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Original Research

Racing Induces Changes in the Blood Concentration of Serum Amyloid A in Thoroughbred Racehorses

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ABSTRACT

Intensive exercise results in the increased blood concentration of the acute phase proteins in horses competing in some sport disciplines. In this study, the blood level of serum amyloid A (SAA) was analyzed in Thoroughbred racehorses during 5 days after completion of the race. Samples were collected from 25 healthy Thoroughbred horses beginning with 4 hours after the race and repeated daily up to the fifth day after the race. Serum amyloid A analysis was performed using commercial enzyme-linked immunosorbent assay kit, and the results were presented as median, interquartile range (IQR), and range. Data were analyzed using Friedman's nonparametric analysis of variance. The acute phase response (APR) was reflected by an increased SAA level after the race, reaching significantly higher concentrations on days 1 ($P < .001$) and 2 ($P = .005$) and falling below the level of the first sample on day 5 ($P = .006$). The median peak concentration observed on day 1 after the race was 3.84 mg/L (IQR, 2.32 to 8.86). Racing induces minute changes in SAA concentration typical for the exercise-induced APR; however, the significance of this reaction in the context of horse health and fitness remains unclear.

1. Introduction

Strenuous exercise causes the release of the proinflammatory agents such as cytokines, prostaglandins, and acute phase proteins (APPs) into the bloodstream in horses, dogs, and humans [1–7]. Acute phase proteins are a group of blood proteins whose concentrations decrease or increase in animals subjected to external or internal challenges. The origin of acute phase response (APR) can be attributable to infection, inflammation, surgical trauma, or other causes and the purpose of the response is to restore homeostasis and to remove the cause of its disturbance [8]. The main APP in horses is serum amyloid A (SAA), the blood protein whose concentration can increase up to a 1,000 times in response to tissue-damaging factors [9–15]. Owing to its fast and explicit reaction to inflammatory stimuli, SAA is considered a reliable tool for health assessment in horses, more sensitive than the total white blood cell count [16]. Recognizing the effect of different types of exercise on the SAA is extremely important for the correct analysis of this biomarker, especially in sport horses regularly subjected to training.

The first reports on changes in SAA blood concentration induced by heavy exercise came from endurance horses participating in the long distance rides (120 and 160 km) [5]. These findings stood in line with observation previously made in human ultramarathon and triathlon competitors [1,2]. Moreover, high-intensity exercise of moderate length, such as sled racing in Alaskan dogs and 2-day eventing in sport horses, did also result in minor but significant increase of the C-reactive protein and SAA,

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respectively [4,17]. Occurrence of a similar SAA reaction has not been confirmed in racehorses after training or racing [18,19], even though elevated expression of the proinflammatory cytokine genes was reported in 2-year-old Thoroughbred racehorses after the training session [6]. The results of our preliminary study [20] suggested that the change in SAA level in Thoroughbred racehorses can be detected in 24 to 48 hours after the race and, therefore, could not have been observed in the studies examining SAA level immediately after exercise [18,19]. However, the other study comparing the concentrations of APPs in standardbred trotters before and 2 days after the race did not indicate the SAA response [21].

In this study, we aimed to test the hypothesis that racing induces changes in blood SAA concentration in Thoroughbred racehorses within 5 days after completion of the race. Determining the exact time frame of this reaction could facilitate the interpretation of control SAA measurements in racehorses during the racing season.

