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Effect of increased adiposity on insulin sensitivity and adipokine concentrations in different equine breeds adapted to cereal-rich or fat-rich meals

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ABSTRACT

The relationships between diet, obesity and insulin dysregulation in equids require further investigation due to their association with laminitis. This study examined the effect of dietary glycaemic load and increased adiposity on insulin sensitivity and adipokine concentrations in different equine breeds. Equal numbers of Standardbred horses, mixed-breed ponies and Andalusian horses were provided with ad libitum hay plus either cereal-rich (CHO; $n = 12$), fat-rich (FAT; $n = 12$) or control (CON; $n = 9$) meals over 20 weeks. The isocaloric CHO and FAT diets were fed to induce obesity by gradually increasing the supplementary feeds to provide 200% of daily digestible energy requirements by Week 20. The CON group were fed a basal ration only and maintained moderate body condition.

At Week 20, the CHO and FAT groups demonstrated significantly increased body condition score, bodyweight, total body fat mass and plasma leptin concentrations compared with the CON group ($P < 0.001$). The CHO group had lower insulin sensitivity (SI; $P < 0.001$) and higher acute insulin response to glucose ($P = 0.002$) than the CON group. In contrast, the FAT group was no different to the control group. Ponies and Andalusians had lower SI values compared with Standardbreds, regardless of diet group ($P = 0.001$). Adiponectin concentrations were similar between the FAT and CON groups, but were significantly lower in the CHO group ($P = 0.010$). The provision of cereal-rich meals appeared to be a more important determinant of insulin sensitivity than the induction of obesity per se. Whether hypoadiponectinaemia is a cause or consequence of insulin dysregulation warrants further investigation.

Introduction

Laminitis associated with insulin dysregulation is an important cause of morbidity in domestic equine populations (Harris et al., 2006; Katz and Bailey, 2012). Insulin dysregulation is an umbrella term that refers to insulin resistance, fasting hyperinsulinaemia and/or exaggerated insulin responses to oral carbohydrates (Frank and Tadros, 2014). Together with obesity (generalised or regional adiposity), insulin dysregulation is considered to be a central component of equine metabolic syndrome (EMS), the clinical phenotype of many equids predisposed to pasture-associated laminitis (Frank et al., 2010). Pasture-associated laminitis also occurs in non-obese horses and ponies (Bailey et al., 2007; Geor, 2010); therefore, the link between obesity and insulin dysregulation requires further investigation. Other aspects of EMS that warrant additional study include alterations to adipokines (adipose-derived hormones such as leptin and adiponectin) and proinflammatory cytokines (Burns et al., 2010; Caltabilota et al., 2010; Wooldridge et al., 2012; Wray et al., 2013).

An apparent association between the induction of obesity and the development of hyperinsulinaemia and insulin resistance was demonstrated in a controlled study of Arabian geldings (Carter et al., 2009a). These changes occurred when horses were provided with multiple 'sweet feed' (cereal-rich) meals per day. The role of diet in the development of insulin dysregulation is an important consideration, because the adaptation of horses to 'sweet feed' meals can induce insulin resistance independent of obesity (Hoffman et al., 2003; Treiber et al., 2005). There is also evidence that weight gain can occur without reduced insulin sensitivity when horses and ponies are provided with relatively low-glycaemic rations (Quinn et al., 2008; Bamford et al., 2016). Additionally, a once-daily oral glycaemic load appeared to improve insulin sensitivity in a group of horses and ponies (Bamford et al., 2016). Therefore, multiple daily episodes of hyperinsulinaemia may be necessary for insulin resistance to develop because of chronic over-stimulation of insulin receptors (Kronfeld et al., 2005; Suagee et al., 2011). The breed of animals studied is another consideration, as differences in the innate insulin sensitivity of different breeds can influence the insulinaemic

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Table 1
Proximate analysis and ingredient composition of the study diets at Week 20.

