ORIGINAL ARTICLE

Dietary impact on circulating glucose profiles in the white rhinoceros

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Summary

Excess dietary glucose may be a factor in several captive wildlife diseases and reproductive abnormalities. The first step in understanding the health consequences of diets high in glucose is to characterize how dietary glucose concentrations change circulating glucose profiles. We adapted the glycemic index approach to detect differences in blood glucose concentrations in white rhinos in response to different meals. Six white rhinos were fasted overnight then randomly assigned to be fed 5 kg of grass hay and one of five meals varying in digestible energy (DE) availability and source (10% DE glucose, 5% DE glucose, 10% DE pelleted horse feed, 10% DE lucerne hay, 10% DE grass hay). After eating, the blood glucose response peaked 45–90 min later and remained elevated up to 180 min. Area under the curve results demonstrated that the blood glucose response was not different between diets. However, at 90 min, serum glucose levels in rhinos eating the 10% lucerne hay diet were significantly lower than the 5% glucose and 10% glucose diets but not the 10% pellet nor 10% grass hay diets. The changes in blood glucose responses to different diets were similar in magnitude to reported domestic horse profiles but are higher than predicted by allometric scaling. We conclude that the grass hay, lucerne hay and low glycemic index horse pellets fed in this study resulted in similar blood glucose responses in white rhinos. The validation of the methodology used in this study is a first step towards elucidating the relationship between glucose, obesity, health and reproduction in rhinos.

Introduction

Diets for wildlife in captivity are often nutrient-rich approximations of poorly understood wild diets (Dierenfeld, 1997; Jordan, 2005). Availability and convenience dictate that captive wildlife species are commonly fed commercial domestic livestock foods. The dietary requirements of wildlife, however, may differ from the production goals of livestock feeding. Domestic animal diets are largely developed for rapid weight gain or high performance and can be energy dense. Such diets are potentially a problem for captive wildlife because they may cause hyperglycaemia, weight gain and obesity, because the caloric energy needed for survival and reproduction in captivity is less than in the wild (Crissey, 2005).

Animals in captivity may experience chronic activation of the hypothalamic-pituitary axis, i.e. distress (Carlstead and Brown, 2005; Linklater et al., 2010). Distress can also lead to obesity by activating

chemical signals, such as neuropeptide Y which is released by the sympathetic nervous system and stimulates fat accumulation (Kuo et al., 2007). In primates, these triggers lead to metabolic syndrome, which is a proinflammatory state characterized by increased visceral fat, elevated blood pressure, hyperlipidemia, and impaired glucose tolerance (Shively et al., 2009). A similar metabolic pathway may exist in rhinos. Excess dietary energy may also, in turn, increase glucocorticoid release and insulin insensitivity, as has been shown in equids (Hoffman et al., 2003).

The triad of obesity, stress, and hyperglycemia leads to insulin resistance and the development of laminitis, endotoxemia, hyperlipemia, osteochondrosis, exertional rhabdomyolysis and metabolic syndrome in horses (Hoffman et al., 2003; Pratt et al., 2006; Treiber et al., 2006a,b; Firshman and Valberg, 2007). Maternal diets high in glucose are also detrimental to offspring health and reproductive function (Rhind, 2004; Vick et al., 2006, 2007). Knowledge of the glucose response to different diets in equids might be adapted to understand disease and poor fertility in other Perissodactyla such as the white rhinoceros (Ceratotherium simum: Rhinocerotidae, L.) because they are also grazers (reviewed in Clauss and Hatt, 2006).

The global captive white rhinoceros population is approximately 800 animals and not self-sustaining because of low fertility and long periods of reproductive senescence, especially amongst captive born females (Hermes et al., 2006; Roth, 2006; Emslie, 2008). The reproductive pathologies contributing to poor fertility in captive white rhinos include polycystic ovaries, uterine tumours, extended luteal phases or cycle lengths, and may be partially caused by dietary imbalances, including overcondition (Patton et al., 1999; Hermes et al., 2005; Swaisgood et al., 2006; Morrow et al., 2008).

