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Increased inflammation and decreased insulin sensitivity indicate metabolic disturbances in zoo-managed compared to free-ranging black rhinoceros (*Diceros bicornis*)

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ABSTRACT

Black rhinoceros (rhinos) living in zoos express a host of unusual disease syndromes that are associated with increased morbidity and mortality, including hemolytic anemia, rhabdomyolysis, hepatopathy and ulcerative skin disease, hypophosphatemia and iron overload. We hypothesized that iron overload is a consequence and indicator of disturbances related to inflammation and insulin/glucose metabolism. The objectives of this study were to: (1) generate the first baseline information on biomarkers of inflammation (tumor necrosis factor alpha [TNFα], serum amyloid A [SAA]), insulin sensitivity (insulin, glucose and proxy calculations of insulin sensitivity), phosphate and iron stores (ferritin) using banked serum from free-ranging black rhinos; and (2) then compare serum biomarkers between zoo-managed ($n = 86$ individuals) and free-ranging ($n = 120$) animals. Enzyme immunoassays were validated for serum and then biomarker levels analyzed using mixed models while controlling for sex, age and year of sample collection. Concentrations of TNFα, SAA, insulin and insulin-to glucose ratio were higher ($P < 0.05$) in black rhinos managed in *ex situ* conditions compared to free-living counterparts. Findings indicate that the captive environment is contributing to increased inflammation and decreased insulin sensitivity in this endangered species.

1. Introduction

The African black rhino (*Diceros bicornis*) is critically endangered, having declined by >90% from 100,000 individuals in the 1960s to ~5000 animals today, largely due to poaching (Emslie, 2013). The worldwide *ex situ* population of ~250 black rhinos (Emslie, 2012) also is under threat, chiefly due to a unique vulnerability to unusual disease syndromes. These maladies include hemolytic anemia (Miller and Boever, 1982; Paglia, 1993; Paglia et al., 1986), rhabdomyolysis (Murray et al., 2000), hepatopathy (Paglia and Tsu, 2012) and ulcerative skin disease (Munson et al., 1998), which contribute to morbidity and mortality (Carlstead et al., 1999; Dennis, 2004; Dennis et al., 2007). Iron overload, or hemosiderosis, is one of the most commonly reported conditions, commonly diagnosed by measuring serum ferritin and iron, total iron binding capacity and transferrin saturation (Kock et al., 1992). At necropsy, abundant iron stores are found in many internal organs, including liver and spleen (Clauss and Paglia, 2012). We suspect that iron overload may be an indicator of underlying metabolic disorders.

Similar to humans, rodents and domestic animals, a sedentary lifestyle and increased fat repositories may create metabolic disturbances, including chronic inflammation, insulin resistance, iron overload, hypophosphatemia and hepatic disease (Haap et al., 2006; Rajala and Scherer, 2003; van Dijk et al., 2003; Xu et al., 2003; Yanoff et al., 2007). The horse, the closest domestic relative of the rhinoceros (Vaughan et al., 2000), commonly develops inflammation and insulin resistance with increased lipid deposits that result in a host of disease states (Field and Jeffcott, 1989; Johnson, 2002; Vick et al., 2006, 2007).

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Growing evidence suggests a correlative relationship between inflammation and insulin resistance (Rajala and Scherer, 2003; Ramos et al., 2003; Xu et al., 2003). Inflammatory cytokines, such as tumor necrosis factor alpha (TNFα), play a key role in regulating insulin/glucose homeostasis by inhibiting insulin signaling and glucose uptake, causing hyperglycemia and compensatory hyperinsulinemia (Hotamisligil et al., 1996; Lang et al., 1992). Through these mechanisms, cytokines are directly implicated in the development of insulin resistance. In humans, circulating acute phase protein and inflammatory cytokine concentrations are increased in overweight individuals and are even greater in obese patients with insulin resistance (Xu et al., 2003; Yang et al., 2006).

Interactions among inflammation, insulin/glucose metabolism and iron metabolism are complex (Fig. 1). Inflammation and insulin resistance adversely influence iron metabolism in humans. Iron overload has also been documented in the horse (Lavoie and Teuscher, 1993; Pearson et al., 1994; Smith et al., 1986), and increased serum ferritin has been associated with increased serum insulin and insulin resistance (Kellon, 2006; Nielsen et al., 2012). Inflammatory molecules, such as TNFα and acute phase proteins, divert iron from red blood cells by up-regulating ferritin expression and enhancing iron sequestration in tissue (Balla et al., 2007; Hirayama et al., 1993; Yanoff et al., 2007). Insulin also contributes to iron storage by stimulating cellular iron uptake and up-regulating ferritin synthesis (Davis et al., 1986; Yokomori et al., 1991). The relationship between iron and insulin/glucose metabolism also can be bi-directional. Iron interferes with insulin's inhibition of glucose production by the liver, thereby increasing serum glucose and augmenting hyperinsulinemia (Dandona et al., 1983; Niederau et al., 1984; Schafer et al., 1981). Iron stores also are positively correlated with insulin resistance in humans, even without significant iron overload (Fernandez-Real et al., 1998; Jehn et al., 2004; Jiang et al., 2004; Schafer et al., 1981; Tuomainen et al., 1997).