	Hay	Supplementary feed		
		CHO	FAT	CON
Energy				
DE (MJ/kg feed, DM basis)	7.1	12.4	16.4	9.4
DE (as fed; MJ/100 kg BW)		13.1	13.1	3.8
Nutrient (%)				
CP	7.7	15.6	14.7	11.9
ADF	46.0	22.1	27.3	37.9
NDF	75.8	33.1	38.7	58.6
NSC	9.2	35.9	5.9	18.4
WSC	7.3	5.3	5.5	11.4
Starch	1.8	30.6	0.4	7.0
Fat	1.8	4.0	27.8	3.8
Ash	5.5	5.0	5.9	5.7
Ingredient (g/100 kg BW)				
Soyahull pellets		300	300	200
Chaff		300	300	200
Micronised maize		455	0	0
Fat supplement		0	200	0
Vitamin/mineral supplement		6	6	6

Proximate analysis performed at Equi-Analytical Laboratories. Hay was sourced from a single batch for the duration of the study. Animals were fed either cereal-rich (CHO), fat-rich (FAT) or control (CON) supplementary feeds divided into 2 daily meals. DM, dry matter; DE, digestible energy; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; NSC, non-structural carbohydrate; WSC, water soluble carbohydrate.

response of an individual to oral non-structural carbohydrates (Bamford et al., 2014).

We aimed to examine the relative influence of a prolonged twice-daily dietary glycaemic load, compared with an isocaloric intake of vegetable fat, on insulin sensitivity and adipokine concentrations after the induction of obesity in horses and ponies. In addition, the metabolic responses of different equine breeds were compared by enrolling three groups with previously-documented differences in innate insulin sensitivity: Standardbred horses, mixed-breed ponies and Andalusian horses (Bamford et al., 2014). We hypothesised that animals gaining weight on a cereal-rich diet would demonstrate lower insulin sensitivity than animals that gained weight on a fat-rich diet.

Materials and methods

Animals

Eleven Standardbred horses (9.5 ± 1.8 years, 457 ± 8 kg, body condition score [BCS] 5.0 ± 0.2), 11 mixed-breed ponies (9.0 ± 1.2 years, 305 ± 17 kg, BCS 5.3 ± 0.3) and 11 Andalusian-cross horses (8.3 ± 1.2 years, 475 ± 17 kg, BCS 5.5 ± 0.2) were studied. No animals demonstrated evidence of pituitary pars intermedia dysfunction when screened with a low-dose dexamethasone suppression test (McFarlane, 2011), nor did they have clinical or radiographic evidence of prior laminitis. They were kept in large dry lot paddocks with ad libitum access to fresh water and hay for at least 8 weeks prior to the study. Routine hoof trimming, dental prophylaxis and anthelmintic treatments were provided as appropriate. The use of animals in this study was approved by the University of Melbourne Animal Ethics Committee (ID 1011918).

Study design and diets

Animals were blocked by breed and randomly assigned to one of three diet groups: a cereal-rich diet (CHO), a fat-rich diet (FAT) or a control diet (CON). The CHO and FAT groups contained 12 animals (four of each breed) and received a hypercaloric ration to induce obesity. The CON group contained nine animals (three of each breed) and received only the basal ration.

Over a 20-week study period, all animals were provided with ad libitum access to fresh water and the same hay in dry lot paddocks. Diet groups differed in the type and amount of complementary feed provided in twice-daily meals (fed at 08:00 and 16:00) on each day of the study period (Table 1). To facilitate the individual provision of meals, animals were fed in separate yards along the perimeter of the dry lot paddocks. All meals contained a base ration of soaked soyahull pellets (Maxisoy, Energreen Nutrition) and lucerne chaff, with a balanced vitamin and mineral supplement (60 mg/kg BW; Ranvet) added to the morning meals. Animals in the CHO group received additional energy in the form of micronised maize (Micrmaize, Hygain).

