Journal of Equine Veterinary Science 42 (2016) 1–6

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

The Effect of Aging on Biochemical Markers in Equine Serum

Mariko Mochizuki [a,*], Fumiko Minowa [b], Chikako Ishimoto [c], Azusa Gin [d], Katsumi Ishioka [d], Kimihiro Okubo [e]

[a] Department of Applied Science, School of Veterinary Nursing and Technology, Nippon Veterinary and Life Science University, Musashino, Tokyo, Japan
[b] Minowa Horse Clinic, Oota, Tokyo, Japan
[c] Shizuoka Veterinary Medical Association, Kikugawa, Shizuoka, Japan
[d] Department of Veterinary Nursing, School of Veterinary Nursing and Technology, Nippon Veterinary and Life Science University, Musashino, Tokyo, Japan
[e] Department of Otolaryngology, Graduate School of Medicine, Nippon Medical School, Tokyo, Japan

ARTICLE INFO

Article history:
Received 12 January 2016
Received in revised form 29 March 2016
Accepted 29 March 2016
Available online 4 April 2016

Keywords:
Aging
Pony
Thoroughbred
Triglyceride
Oxidative stress

ABSTRACT

In this study, we investigated the effect of aging on biochemical markers in serum obtained from Thoroughbred horses (n = 30) and ponies (n = 15). Aging was observed to have an effect on albumin, total bilirubin, thyroxine, and iron levels. The triglyceride concentration in ponies was found to be significantly higher than in Thoroughbreds. Furthermore, an age-related increase in triglycerides was only observed in ponies. There was no aging effect on diacron-reactive oxygen metabolites and biological antioxidant potential concentration. A significant negative correlation between age and bilirubin, known as a strong antioxidative substance, was obtained in both the Thoroughbred and pony groups. However, the distribution area of data was different due to higher bilirubin concentrations in Thoroughbreds. The serum iron levels were correlated with serum triglyceride levels in ponies. We propose that the metabolism of ponies differs from that of Thoroughbreds and that careful breeding and appropriate exercise are required for aging ponies.

1. Introduction

There are many interesting studies into disease status, nutrition management, and exercise regimens for aged horses because there are many owners with aging horses. For example, McKeever et al [1] suggested that thermoregulation and cardiovascular function in horses are decreased by aging. In a study of endocrine responses to exercise in old horses, a significant difference ($P < .05$) between older horses and younger horses was suggested from several data such as plasma aldosterone and renin [2]. Ralston et al [3] suggested that mean corpuscular volume in aging horses (n = 14, ≥20 years old) was greater ($P < .01$) than that in young horses (n = 12, ≤5 years old). The importance of training for reversing aging effects was also suggested in a study investigating maximal heart rate [4]. The importance of exercise for older horses was also summarized in a thorough review [5]. We also found an interesting study on the effect of aging on biochemical markers in the serum of pure-bred Arabian mares in Turkey [6]. In this study, 11 biochemical parameters and four serum mineral levels were reported. Age-related differences were suggested for most of the parameters such as bilirubin, total protein, and calcium. However, when compared to studies in Europe and the United States, studies in Japan were thought to be centered on racehorses. Thus, we believed that it was also important to investigate riding horses, including ponies, in Japan.

Oxidative stress (OS) and antioxidant status of horses has been evaluated using several markers. Smarsh et al [7] suggested that a single dose of various nutraceuticals such

* Corresponding author at: Mariko Mochizuki, Department of Applied Science, School of Veterinary Nursing, Nippon Veterinary and Life Science University, 1-7-1 Kyounan, Musashino, Tokyo 180-8602, Japan.
E-mail address: m-mochi@nvlu.ac.jp (M. Mochizuki).

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http://dx.doi.org/10.1016/j.jevs.2016.03.011

as black tea, cranberry, orange peel, and ginger extract does not produce an effect on either OS or antioxidant status. Andriichuk et al [8] suggested that OS in horses is decreased by exercise. A relationship between oral vitamin E supplementation and OS has also been reported [9]. Recently, the development of an instrument to easily analyze OS levels (diacron-reactive oxygen metabolites [d-ROMs]) and biological antioxidant potential (BAP) has led to an increase in studies describing OS and antioxidants in animals. In addition, there are studies related to training [10] and reproduction [11] in horses as well as interesting studies related to equine diseases. For example, OS is thought to play a role in various equine respiratory diseases, and higher d-ROM levels were found in horses diagnosed with *Rhodococcus equi* infection [12] and pneumonia [13]. We sought to investigate the relationship between age and several serum biochemical markers including d-ROMs and BAP concentration.

