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A POTENTIAL LINK BETWEEN INSULIN RESISTANCE AND IRON OVERLOAD DISORDER IN BROWSING RHINOCEROSES INVESTIGATED THROUGH THE USE OF AN EQUINE MODEL

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Abstract: Iron overload disorder afflicts captive rhinoceros but has not been documented in the wild. The specific cause for the disorder has not been identified but is likely associated with diet and management. Compared with wild counterparts, captive rhinoceros eat diets containing more iron, have greater fat stores, and exercise less. It has been suggested that the problem may be linked to development of insulin resistance in the captive population. Given that controlled experiments with sufficient numbers of rhinoceros are logistically not possible, an equine model was used to look for a relationship between iron status and insulin resistance; the nutritional requirements of horses are used as a guide for rhinoceros, because they have similar gastrointestinal tracts. Sixteen horses were tested to determine blood insulin responses to an oral drench of dextrose (0.25 g/kg bodyweight) and a meal of pelleted corn (1.5 g/kg bodyweight). Fasting blood samples were taken 30 and 0 min before administration. Further blood samples were taken every 30 min for 4 hr after administration to determine peak insulin and total area under the insulin curve (AUC). Fasting samples were tested for serum ferritin concentrations. Correlations were determined between ferritin and peak insulin concentrations and insulin AUC after administration of oral dextrose and pelleted corn. The strongest correlation was between ferritin and insulin AUC after dextrose administration (r=0.61; P=0.01) followed by AUC after feeding a meal of pelleted corn ($r = 0.60$; $P = 0.01$), with the correlation for peak insulin being 0.53 ($P = 0.03$) after dextrose administration and 0.56 ($P = 0.02$) after pelleted corn. When evaluating responses by gender, a significant correlation existed only for females, influenced by one insulin resistant individual. These data suggest a potential link between insulin resistance and body stores of iron and also suggest that approaches to reduce the susceptibility to insulin resistance should be incorporated into management of captive browsing rhinoceros.

Key words: Diceros bicornis, equine, ferritin, insulin, iron, rhinoceros.

INTRODUCTION

Browsing rhinoceros species kept in captivity may become iron overloaded, with such overload being associated with multiple clinical disorders.[17] Normal concentrations of iron in plants favored by wild black rhinos can be fairly low; one study reported a range from 9 to 13.6 ppm.[12] It has been speculated that that iron accumulation is an adaptation to a low-iron diet.[2] Excess iron accumulation is a health concern necessitating a search for ways to decrease iron intake or absorption or to deal with the associated health concerns.[4,8]

One concern with excess iron accumulation is the apparent link to insulin resistance. In humans, a positive correlation has been shown between serum iron and the insulin resistance index, as well as with blood glucose concentrations.[26] Researchers suggest that an increased intake of dietary iron is an important risk factor in the development of insulin resistance.[1,13,26] Researchers suggest that high insulin levels, as occur with insulin resistance, can contribute to iron overload by stimulating cellular iron uptake and upregulating ferritin synthesis, with serum ferritin considered to be an indirect measure of stored iron.[1,7,27] Iron can also contribute to insulin resistance by interfering with insulin's inhibition of glucose production by the liver, thus increasing serum glucose with a resultant raised blood insulin level.[6,15] The relationship between the accumulation of iron and insulin resistance reported in humans[9] may be present in rhinos. Insulin/glucose ratios were found to be greater in captive rhinos than in free-ranging ones,[23] and it is suggested that the management, husbandry, and diet of black rhinos in captivity may play a role in the development of insulin resistance.

Testing rhinos to determine if they are insulin resistant would be challenging due to the limited sample size available and the difficulties associated with performing an insulin response trial.[10] Hence, even though it is recognized that high

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ferritin concentrations are present in captive black rhinos,[8] an association with insulin resistance cannot be confirmed. Testing of horses to determine insulin resistance is routinely done, and its association with obesity and lack of exercise in horses is recognized.[11] Although insulin resistance is commonly found in horses, a relationship between insulin resistance and ferritin has not been previously explored. Thus, the hypothesis for this pilot study is that insulin concentrations following a glycemic challenge in horses are positively correlated to serum ferritin concentrations. Given that the established nutritional requirements of horses[16] are used as a guideline for rhinos due to their similar gastrointestinal tracts, the purpose of this study was to determine if an association between high ferritin concentrations and insulin resistance exists in horses, thus providing stronger evidence for a potential link between insulin resistance and iron overload in rhinos. If so, this knowledge could suggest approaches to manage insulin resistance in rhinos based on approaches used in horses, and thereby managing iron overload.

