Serum Zinc Levels and Their Relationship with Diseases in Racehorses

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(Received 18 March 2012/Accepted 10 August 2012/Published online in J-STAGE 28 August 2012)

ABSTRACT. Zinc is one of the essential microelements involved in the regulation of enzyme activity, as well as metabolism of nucleic acid and proteins. There have been few reports on equine serum zinc concentrations during the training period, and little is known about the relationship between serum zinc levels and diseases in horses. In this study, we measured serum zinc levels in healthy Thoroughbred racehorses, as well as in other horses, under general disease or training conditions. The reference value for serum zinc levels in Thoroughbred horses was 41–79 µg/dl. There were no differences in serum zinc levels due to sex or age. Significant decreases in serum zinc levels were observed after training, but serum zinc levels did not vary with intensity of sweating. Serum zinc levels were lower in horses clinically diagnosed as having shipping fever (36.3 ± 2.7 µg/dl), fever (45.3 ± 3.0 µg/dl) and cellulitis (44.0 ± 3.4 µg/dl), as compared to control values (59.7 ± 9.7 µg/dl). They also tended to decrease in experimentally infected horses one day after inoculation. Changes in serum zinc levels reached nadir one day after surgical invasion, except for a horse that experienced complicating shock. These results suggest that zinc is a serological indicator of inflammatory status in Thoroughbred horses.

KEY WORDS: exercise, inflammation, racehorse, sweat, zinc.

Zinc is known to be an important factor in the regulation of more than 200 enzymes in vivo, and is associated with various functions, including fetal development, growth, tissue repair, ossification, reproductive system, appetite and the immune system [30]. The main zinc deficiency disorders in humans include taste and dermatological disorders, as well as failure to thrive [28]. There are many factors that have an influence on zinc metabolism. Yanagisawa summarized the major causes of zinc deficiency as follows: inadequate intake (low zinc-containing diets, intravenous alimentation, etc.); malabsorption (malabsorption syndrome, drugs with chelating activity, etc.); excess loss (loss into digestive fluid with diarrhea, increased urinary elimination with diabetes mellitus, renal disease, enhanced catabolism, etc.); increased demand (pregnancy, enhanced anabolism, etc.); and unexplained [38].

The conventional procedure for measuring zinc levels (atom absorption method) is complex and difficult to apply in clinical settings. However, the relationship between serum zinc levels and hepatic failure [22, 35], gastric mucosal disorder [25], chronic renal failure [20] and diabetes [8] has recently been clarified by the development of a simple automated biochemical method.

In horses, associations between zinc deficiency and anorexia, reduced growth rate, parakeratosis, alopecia [11], osteochondrosis [10] and white line disease [12] have been reported. Previous studies have reported various equine reference values, including 111.2 ± 45.8 µg/dl on pasture and 170.0 ± 52.3 µg/dl in stable [34], 47 ± 11 µg/dl on pasture and 47 ± 9 µg/dl in stable [6], 58.8–66.0 µg/dl on pasture [18], 65.4 ± 9.8 µg/dl [33], 46.0–59.0 µg/dl [5] and 42.0–87.0 µg/dl [21]. These results suggest large differences in horse management. In addition, it is known that horses produce great quantities of sweat, which contains large amounts of minerals, during exercise [15], but there have been few reports on serum zinc levels in Thoroughbred racehorses, and associations with disease are presently unknown.

In this study, we examined: 1) reference zinc values in racehorses; 2) the influences of exercise intensity and degree of hidropoiesis on the clinical application of zinc testing; and 3) the relationship between serum zinc levels and various disease states.

MATERIALS AND METHODS

Samples from healthy horses: In order to determine the reference values for serum zinc levels in Thoroughbred horses, 210 clinically healthy Thoroughbreds (120 males, 7 geldings and 83 females) under rest conditions in February 2008 at the Japan Racing Association (JRA) Miho training center were used in this study. Horses were also...
divided by age (2 in the 2-year-old group, 105 in the 3-year-old group, 50 in the 4-year-old group, 24 in the 5-year-old group, 16 in the 6-year-old group, 8 in the 7-year-old group, 2 in the 8-year-old group, 2 in the 9-year-old group and 1 in the 11-year-old group) in order to assess age-related differences. Blood was collected from the jugular vein at 10:00 a.m. In addition, to compare the effects of training stage, 391 healthy Thoroughbred racehorses belonging to 24 stables at the JRA Miho training center in May 2009 were used. We conducted a survey by recording the training intensity (1, walk or trot; 2, canter; 3, gallop) and the degree of sweating (1, no sweating; 2, sweating only under the saddle; 3, sweating from head to hip; 4, sweat dripping from abdomen).

