Effect of increased adiposity on insulin sensitivity and adipokine concentrations in horses and ponies fed a high-fat diet, with or without a once daily high-glycaemic meal

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Summary

Reasons for study: The relative influence of obesity and the adaptation to high-glycaemic diets on the development of insulin dysregulation in equids is unclear.

Objectives: To determine whether increased adiposity *per se* is responsible for the decreased insulin sensitivity (SI) often observed in obese horses, or whether a dietary glycaemic response is critically important.

Study design: Randomised controlled trial.

Methods: Eighteen horses and ponies were studied over a 20-week period. They received *ad libitum* hay, plus either a high-fat (low-glycaemic) diet (FAT; n=6) or a similar (isocaloric) diet containing 1.5 g/kg bwt once daily glucose (GLU; n=6) to induce obesity. A third group received a control ration (CON; n=6). Adiposity was monitored using body condition score (BCS), and total body fat mass percentage (TBFM) was determined using a deuterium oxide dilution technique. Insulin sensitivity was assessed using a frequently-sampled intravenous glucose tolerance test. Plasma concentrations of glucose, insulin, leptin, adiponectin, tumour necrosis factor- α (TNF- α) and serum amyloid A (SAA) were measured.

Results: The FAT and GLU groups became obese ($BCS \geq 7$), whilst the CON group maintained moderate condition ($BCS \leq 6$). Total body fat mass and leptin concentrations were increased in the FAT and GLU groups compared with the CON group ($P < 0.001$ and $P = 0.003$, respectively). Values for both insulin-dependent (SI) and insulin-independent (Sg) glucose disposal were higher in the GLU group compared with the FAT and CON groups ($P = 0.006$ and $P = 0.03$, respectively). There were no differences in adiponectin, TNF- α or SAA between groups (all $P \geq 0.4$).

Conclusions: Increased adiposity did not decrease insulin sensitivity in either the FAT or GLU diet groups, suggesting that obesity *per se* might not be responsible for the lower SI values reported in previous studies. Contrary to expectations, once daily glucose appeared to increase insulin sensitivity. Further work is required into the dietary causes of insulin resistance in equids.

Introduction

Pasture-associated laminitis is a significant cause of morbidity in domestic horse populations worldwide [1], with obesity or recent weight gain recognised to be a major risk factor [2,3]. Obese horses and ponies (exhibiting generalised or regional adiposity) often have evidence of insulin dysregulation – a recently proposed term that encompasses insulin resistance (IR), fasting hyperinsulinaemia, and exaggerated insulin responses to oral carbohydrates [4]. However, the association between obesity and insulin dysregulation in horses remains incompletely understood. It has been suggested that alterations to adipose tissue function in the obese state contributes to IR through the production of proinflammatory cytokines and biologically active hormones (adipokines) [5]. Leptin and adiponectin are two adipokines that are postulated to play a role in the pathogenesis of insulin dysregulation and warrant further investigation [6,7].

In a previous study that induced obesity in a cohort of Arabian horses, reduced insulin sensitivity (SI) and increased acute insulin response to glucose (AIRg) were reported when glucose and insulin dynamics were assessed using a frequently-sampled intravenous glucose tolerance test (FSIGT) [8]. These changes occurred when animals were provided with ‘sweet feed’ meals containing large

amounts of non-structural carbohydrates (NSC). The adaptation of non-obese horses to ‘sweet feed’ meals has also been shown to decrease SI [9,10]. Diets that induce repeated episodes of hyperglycaemia and hyperinsulinaemia might contribute to IR through the down-regulation of chronically stimulated insulin receptors [11,12]. Therefore, the relative influence of increased adiposity and diet-associated hyperinsulinaemia on the development of insulin dysregulation remains unclear.

An improved understanding of the pathogenesis of equine insulin dysregulation could aid in the identification of ‘at risk’ individuals before the onset of laminitis [13]. The purpose of this study was to determine whether increased adiposity *per se* is responsible for the altered glucose and insulin dynamics observed in obese horses and ponies. We aimed to induce obesity by providing either a high-fat (low-glycaemic) diet, or a similar diet containing a once daily high-glycaemic meal. We hypothesised that SI (assessed using a FSIGT) would be reduced in horses and ponies after the induction of obesity, and that SI would be even lower in the animals that consumed the high-glycaemic meals.

Materials and methods

Animals and groups

Eighteen horses and ponies (six Standardbred horses [452 ± 17 kg], six mixed-breed ponies [290 ± 38 kg], six Andalusian-cross horses [446 ± 25 kg]) aged 5–19 years were used in this study. These breeds were chosen to provide a range of initial SI values [14]. Insulin sensitivity was not assessed prior to enrolment and no individuals had evidence of previous laminitis (including radiography). All animals were in moderate body condition at the outset of the study (median [range], 4.9 [3.8–5.8] out of 9) [15,16], and were determined to be free from pituitary pars intermedia dysfunction by clinical examination and the results of a low-dose dexamethasone suppression test [17]. The horses and ponies were kept in dirt paddocks and maintained on *ad libitum* pasture hay for at least 6 weeks prior to the study. All animals received routine anthelmintic, dental and farriery treatments as appropriate.