2. Materials and Methods

2.1. Animals and Sample Preparation

Blood samples were collected from 45 animals at the equestrian club of Shizuoka Prefecture in Japan on August 19, 2015. The animals were classified into two groups of Thoroughbreds (gelding/female: n = 24/6) or ponies. Three different species of pony were investigated, including Haflinger (n = 1/0), Welsh Pony (n = 2/1), and Welsh part-bred (n = 1/7, male; n = 3). Although the horses were fed a hay diet, hay cubes, and various pellets, the type and dosage of feed differed depending on the horse's age, exercise regimen, and constitution. The animals were given drinking water ad libitum. On the day of sampling, animals were fed at 6:00 and then pastured between 7:30 and 10:00. Sampling was conducted between 10:30 and 12:00. Because the equestrian club opened at 13:00, the animals were not used for horseback riding in the morning. Animals were divided into two groups for this study, and blood samples were randomly collected to avoid the effect of factors such as breed and age. The study was conducted following the ethical policies of experimental animals of Nippon Veterinary and Life Science University (approval number, 27S-8).

Approximately 20 mL of blood was collected from horses and ponies into vacuum blood collection tubes (Venoject II, Terumo Corporation, Tokyo, Japan) and transported the same day to Shizuoka Institute of Science and Technology on ice. The samples were centrifuged at 3,000 rpm for 15 minutes, and then, sera were transferred into Eppendorf tubes and transported to our laboratory on ice. The transported samples were stored at −30°C until analysis.

2.2. Sample Analysis

The analysis of d-ROMs and BAP was carried out using a free radical analyzer (Free Carpe Diem, Diacron International srl., Grosseto, Italy). Frozen samples were transported to the laboratory for analysis (Monolis Inc, Tokyo, Japan), and biochemical markers were then analyzed. The concentrations of total protein, albumin, total bile acid, lipase, blood urea nitrogen (BUN), creatinine, triglyceride, total cholesterol, total bilirubin, glucose, amylase, magnesium (Mg), iron (Fe), copper (Cu), and zinc (Zn) were analyzed using an auto-analyzer (JCA-BM2250, JEOL Ltd, Tokyo, Japan). The concentrations of thyroxine (T4) and cortisol were analyzed using a CLEIA method (Immulyze 2000, Siemens Healthcare GmbH, Erlangen, Germany).

2.3. Statistical Analysis

The levels of biochemical markers in serum are represented as means ± standard error of the mean, and statistical comparisons were performed using a Mann–Whitney *U* test. Significance of the correlation was tested by Spearman's rank-correlation coefficient. The equality of variance was tested by Levine's test for equality of variance. Analyses were performed using Japanese language version software, Lotus 2001 (Lotus Development Corp, Tokyo, Japan), Excel 2010 (Microsoft Japan Co, Ltd, Tokyo, Japan), and SPSS statistics 19 (SAS Institute Inc, Tokyo, Japan).

3. Results

3.1. Comparison of Biochemical Markers in Thoroughbreds and Ponies

A comparison of biochemical markers in the Thoroughbred and pony groups is shown in Table 1. The levels of triglyceride ($P < .01$) and Fe ($P < .05$) in the pony group were significantly higher than those in the Thoroughbred group. In contrast, total bilirubin ($P < .001$), amylase ($P < .01$), and BUN ($P < .01$) levels in the Thoroughbred group were significantly higher than in the pony group.

3.2. The Effect of Aging on Various Biochemical Markers

A significant decrease associated with age was also observed in the bilirubin concentration in serum ($r = -0.402$, $P < .01$) (Table 1). However, the distribution area between Thoroughbred ($r = -0.644$, $P < .01$) and pony ($r = -0.582$, $P < .05$) groups was markedly different due to a significantly higher bilirubin concentration in Thoroughbreds (Fig. 1). The difference between Fe levels in the Thoroughbred and pony groups was also investigated. Although there was a significant correlation between serum Fe concentration and age ($r = 0.544$, $P < .01$), the distribution between the Thoroughbred ($r = 0.439$, $P < .05$) and pony ($r = 0.733$, $P < .01$) groups was different due to a higher Fe concentration in ponies (data not shown). On the other hand, a significant increase in lipase ($r = 0.570$, $P < .01$) was observed (Thoroughbred, $r = 0.540$, $P < .01$; pony, $r = 0.557$, $P < .05$) (Fig. 2). Although there was no effect of aging on triglyceride concentration in serum from the Thoroughbred group ($r = -0.022$, $P = .906$), an increased triglyceride concentration ($r = 0.764$, $P < .001$) dependent on age was observed in pony serum (Fig. 3). In the pony group, triglyceride levels correlated with lipase ($r = 0.693$, $P < .01$) (Fig. 4) and Fe ($r = 0.640$, $P < .05$) concentrations (Fig. 5). Similar relationships were not observed for lipase ($r = 0.008$, $P = .968$) or Fe ($r = 0.201$, $P = .288$) in Thoroughbreds. Although