Tf, Hp, Cp, IgA, IgG, Ant, and acidic Gp.

2.4. Statistical Analysis

Analysis of variance (Proc GLM) was realized with a model, including the fixed effect of time, sex, and the simple interactions. Data were expressed as the mean \pm standard deviation. The means were compared by the Student-Newman-Keuls test of the SAS (SAS Institute Inc, Cary, NC, 2012). Differences were considered significant with $P < .05$.

3. Results

The results are summarized in Table 1. The concentrations of total serum protein and APPs determined before the contest were similar to the values reported previously by other authors in healthy horses [12]. In both geldings and mares, APPs concentrations did not change in response to exercise.

Significant differences in the concentrations of total serum protein, Alb, Hp, IgA, and IgG between genders were noted.

4. Discussion

This is the first study evaluating the effect of marcha exercise on the APR of Mangalarga Marchador horses. A reaction to physical exercise analogous to the APR observed in inflammatory conditions has been reported in many species, including equines [9,15,19–21]. In the present study, the marcha exercise did not trigger an APR because the levels of APPs (Alb, Tf, Hp, Cp, IgA, IgG, Ant, and Gp acid) remained unchanged. Likewise, a previous study demonstrated that after the end of limited-distance and long-distance endurance rides (34 and 60 km), the concentrations of APPs did not change significantly [22]. The physical effort of racing for 1,600–2,500 m also was not able to cause alterations in the levels of acute-inflammation markers in Standardbred horses [23] and Standardbred trotters [24].

Download English Version:

<https://daneshyari.com/en/article/8483188>

Download Persian Version:

<https://daneshyari.com/article/8483188>

[Daneshyari.com](https://daneshyari.com)