MATERIALS AND METHODS

Test subjects

Sixteen Arabian horses, specifically four young geldings (414 ± 3 kg; 28 ± 1 mo), four young fillies (397 ± 3 kg; 28 ± 1 mo), four mature geldings (480 ± 2 kg; 14.3 ± 0.5 yr), and four mature mares (466 ± 2 kg; 14.1 ± 0.5 yr), were maintained on pasture with access to a trace-mineralized salt block. Horses received light exercise for approximately 1 hr either through horsemanship classes (mature horses) or through training classes (young horses), 3–4 days per week for the duration of the study. Horses were supplemented with grass hay but received no concentrate to assure they were on what would be considered a low-glycemic–response diet.

Treatments and testing procedures

A control treatment consisted of dextrose administered at a dose of 0.25 g dextrose/kg of bodyweight in a 50% oral drench (with water). An exaggerated insulin response was expected from pelleted corn administered at the rate of 1.5 g/kg of bodyweight on an as-fed basis of their weight. Testing for blood insulin level response to treatment was separated by 1 wk with treatment randomized. The afternoon prior to testing, the horses were brought into stalls from pasture, weighed on a digital scale, and fed 0.5 % bodyweight in timothy hay. All hay provided was consumed promptly with no refusals. Horses had ad libitum access to water throughout the study. Horses were stalled individually until sampling was completed. Sampling was conducted the following morning after a 12-hr fast to normalize blood glucose and insulin concentrations.[18] The morning of sampling, a 14-cm, 14 G catheter was placed into either the left or right jugular vein of each horse. An extension set with a stopcock was placed onto the catheter and sutured into place. The catheters were in place 30 min prior to the first blood sample. After the final blood sampling of each test, the catheters were removed and the horses were returned to pasture. The protocol was approved by the Michigan State University Institutional Animal Care and Use Committee (AUF# 09/06-117-0).

Sample handling

Blood samples were taken at –30, 0 (just prior to administering the appropriate treatment), 30, 60, 90, 120, 150, 180, and 240 min after treatment. After each blood sample, the catheter was flushed with 10 mL of heparinized saline (10 units heparin/mL saline). The blood was injected into vacutainer tubes. Tubes were placed on ice until they could be centrifuged at 3,000 *g* for 25 min (serum tubes). Serum was stored at –20° C until analyzed. Frozen fasting samples (pretreatment) were sent on dry ice to the Endocrinology Laboratory at the Cleveland Metroparks Zoo for analysis of serum ferritin.

Sample analyses

Insulin was analyzed using a commercially available radioimmunoassay kit (Coat-A Count Insulin, Siemens Medical Solutions Diagnostics, Berkeley, California 94702, USA) as directed by the package insert.

The ferritin assay was developed based on a modified protocol.[21] Serially diluted horse serum samples displayed parallelism with the standard curve at dilutions ranging from 1:2 to 1:100. Recovery of serum diluted 1:25 and spiked with 5 and 30 ng/mL standard averaged >90%.

Insulinemic response calculations

Insulin response calculations were determined by averaging the responses at 30 and 0 min before feeding for a baseline and then using the trapezoidal method of numerical integration[25] to calculate the area under the curve (AUC) above baseline values. The peak serum insulin concen-

Table 1. Serum ferritin (ng/mL), area under the curve (AUC) of insulin (μIU/mL*min), and peak insulin concentrations (μIU/mL) after administration of either 0.25 g dextrose/kg of bodyweight or 1.5 g pelleted corn/kg of bodyweight on an as-fed basis.

Horse gender	Age (yr)	Ferritin	Peak insulin (dextrose)	AUC insulin (dextrose)	Peak insulin (pelleted corn)	AUC insulin (pelleted corn)
Male	3	130	18	1,305	30	3,094
Male	3	252	6	432	17	1,897
Male	3	274	20	1,030	46	5,646
Female	3	285	26	1,710	50	6,937
Female	>10	331	90	7,093	270	14,053
Female	3	347	13	716	37	4,090
Male	3	400	8	307	38	5,004
Female	3	458	44	3,786	67	10,886
Male	>10	461	141	12,097	314	37,898
Male	>10	489	76	7,524	97	304
Male	>10	493	126	517	206	28,803
Female	>10	554	44	5,079	84	13,640
Female	3	571	43	3,038	42	6,013
Female	3	654	34	3,004	41	5,714
Male	>10	684	46	3,398	91	9,520
Female	>10	882	152	18,632	667	71,626

trations above baseline were identified from the sample points.

Statistics

Statistics were performed using SAS 8.2 (SAS Institute Inc., Cary, North Carolina 27513, USA), with PROC CORR used to evaluate the relationship between serum ferritin and both peak insulin and insulin AUC with Pearson's correlations reported. Significance was considered at $P \leq 0.05$, and trends were explored at $P \leq 0.1$.