Between July and September 2008, we obtained serum from disease-affected horses (16 with shipping fever, 13 with fever, 10 with cellulitis and 5 with tying-up syndrome), and from horses before and after undergoing surgery with general anesthesia (4 undergoing arthroscopy, 2 undergoing celiotomy and 1 undergoing castration) at the JRA Miho training center. In addition, we obtained serial serum samples collected from 6 Thoroughbred horses before and after experimental infection with Streptococcus equi subsp. zooepidemicus in the lung lobe as part of another research project [14] that approved by Animal Use and Care Committee and Animal Welfare and Ethics Committee of the Japan Racing Association’s Equine Research Institute. Concentrations of traditional inflammation markers in horses such as serum amyloid A (SAA) and fibrinogen (Fbg) in these serial serum samples were already reported by Hobo et al. [14].

Measurement of serum zinc: Zinc was measured using the “Acurus Auto Zn” (Shino-Test Corporation, Tokyo, Japan), which is based on the colorimetric method and utilizes an automated analyzer (Hitachi 7700 series; Hitachi High-Technology Corporation, Tokyo, Japan). Figurashi et al. reported that “Acurus Auto Zn” does not require a deproteinization procedure, and shows good reproducibility and good correlativity with the atomic absorption method [13].

Statistical analysis: All data are shown as means ± SD. Reference values were calculated as means ± 1.96 SD. Results were subjected to one-way ANOVA to estimate the effects of age, sex, amount of sweat and exercise intensity. Following significant effects on ANOVA, the significance of differences between groups was determined by a post-hoc Turkey-Kramer HSD test. Dunnett’s test was also used to compare disease groups with controls, and post-exercise, post-surgery or post-infection challenge with baseline values. All data were analyzed using the computer software package JMP (SAS Institute Inc., Cary, NC, U.S.A.). Values of P<0.05 were considered to be significant.

RESULTS

Reference values: Serum zinc levels were 59.7 ± 9.7 µg/dl (n=391) in Thoroughbred racing horses, and the reference value was estimated to be 41–79 µg/dl. There were no significant differences in serum zinc levels among males (60.28 ± 10.13 µg/dl), geldings (62.86 ± 5.93 µg/dl) or females (58.64 ± 9.36 µg/dl). There were also no differences in zinc levels based on age: 2-year-old group, 55.5 ± 7.8 µg/dl; 3-year-old group, 60.6 ± 9.5 µg/dl; 4-year-old group, 60.3 ± 10.3 µg/dl; 5-year-old group, 56.0 ± 7.6 µg/dl; 6-year-old group, 57.0 ± 11.9 µg/dl; 7-year-old group, 57.3 ± 9.3 µg/dl; 8-year-old group, 67.0 ± 5.7 µg/dl; 9-year-old group, 66.0 ± 11.3 µg/dl; 11-year-old group, 68.0 µg/dl.

Effects of exercise and sweating: Serum zinc levels in exercise group 1 were 61.6 ± 7.2 µg/dl, in group 2 were 59.6 ± 6.7 µg/dl and in group 3 were 55.0 ± 8.0 µg/dl. There was a significant difference in zinc levels between groups 3 and 1 (P<0.01) and between groups 3 and 2 (P<0.01), but no significant difference between groups 1 and 2. On the other hand, there were no significant differences in serum zinc levels among the sweat categories (1, 2 and 3); 60.9 ± 6.8 µg/dl in group 1, 59.8 ± 7.3 µg/dl in group 2, and 59.9 ± 7.4 µg/dl in group 3.

Relationship between zinc levels and disease: Serum zinc levels were significantly lower in horses affected by shipping fever (36.3 ± 2.7 µg/dl), fever (45.3 ± 3.0 µg/dl) and cellulitis (44.0 ± 3.4 µg/dl), as compared with control horses (59.7 ± 9.7 µg/dl) (P<0.01) (Fig. 1). However, there were no differences between horses with tying-up syndrome (65.7 ± 3.4 µg/dl) and controls.