In nature, white rhinos live in highly seasonal environments. Warm wet summers of southern and eastern Africa provide an abundance of high quality forage, while during the cool dry winters, grass can be scarce and of poorer quality (Perrin and Brereton-Stiles, 1999). White rhinos apparently compensate for the reduced quality of nutrition in grass in the winter dry season by living off stored body fat (Shrader et al., 2006). Thus, body condition in wild white rhinos cycles annually. Seasonal changes in body condition have already been correlated to reproductive status in other Perissodactlya (Gastal et al., 2004; Lemma et al., 2006). If white rhino disease syndromes and repro-ductive failure are related to sustained higher energy diets in captivity, some of the observed health problems might be resolved by reducing energy intake, as has been the case for horses (Treiber et al., 2006b). In Shetland ponies, for example, restricting caloric intake to induce weight loss rate of 1% of ideal body weight per week for 17 weeks reversed insulin resistance (Van Weyenberg et al., 2008).

Wild white rhinoceros select a wide variety of grasses to eat that vary seasonally in their availability and nutrient value (Shrader et al., 2006; Arsenault and Owen-Smith, 2008; Waldram et al., 2008). Wild diets contain high levels of crude fiber (e.g. 36%), and low to moderate protein (e.g. 4.5–14.9% dry biomass; Owen-Smith, 1988; Kiefer, 2002). Captive rhino diets, however, are based on a few species of mono-cultured grass and legume hays supplemented with local browse, pelleted concentrates, fruits, and vegetables (Dierenfeld, 1996). Dietary recommendations for captive white rhinoceros suggest up to one-third of their daily caloric intake can be delivered in commercially available concentrated pellets, with no specific differentiation between grain- or forage-based pellets (Dierenfeld, 1996; Lintzenich and Ward, 1997). More recently, Clauss and Hatt (2006) advocate an even lower percentage of pellets in diets for captive rhinos, and warn particularly against the use of grain-based products. Processing of grains – including initial grinding to incorporate ingredients into pellets – but also the heat generated in the pelleting or extrusion processes, can increase digestibility of carbohydrates and affect glycemic index and glucose response to ingredients. Published feeding recommendations discourage the use of sugary produce (such as ripe bananas, melons, sweet potatoes, sweet corn); nonetheless, many institutions still regularly provide fresh produce or use it as a seemingly innocuous training or enrichment reward for rhinos (authors' observations). Thus, although not necessarily providing glucose per se, captive diets may provide higher available energy, as well as readily digestible carbohydrates, throughout the year than levels to which rhinos are physiologically adapted.

Despite its potential importance and possible link to health and reproduction, almost nothing is known about circulating glucose concentrations in rhinoceros fed diets varying in glucose availability. The objective of this study was to determine the duration and magnitude of the blood glucose response to standard rhino diet ingredients, and to high and low glucose concentrations.

Materials and methods

Meals representing diets varying in digestible energy content were fed to individual rhinos as an experiment to measure dietary influences on circulating blood glucose concentrations. The standard diet for the rhinos in this study included 30 kg grass hay, 2.5 kg lucerne hay daily, with 1 kg horse pellets or 1 kg chopped carrots and apples supplement per animal per week. This diet contained approximately 325 MJ digestible energy (DE) per day, calculated utilizing DE values for foods based on values derived from domestic horses (Kiefer, 2002; Clauss and Hatt, 2006). For each of the five diet trials, a percentage of the rhinos' daily estimated digestible energy intake was fed as either 10% DE glucose powder (1.76 kg, mixed to paste consistency with water and layered on top of grass hay: Healtheries, Auckland, New Zealand), 5% DE glucose powder (0.88 kg), 10% DE pelleted grain-based horse feed (2.1 kg; NRM, Auckland, New Zealand), 10% DE lucerne hay (3 kg; *Medicago sativa*) or 10% DE grass hay (5 kg; *Lolium* spp.) for the first meal of the day (Table 1). Each rhino received each meal over the course of the study. Additional grass hay was made available (up to 5 kg total) to rhinos during the 3-h period of blood sampling, and as a training aid. Meals were fed once to each rhino in a random sequence (ranging from 1 to 90 days between meals) and no acclimation period was performed. At approximately 8:00 hours each morning, the rhino was fed one of the meals and blood glucose measured immediately before feeding and every 45 min