RESULTS

Serum ferritin concentrations ranged from 130 to 882 ng/mL (Table 1). As hypothesized, ferritin was positively correlated with both the peak insulin and the AUC for insulin after challenges with both dextrose and pelleted corn. The strongest correlation was between serum ferritin and the insulin AUC after the dextrose administration ($r = 0.61$; $P = 0.01$) followed by the AUC after feeding a meal of pelleted corn ($r = 0.60$; $P = 0.01$), with the correlation for the peak insulin being 0.53 ($P = 0.03$) after dextrose administration and 0.56 ($P = 0.02$) after the meal of pelleted corn.

When evaluating only the young horses, there were trends for positive correlations between serum ferritin and the insulin AUC ($r = 0.70$; $P = 0.052$) and peak insulin ($r = 0.65$; $P = 0.08$) after dextrose administration but not between ferritin and the insulin AUC ($r = 0.50$; $P = 0.21$) and peak insulin ($r = 0.34$; $P = 0.41$) after a meal of pelleted corn. For the older horses, there was only a trend for a positive correlation between serum ferritin and the insulin AUC after receiving a meal of pelleted corn ($r = 0.64$; $P = 0.09$).

Additionally, no significant correlations were found when evaluating only the male horses, but they were present for the females. The strongest correlations were between ferritin and the insulin AUC after the meal of pelleted corn ($r = 0.74$; $P = 0.04$) and dextrose administration ($r = 0.73$; $P = 0.04$), with trends noted for the correlation between ferritin and peak insulin after dextrose administration ($r = 0.63$; $P = 0.09$) and after the meal of pelleted corn ($r = 0.62$; $P = 0.1$).

DISCUSSION

Although the digestive tracts may appear similar between the horse and black rhinoceros, the type of diet ingested in nature is markedly different; the black rhinoceros is a browser, while the horse is a grazer, which presumably might have an effect on iron availability in the diet. Regardless, the positive correlation between serum ferritin, an indicator of iron stores in the body, and the insulin response to a glycemic challenge suggest that a relationship between iron accumulation and insulin insensitivity may exist in horses. It was interesting to note the positive correlation in the females but not the males. Granted, the power for detecting significance was greatly reduced, because the number of animals evaluated when limited to a single gender was halved. Hence, the results should not be

interpreted as not being applicable to males, because the failure to detect a significant correlation was simply due to a lack of power. Additionally, although there were several trends for significant positive correlations in various measurements for the younger horses, there was only a trend for one positive correlation in the older horses. This may have been due to all the younger horses' being of similar age (within a few months), whereas the ages of the older horses had greater variation. Additionally, there were nine young horses and only seven older horses, so greater statistical power was available to analyze young horse data. Regardless, due to low animal numbers, these results should be interpreted with caution and additional studies to control for such factors as age and gender are warranted. Additionally, it should be noted that the horse with the highest serum ferritin concentration and the highest insulin responses for all measurements aided in achieving significant correlations. This horse had previously been confirmed as insulin resistant. Inclusion of the data, clearly an outlier, was deemed prudent, because it alone suggests but does not prove a link between serum ferritin and insulin resistance. Although the results cannot prove that a relationship between serum ferritin and insulin response also exists in rhinos, it may be suggestive of such. Insulin resistance in rhinos, if shown to be present, could contribute to the development of iron overload disorder, and approaches to reduce the possibility for the development of insulin resistance in rhinos would be prudent.

Approaches to the management of insulin resistance that have been used in horses may need to be considered for use in browsing rhinos. Besides altering diets to decrease soluble carbohydrates, supplementation with prebiotic fibers, such as short-chain fructo-oligosaccharides, has improved insulin sensitivity in several species, including horses.[19]

In horses, obesity is a contributing factor,[24] and exercise has been shown to increase insulin sensitivity.[14] The same is true in humans.[5,20,22] With captive rhinos, exercise is typically limited.[3] The feeding program of captive rhinos is normally designed to ensure a steady supply of nutrients to ensure that weight loss does not occur. In horses, insulin sensitivity increases with weight loss.[11] Seasonal weight loss is probably a normal part of the lifecycle in the wild. These weight fluctuations are typically lost when animals are fed to maintain an appropriate appearance. Lack of exercise combined with constant bodyweight, particularly if the weight exceeds the ideal, may contribute to these animals' becoming insulin resistant and susceptible to health issues that accompany such a state.

CONCLUSIONS

There was a positive correlation between serum ferritin, an indicator of stored iron in the body, and insulin response to a glycemic challenge in this pilot study using horses. This suggests a similar link may exist in rhinos. Excess fat stores and lack of exercise in rhinos may contribute to the development of insulin resistance, as they do in horses, and increase the incidence of iron overload disorder.

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