Inflammation and insulin resistance in humans also can contribute to hypophosphatemia (Gaasbeek and Meinders, 2005). Exogenous administration of cytokines causes marked decreases in serum phosphate levels (Barak et al., 1998), and insulin also stimulates cellular phosphate absorption, thereby decreasing serum phosphate availability. Like iron, hypophosphatemia can inhibit glucose uptake, exacerbating hyperglycemia and hyperinsulinemia (Haglin et al., 2001). Symptoms resulting from hypophosphatemia include hemolytic anemia, rhabdomyolysis, liver failure and neurological manifestations, including encephalopathy (Gaasbeek and Meinders, 2005; Nanji and Anderson, 1985).

These disease conditions in humans are similar to those afflicting captive black rhinos, including rhabdomyolysis, liver disease and neurological symptoms (Dennis, 2004; Paglia et al., 2001). Hemolytic anemia is cited as a leading cause of death in captive black rhinos with a 75% mortality rate (Miller and Boever, 1982; Paglia et al., 1996). Hypophosphatemia also has been documented in multiple cases of black rhino illness (Murray et al., 2000; Paglia, 1993; Paglia et al., 1996). A survey of 88% of black rhinos held in North American zoological institutions from 1930 through 2001 revealed that 73% of captive-born animals died before reproducing, and 77% of these were <6 y of age (i.e., prepubertal; Dennis, 2004).

Our objective was to establish and compare values for inflammatory status and insulin sensitivity related to phosphate and iron stores in zoo-housed versus wild black rhinos. Determining reference intervals for these parameters for the first time from wild black rhinos was of particular importance. As the phenomena of inflammation and insulin resistance are complex (Malle and De Beer, 1996; van Deventer et al., 1990), we focused on multiple serum biomarkers. One priority was TNFα, which is well established to increase in circulation with inflammation (Hesse et al., 1988; Ramos et al., 2003; van Deventer et al., 1990) and play a role in insulin resistance, with concentrations elevated in overweight humans, rodents and horses (Lang et al., 1992; Ramos et al., 2003; Ruan et al., 2003; Vick et al., 2007). A second priority was serum amyloid A (SAA), an established biomarker of inflammation expressed by human adipocytes and correlated with body mass index (Malle and De Beer, 1996; O'Brien and Chait, 2006; Sasaki et al., 2003; Suganami et al., 2005). SAA is known to stimulate lipolysis, thereby contributing to insulin resistance (Malle and De Beer, 1996; O'Brien and Chait, 2006; Sasaki et al., 2003; Suganami et al., 2005) and is elevated in patients with iron overload (Kirk et al., 2001). We measured insulin and glucose as indicators of insulin sensitivity, while taking advantage of reference metrics in healthy, insulin resistant and equine metabolic syndrome horses (Keen, 2013; Nadeau et al., 2006; Suagee et al., 2013), another Perissodactyla species. Finally, we examined serum inorganic phosphate and serum ferritin, an established marker of iron stores in mammals, including the black rhino (Fernandez-Real et al., 1998; Smith et al., 1984; Tuomainen et al., 1997). We hypothesized that black rhinos maintained in zoological conditions exhibit increased concentrations of inflammatory markers and decreased insulin sensitivity compared to free-ranging counterparts.

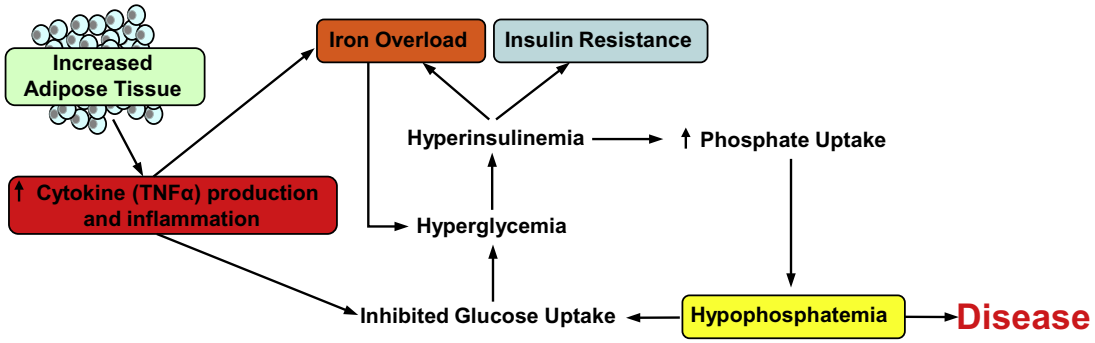

Fig. 1. Schematic of the hypothesized endocrine cascade leading to disease in zoo-managed black rhinos. Excessive adipose tissue creates a pro-inflammatory state and overproduction of cytokines that inhibit insulin-stimulated glucose uptake, thereby inducing hyperglycemia, consequent hyperinsulinemia and insulin resistance. Inflammation also diverts iron from red blood cell production into tissue storage. Hyperinsulinemia further contributes to iron overload by stimulating cellular iron uptake and up-regulating ferritin synthesis. Excess iron interferes with insulin's inhibition of glucose production by the liver, thereby increasing serum glucose and augmenting hyperinsulinemia. Insulin also stimulates cellular phosphate absorption, decreasing serum phosphate availability. The resulting hypophosphatemia exerts negative feedback by inhibiting glucose transport, exacerbating hyperglycemia and hyperinsulinemia. In humans, hypophosphatemia is correlated with hyperinsulinemia, insulin resistance and metabolic syndrome as well as diseases similar to those documented in the black rhino, including hemolytic anemia, rhabdomyolysis and encephalopathy (Gaasbeek and Meinders, 2005; Haap et al., 2006; Nanji and Anderson, 1985).