thereafter for 3 h (five blood samples total). The grass hay and lucerne hay were sub-sampled (200 g) for nutrient analysis and DE calculated using pre-calibrated near infrared spectroscopy at a commercial laboratory (Pagan, 1998; New Zealand Laboratory Services, Hamilton, New Zealand; Table 1). Nutrient composition, including DE, for the pelleted horse feed was provided by the manufacturer.

Experiments were conducted over ten months from May 2008 to February 2009 on six white rhinoceros (one male and three females at Hamilton Zoo and two males at Auckland Zoo, New Zealand). All rhinos were 5- to 20-year-old adults trained for blood collection by venipuncture of the pinea. Training required 3 months and was accomplished prior to initiating the feeding experiments. Blood was aspirated with a 1 ml syringe and a 23 or 25 gauge needle or the skin was pricked with a needle to draw blood onto the surface. Blood glucose concentrations were measured immediately using a handheld glucose meter (Accu-Chek, Roche Diagnostics, Auckland, New Zealand). To validate the accuracy of the glucose meter, one blood sample from each rhino was drawn into a fluoride oxalate tube, stored on ice and sent within four hours to a commercial laboratory (Gribbles Veterinary Pathology, Auckland, New Zealand) for comparison.

Blood glucose results are reported as averaged mmol/l ± standard error of the mean. The glucose values from the handheld meter and the laboratory analysis were correlated using a Pearson's correlation test in SPSS (2007). Area under the curve (AUC) glucose profiles were calculated using the zeroed incremental trapezoid method as in Wolever (2004) and Gordon et al. (2007). The glucose value at time zero was considered baseline for each animal and subsequent values were zeroed against it, then the means were compared by repeated measures ANOVA, and *post hoc* *t*-tests.

To compare the effects of time and treatment, repeated measures analysis of variance for the five diets (sampled at 0, 45, 90, 135, 180 min) were performed using General Linear Model procedures. Levene's test of equality of error variances was performed and indicated that variances were not equal therefore the Tamhane's test was used in *post hoc* univariate tests amongst time and treatments. All tests were considered significant at the 0.05 level. We considered a p < 0.1 as a statistical trend requiring closer examination. This study was performed with the approval of the Victoria University of Wellington Animal Ethics Committee (protocol no. 2006R27).

Table 1 Nutritional constituents of meals used in this study

	NRM Pellets*	Grass hay	Lucerne hay
% DM	88.0	88.3	87.2
CP	12.5	6.7	15.3
CF	7.0	–	–
NDF	37.0	78.7.	56.1
ADF	12.0	45.1	44.6
DE	12.0	6.6	7.5

DM, dry matter; CP, crude protein; CF, crude fat; NDF, non–detergent fibre; ADF, acid detergent fibre.

All constituents are expressed as percent (%), except digestible energy (DE) is MJ/kg.

*The pellet ingredients included unspecified amounts of wheat, maize, oats, wheat by-products, maize by-products, barley, barley by-products, extracted soyabean meal, rapeseed meal, peas, lucerne meal, molasses, vegetable oils, limestone, dicalcium phosphate, salt, KER vitamins and minerals premix (information provided by manufacturer, NRM, Auckland, New Zealand).

Results

The diets were all consumed within 45 min of presentation. The rhinos had different reactions to the glucose paste; some ate the hay around it and consumed it last. Others ate the glucose paste preferentially even to the extent of licking remnants of the paste from the concrete floor afterwards. The paired glucose meter and laboratory blood glucose values ($n = 6$) were positively correlated ($r = 0.96$; $p < 0.01$). Glucose meter values were, on the average, $11 \pm 0.3\%$ lower than the laboratory values, similar to previous comparisons in the horse (Russell et al., 2007).