There were no significant differences between zinc levels pre- and post-surgery with general anesthesia. However, all serum zinc levels decreased on the day after surgery, and then rose to normal by the second day, except in one case that experienced postoperative shock (Fig. 2).

In the pneumonia infection model, serum zinc levels showed a sharp decrease on the day after bacterial inoculation (P<0.01), followed by a gradual rise to normal levels. There were no significant differences in zinc levels at any time points (before inoculation, on the day of inoculation, and at 2–6 days after infection) (Fig. 3). In contrast to these changes in serum zinc level, the traditional inflammation markers such as SAA and Fbg increased gradually, peaking at day 4.

DISCUSSION

Zinc is absorbed in the small intestine. Many feedstuffs contain 15–40 mg/kg DM, and the digestibility was calculated to be 20.8% in horses [26]. Inorganic salts possess a higher availability than phytate salts of zinc in cereal grains and oilseed meals. The zinc requirement is 461 mg/day in exercised horses, and 274 mg/day in sedentary horses, as digestibility in sedentary horses decreases from 25 to 14% during exercise [16]. The endogenous loss in Zn is between 50 and 70 mg/day for a 500–550-kg horse [16, 26] or 0.1–0.13 mg/kg BW daily. In humans, the reference value for serum zinc levels is reported to be 84–159 µg/dl [38] or 104 ± 14 µg/dl [23]. In addition, zinc levels are higher in males than in females, and decrease with age [27].

The present results showed reference values from 41 to 79 µg/dl in Thoroughbred racehorses. Previous reports have shown various serum zinc levels (mean ± SD), 111 ± 45 µg/dl (pasture), 170 ± 54 µg/dl (stable) in Thoroughbreds [34, 47 ± 11 µg/dl (pasture), 47 ± 9 µg/dl (stable) in Thorough-
breds [6], 65.4 ± 9.8 µg/dl in Icelandic horses [33], 58.8–66.0 µg/dl (pasture) in Icelandic horses [18], 46.0–59.0 µg/dl in Warmbloods [5] and 42.0–87.0 µg/dl in various breds [21].

One reason for this difference may be environmental differences, such as weather, feedstuffs, grazing times, exercise intensity or daily routine. Previous reports have shown significant differences between farms [18] and/or stables [34]. In this study, there was a significant difference in serum zinc levels only between the highest (65.5 ± 8.9 µg/dl) and lowest (54.9 ± 4.9 µg/dl) levels seen among the 24 stables. This suggests that the environment in the training center is similar among stables. In human athletes, blood zinc levels are lower due to increased zinc losses in sweat [7], and increased urinary excretion [29]. Although we could not conclude that there were no age differences, age and sex did not appear to significantly affect serum zinc in racehorses.

In this study, we were unable to demonstrate seasonal changes in serum zinc levels. Stubey et al. showed no significant differences between months in mean values of stabled Thoroughbreds [34]. Gromadzka and Biricik studied the seasonal changes in serum zinc in mares; Gromadzka reported high levels in autumn and winter in Shetland ponies [9], but Biricik reported high levels in summer, with no significant differences, and no relationship between seasonal changes and serum zinc (r=0.112) in Warmbloods [5]. Because the results of these reports differ, this suggest that these differences are not due to seasonal variations, but rather to changes in intake, probably resulting from changes in the composition of pasture grass or increased mineral dosage in late pregnancy. At training centers in Japan, horses are given little fresh grass, while concentrated feeds and hay are used throughout the year.

There were no differences based on sweating, but a significant difference was seen with exercise intensity. Inoue et al. reported that serum zinc levels temporarily increase during treadmill exercise, with levels returning to normal one hour after exercise [17]. Lukaski et al. reported that zinc levels are also elevated immediately after exercise in humans [19]. This increase is not considered to be due to hemoconcentra-

Fig. 1. Concentration of serum Zn in various diseases. The top and bottom of the boxes show the upper and lower quartiles, respectively. The band near the middle of the box shows the median. The ends of the whiskers show the maximum and minimum values for all the data. Values with asterisks are significantly different (* P<0.05, ** P<0.01) from the values in controls.

Fig. 2. Changes in serum Zn pre- and post-surgery. Although there were no significant differences between pre- and post-surgery zinc levels, all serum zinc levels were lower on day 1 and higher on day 2, except in one case, which experienced anesthetic shock on the same day.