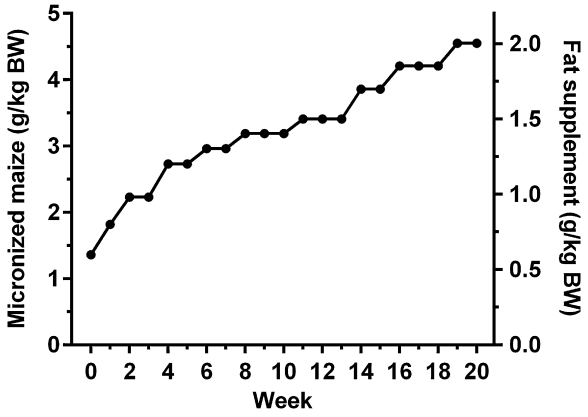

Fig. 1. Amount of micronised maize (left y axis) or fat supplement (right y axis) added to the base ration in the cereal-rich (CHO) and fat-rich (FAT) meals over the study period. The fat supplement consisted of an equal mixture (by weight) of liquid oil and granulated vegetable fats. The total amount of each supplement was divided into twice-daily meals.

The amount of micronised maize added to the base ration was gradually increased over the study period to allow for gastrointestinal adaptation (Fig. 1). The final amount of micronised maize in the diet reached 4.55 g/kg BW (providing 3.34 g/kg BW of additional non-structural carbohydrate), with the total ration providing approximately 200% of daily digestible energy (DE) requirements (NRC, 2007). Animals in the FAT group received an isocaloric amount of supplementary vegetable fat as an equal mix (by weight) of liquid oil (Energy Gold, Kohnke's Own) and granulated (Cool Calories, Buckeye Nutrition) fats. Mirroring the gradual increase in micronised maize for the CHO meals, supplementary vegetable fat was gradually increased in the FAT meals over the study period to allow for gastrointestinal adaptation (Fig. 1). To control for seasonal and environmental influences, animals in the CON group also had ad libitum access to hay and received meals containing the base ration only throughout the study.

Hay consumption was accurately quantified on three separate occasions (Week 0, Week 12 and Week 20) when horses and ponies were kept in individual yards for a 24 h period.

Assessment of adiposity

Bodyweight (BW) was measured weekly using calibrated scales. Percentage change from Week 0 (ΔBW) was calculated to account for differences in average starting BW between breeds. BCS was determined weekly by an experienced observer using a 9-point scale (Henneke et al., 1983; Kohnke, 1992). Regional adiposity along the nuchal ligament was assessed using the cresty neck score (CNS) described by Carter et al. (2009b). Total body fat mass (TBFM) was accurately determined during Week 0 and Week 20 using deuterium oxide (D_2O) dilution (Dugdale et al., 2011). Briefly, a dose of 0.12 g/kg BW D_2O (Cambridge Isotope Laboratories) was administered through a temporary catheter in the left jugular vein. Blood samples (20 mL) were collected by venepuncture of the right jugular vein immediately before and 4 h after D_2O infusion. Syringes were weighed to determine the exact weight of D_2O administered to each animal. Heparinised plasma samples were analysed using gas isotope ratio mass spectrometry (Iso-Analytical). Total body fat mass was determined using previously described calculations (Dugdale et al., 2011).

Assessment of insulin sensitivity

Insulin sensitivity was assessed using a previously described insulin-modified frequently-sampled IV glucose tolerance test (FSIGT) during Week 0 and Week 20 (Hoffman et al., 2003). Briefly, horses and ponies were moved from the dry lot on the morning of testing and IV catheters were placed in the left jugular vein under local anaesthesia. Blood samples were collected 60 min, 45 min and immediately before the infusion of a glucose solution (300 mg/kg BW; 40% weight/volume) through the jugular catheter. Twenty minutes later, an insulin bolus (20 mU/kg BW; Actrapid, Novo Nordisk) was delivered by venepuncture of the right jugular vein. Blood samples (10 mL) were collected 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min after the glucose infusion. Samples were transferred to tubes containing lithium heparin anticoagulant (Vacutainer, BD) and placed on ice until centrifugation.

Blood collection

Blood samples were collected during Week 0 and Week 20 to determine plasma concentrations of glucose, insulin, leptin, adiponectin, tumour necrosis factor-α