Glucose concentrations in the blood rose, peaking at 45–90 min, and remained elevated for up to 180 min after meals with the exception of the 10% lucerne which went below baseline by 90 min (Fig. 1). The magnitude of the response in circulating glucose depended on the energy content of the meal. Raw values ranged from a low of 2.4 mmol/l at 0 min for the 10% grass hay diet to 5.8 mmol/l at 90 min after eating the 10% glucose diet (both values from the same lactating female). The highest averaged values were at 4.95 ± 0.68 mmol/l 90 min after eating the 10% glucose diet. The AUC blood glucose response was not different between diets (RM-ANOVA, $F_{4,26} = 2.005$, $p = 0.205$), and no *post hoc* *t*-tests were significant ($p < 0.05$). The zeroed AUC values (mmol/l ± SEM) were: 6.1 ± 3.3 for 10% glucose powder, 2.2 ± 0.9 for 5% glucose powder, 2.2 ± 1.7 for 10% pellets, 1.7 ± 0.9 for 10% grass hay and 0.5 ± 0.3 for 10% lucerne hay.
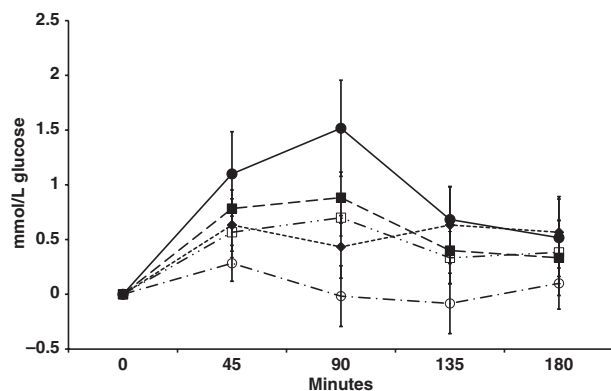

Fig. 1 Zeroed changes in blood glucose values in white rhinos fed meals varying in digestible energy (% of total daily DE intake): 10% DE lucerne hay (○), 10% DE glucose (●), 5% DE glucose (■), 10% DE pellets (◆), 10% DE grass hay (□) (*n* = 6; ±SEM). For each meal, an additional 5 kg of grass hay was provided *ad libitum*.

For the time and treatment effects, there was a significant effect of time (ANOVA, $F_{4,100} = 11.713$, $p < 0.001$) and a weak interaction between time and treatment (ANOVA, $F_{16,100} = 1.593$, $p = 0.084$) so *post hoc* tests were performed to investigate differences between treatments at individual time periods. No differences were detected at 0, 45, 135 or 180 min between diets. However, at 90 min, differences between diets were detected (ANOVA, $F_{4,25} = 5.633$, $p < 0.01$). The 10% lucerne hay diet was significantly lower than both the 10% glucose (mean difference = -1.33 ± 0.32) and the 5% glucose diets (mean difference = -0.87 ± 0.23).

Discussion

The AUC analysis demonstrated no significant differences between diets. The magnitude of the response is 40% lower compared than that reported in the horse, but our sampling time was not as frequent or as long (i.e. Rodiek and Stull, 2007). Allometric theory suggests that rhinos would have lower circulating glucose concentrations in relation to horses (Umminger, 1975; Gordon et al., 2007; Kjeld and Olafsson, 2008). Using Kjeld and Olafsson's equation, with the rhinos' body weights of 1400–2050 kg, we would expect glucose measurements to range from 1.29 to 1.8 mmol/l. Our results were higher (2.8–5.4 mmol/l), but do fall within the reported range in the International Species Information System (ISIS) (2003) MedArks records for white rhinos (1.10–14.1 mmol/l, *n* = 98). Information on wild white rhino glucose values is limited to animals already stressed by capture and anesthesia. In 84 blood samples drawn from 16 white rhinos immobilized over a 2-day period, Seal et al. (1976) found glucose measurements of 35–117 mg/dl (equivalent to 1.94–6.5 mmol/l). These values are also similar to our results, though it is not clear how the blood samples were handled in the field, which could affect assay sensitivity. The glucose profile of nonstressed, wild white rhinos is unknown, but our data and other reported values (both captive and wild) appear to indicate relatively high circulating glucose as an atypical 'norm' for such a large animal.