Fig. 3. Changes in body temperature, SAA, Fbg and Zn in infection model. Data are expressed means ± SD. SAA and Fbg levels increased, while serum zinc levels decreased after inoculation. Peak values for each index were reached on the following days: day 4 for SAA; day 4 for Fbg; and day 1 for Zn. Values with asterisks are significantly different (* P<0.05, ** P<0.01) from the values before bacterial inoculation. The data of the body temperature and concentrations of serum amyloid A (SAA) and fibrinogen (Fbg) were quotations from the study reported by Hobo et al. [14].
increased in the strong exercise group is that exercise intensity was greater than with treadmill exercise (36 km/hr). In previous reports in humans, plasma zinc concentrations decreased after exercise, with the nadir observed at 3 hr [2], 5 days [32] or 70 min after exercise [37]. The disparity in results in the aforementioned studies may be due to variations in the timing of blood draws, fitness status of subjects, and exercise intensity, type and duration, as well as zinc status [37]. In this study, we cannot demonstrate the influence of exercise intensity, diurnal variation or feeding, because the time at which they trained or at which sampling after exercise was not equal. Therefore, exercise influences serum zinc levels, but the effect is small when compared to the reference value range, thus limiting the clinical importance.

Serum zinc levels in shipping fever, fever and cellulitis were lower than in control horses, but the mean was within the reference range. This is due to the large reference range resulting from the large individual differences in sound horses. Tomita et al. reported that some patients showed zinc deficiency symptoms that improved with zinc supplement intake, despite showing normal serum zinc levels, and suggested using a cutoff value as the diagnostic criterion in humans [36]. On the other hand, significant differences were not observed in tying-up syndrome and controls. Five horses with tying-up syndrome showed high creatinine kinase levels (10,506, 12,100, 13,140, 18,308 and 80,833 U/l). The collapse of muscle tissue is the main clinical pathogenesis in tying-up syndrome, but serum zinc did not vary with physiological response on first examination.

Oakes et al. reported that plasma zinc fell by 32 to 44% after elective knee arthroplasty in humans [24], while Auer et al. reported that plasma zinc decreased after localized tissue injury in horses [3]. In the present study, serum zinc levels decreased after surgical invasion, but we cannot exclude the effects of general anesthesia. In acute inflammation, serum zinc levels decrease due to redistribution of zinc from plasma to the liver and lymphocytes [4]. The mechanism by which serum zinc levels are later restored is unclear, but the possible cause of the elevation of serum zinc levels was not due to eating, but resulted from the inflow of zinc stored during inflammation, as in fasting cases after celiotomy, zinc levels were also elevated. A horse in which serum zinc was lower for more than 2 days after surgery developed serious shock symptoms on the same day. These results suggested that serum zinc is a useful marker in postoperative management.

In the infection model, serum zinc levels decreased on day 1. Subsequently, zinc levels gradually returned to pre-infection levels, together with the decrease in body temperature. Traditional inflammation markers, such as SAA and Fbg, increase gradually, peaking at day 4. In contrast, serum zinc levels decline and recover rapidly, with the nadir being observed on day 1. Serum zinc levels decrease due to tissue damage and infection [31]. Shenkin et al. suggested that the decrease in serum zinc is brought about by changes in the concentration of specific tissue proteins controlled by cytokines, particularly interleukin 1, tumor necrosis factor, and interleukin 6 [31]. These results suggest that serum zinc levels reflect the degree of inflammation in horses.

The present study confirmed reference values for serum zinc levels in Thoroughbred racehorses, and also demonstrated a clear decrease in serum zinc levels in horses affected by inflammatory disease, after invasive treatment with general anesthesia, and in bacterial challenge pneumonia models in Thoroughbreds. These results suggest that measurement of serum zinc levels is a potential marker for monitoring the inflammatory state of Thoroughbred racehorses. In addition, we found that serum zinc is more suitable as an index for serial changes in the same horses than evaluation at first examination, because intra-individual differences are large.

ACKNOWLEDGMENTS. We would like to thank Ms. Chieko Itoga, Ms. Harumi Kuno, Ms. Hiroyo Saito, Ms. Mizuho Kawarada and Ms. Naomi Tsukamoto for invaluable technical assistance.

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