2. Materials and Methods

Twenty five privately owned Thoroughbred racehorses aged 2 to 6 years (mean, 2.8 years) stabled in one horse racing facility were used in this study. All horses were trained and competed in flat racing throughout the whole racing season (April to November). The study was performed on the last day of the season, in a dry, cold weather (environmental temperature 3°C), with track elasticity rate 3.8 (in a 1- to 5-point scale). Racing distances varied from 1,200 to 1,600 m and were selected appropriately to the age and fitness of the horse by the horse trainers. Collection of blood samples was approved by the Local Ethic Committee, the trainers, and the owners of the horses. Samples were collected by jugular venipuncture into tubes with no additives (Vacutainer Systems; Becton Dickinson, France) starting 4 hours after completion of the race (day 0). The procedure was repeated daily for the next five consecutive days. In this period, horses were subjected to light exercise in the horse walker. With each sample collection, horses underwent thorough clinical examination performed by the equine veterinarian. All horses recovered well after the race, and none of them showed clinical signs of disease or injury during the time of the study. Blood samples were transported immediately after collection to the laboratory, where they were centrifuged at 4,380*g* for 5 minutes. The collected serum was divided in aliquots, frozen, and stored at −20°C for SAA analysis. Serum amyloid A concentration was measured with the commercial enzyme-linked immunosorbent assay kit (PHASE SAA; Tridelta Ltd, Ireland) previously validated for use in the equine studies [5,11,17,19,20]. The analysis was performed using Friedman's nonparametric analysis of variance. Changes of SAA concentration compared to the first measurement 4 hours after the race were evaluated using Wilcoxon signed-rank tests with a Bonferroni correction applied (given that five mutual comparisons were performed; *P* value from each pairwise comparison was multiplied by five to control for the increased possibility of type I error associated with the multiple comparisons). An overall alfa level of 0.05 was assumed. The analysis was carried out in Statistica 10 (StatSoft Inc).

3. Results

The results were reported as median, interquartile range, and range (i.e., min–max). The significant changes in the mean SAA concentration were noted in the first 5 days after the race ($\chi^2(5) = 71.5$, $P < .001$). Compared with the first measurement obtained 4 hours after the effort, the serum SAA level increased on the day 1 ($P < .001$) and was still significantly higher on the day 2 ($P = .005$), then returned to the level comparable with the initial level on the days 3 ($P = .999$) and 4 ($P = .262$) and fell below the initial level on the day 5 ($P = .006$; Fig. 1). The median peak SAA concentration was observed at day 1 (3.84 mg/L [2.32–8.86]) with the highest individual SAA concentration of 10.15 mg/L.

4. Discussion

The change between the highest and the lowest median SAA concentration, observed in horses on days 1 and 5 after race, was approximately sevenfold. The highest individual concentration was still relatively low comparing with those reported in horses with acute inflammatory diseases of respiratory or gastrointestinal system [13–15]. Such small changes are not likely to be of much relevance in the assessment of horse health based on the interpretation of serum SAA level. The SAA concentration range in healthy horses may differ depending on the population and the method used [5,11,13–15,17,20,22]. The highest SAA level reported of healthy horses was 20 mg/L [22]. The post-exercise changes in horses described in the recent and the previous studies remain far below this level and could not be interpreted as indicative of serious health conditions.

The similar minute difference has been previously reported in endurance and eventing horses tested for SAA concentration before and shortly after the competition or training [5,17,19]. The limitation of our study is the lack of

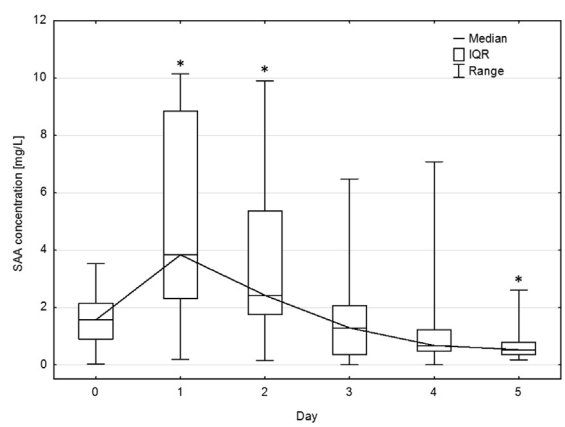

Fig. 1. Median concentration of serum amyloid A in 5 days after the race (boxes stand for the interquartile range (IQR), and whiskers stand for the range). *SAA concentrations significantly different from the baseline ($P < .05$). SAA, serum amyloid A.