In this study, glucose was administered as 'proof of principle' of the methodology and does not necessarily reflect a physiological response to items typically in a rhino diet, although honey, fruit juices and molasses are included in some training/enrichment food lists for the species. Furthermore, fruits (bananas, pineapples, papaya, grapes, melons) and vegetables (sweet corn, pumpkin, potatoes, beets)

are often used for these purposes. Although feeding recommendations discourage their use, quantities of these items fed to large herbivores are not insubstantial in some instances, and must be considered in total diet evaluations. Nonetheless, we did not compare glucose response following 'produce' or sweet feed meals, which would be interesting to do in the future.

Overall, the foods fed in this study show similar glucose responses. The lucerne hay diet contained more than twice the protein content as the grass hay, and was eightfold higher in insoluble fibre concentration than the pelleted feed (Table 1), which may account for the lower peak at 90 min relative to the other diets. Rodiek and Stull (2007) reported that the blood glucose responses in horses fed corn (maize) and oats, both components of the NRM pellets, were seven times higher than peaks following an alfalfa (lucerne) hay meal. We did not observe this; however, the pellets used in this study were specifically formulated to produce a low glycaemic response in horses, and appear to elicit a similar response in white rhinos. Protein can be insulinotropic, and can lower blood glucose concentrations when eaten with carbohydrates (e.g. in humans, Flint et al., 2004). Increased fibre concentrations of the forages may also slow the absorption of glucose into the bloodstream from the small intestine (Rodiek and Stull, 2007). We did not test the high glucose 'treats' that are sometimes fed to rhinos. If high-glucose (sugary) ingredients are utilized for training/enrichment purposes, perhaps they should be fed jointly with higher fibre and/or protein feeds rather than singly – by adding beet pulp, rice bran, soy hulls or browse/forage to the enrichment regimen.

One of the physiological sex allocation mechanisms may be female embryo intolerance of high *in utero* glucose concentrations (Gutierrez-Adan et al., 2001; Cameron, 2004). Changes in maternal energy balance around the time of conception have been associated with changes in birth sex ratios in both cattle and horses (Roche et al., 2006; Cameron and Linklater, 2007), with a positive energy balance resulting in fewer female births. In wild horses, the birth sex ratio can range from 3 to 81% male between mares depending on whether they were in negative or positive energy balance respectively (Cameron and Linklater, 2007). Male-biased birth sex ratios may occur in captive rhino populations, or at least at specific institutions, more than predicted by chance (Dennis et al., 2007b). We have identified rainfall and inferred body condition, as influencing calf sex in wild black rhinos (Berkeley and Linklater, 2010). If this mechanism is also the case for white rhinoceros, then a male birth sex bias might be a consequence of high glycaemic diets.

Glucose profiles in response to different diets have not been reported previously for any large wild ungulate species. Not only do we have limited data on circulating glucose in response to diet, we do not know the consequences of diets high in glucose. Captive diets may underlie a number of pathological syndromes and diseases in rhinos, particularly black rhinos (Dennis et al., 2007a). We demonstrated significant blood glucose variation in response to diet, and higher glucose levels than expected by allometry. We also showed the handheld glucose meter to be a rapid, easy and reliable tool that could be applied more broadly to understand circulating glucose in wildlife with minimal animal handling. Our results suggest that diets of grass hay, lucerne hay, and low glycaemic index horse pellets produce similar glucose responses in white rhinos. Our results support the use of a low glycaemic diet in white rhinos to maintain low circulating glucose concentrations in accordance with existing management guidelines. We recommend further experiments to: (i) compare glucose concentrations in captive and wild rhino diets and (ii) determine the relationship between circulating glucose concentrations, reproductive failure and pathological processes in all species of rhinos.

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