Animals were blocked by breed and randomly assigned to one of three diet groups using a random number generator^a, such that each diet group contained six animals total (two of each breed). Further detail of group composition is included as Supplementary Item 1. An *a priori* estimate of study power indicated that six animals per group were appropriate to detect a 1-unit change in SI with a power of 80%. Investigators were not blinded to diet group allocation during the study.

Study design and diets

The study was conducted over a 20-week period. All animals had free access to fresh water and pasture hay (sourced from a single batch; Table 1) in dirt paddocks at all times. Diet groups differed in the type and amount of supplementary feed (Table 1), with the weight of supplementary feeds adjusted to the body weight of each horse on the first day of each week. Twice daily supplementary meals were provided to each animal in individual yards (4 m x 4 m) at 0800 h and 1600 h on each day of the study, with any refusals recorded. All meals consisted of pelleted soyabean hulls^b (pre-soaked with water according to manufacturer's directions) mixed with lucerne chaff. A vitamin and mineral supplement^c was added to the morning meal. The first diet group (CON group) received the standard meal only. The purpose of this group was to maintain a moderate BCS throughout the study to control for seasonal and environmental influences on Week 20 results. In order to induce obesity, the second diet group (FAT group) received additional energy in the form of a mixture of liquid (canola)^d and granulated (vegetable)^e oils added to the meals. To allow for gastrointestinal adaptation, the amount of fat was gradually increased to 2 g/kg bwt by Week 20. The total ration provided approximately 200% of maintenance digestible energy (DE) requirements [18]. Further detail of the provision of dietary fat is included in Supplementary Item 2. The third diet group (GLU group) received a ration isocaloric to the FAT group to also induce obesity. This group received 1.5 g/kg bwt glucose^f in the morning meal each day to produce a large glycaemic response. The dose of glucose was chosen after pilot studies determined this to be the maximum amount which was reliably consumed in a meal. A once daily glycaemic load was chosen to maximise peak postprandial glucose and insulin concentrations (subsequent glucose-containing meals might have caused a blunted insulin response due to a potential

‘second meal effect’ [19]). The evening meal contained a reduced amount of the fat supplement to ensure that daily DE intake in the supplementary meals was equal between the FAT and GLU groups.

Individual hay consumption was measured at three time-points during the study: Week 0, Week 12 and Week 20. On these occasions, animals were kept in their individual yards for a 24-h period and hay intakes were accurately weighed. Group intakes of hay were estimated throughout the study.

Assessment of glucose and insulin responses

In a pilot study conducted prior to Week 0, postprandial glycaemic and insulinaemic responses to the FAT and GLU meals were evaluated in the six Standardbreds, six ponies and six Andalusians. Blood samples were collected via a jugular catheter at 30 min intervals over a 6-h period following the meal. Postprandial glycaemic and insulinaemic responses to the GLU (n=6) meals were assessed again during Week 12.

Assessment of adiposity

Body condition score (BCS) was assessed each week by a single experienced observer using a 9-point scale [15,16] and body weight was measured using calibrated scales. To account for large differences in body weight between breed groups, percentage change in body weight (ΔBW) from Week 0 values were calculated. The cresty neck score (CNS) described by Carter et al. [20] was used to determine neck crest adiposity. Total body fat mass (TBFM) was determined at Week 0 and Week 20 using a previously described deuterium oxide (D_2O) dilution technique [21]. Briefly, a dose of 0.12 g/kg bwt D_2O^g was administered over 60 s through a temporary catheter in the left jugular vein. Heparinised^h blood samples (20 mL) were collected by venepuncture of the contralateral jugular vein immediately before and 4 h after D_2O administration. Plasma samples were analysed in a commercial laboratoryⁱ by gas isotope ratio mass spectrometry and TBFM was derived using previously described calculations [21].

Assessment of insulin sensitivity

Insulin sensitivity was determined using a FSIGT as previously described [9]. Briefly, horses were removed from the paddock at 0800 h and a catheter was placed in the left jugular vein. Baseline blood samples (10 mL) were collected 60, 45 and 0 min prior to a glucose bolus (300 mg/kg bwt; 40% wt/vol solution) administered through the jugular catheter. Twenty minutes after the glucose bolus, an insulin bolus^j (20 mIU/kg bwt) was administered into the right jugular vein. Blood samples (10 mL) were collected at 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 bolus and immediately transferred into tubes containing lithium heparin anticoagulant^h and placed on ice until centrifugation.

Blood sample collection

Jugular venous blood samples (20 mL) were collected from each animal immediately prior to the morning meals during Week 0 and Week 20. Samples were transferred to tubes^h containing lithium heparin or EDTA and placed on ice until centrifugation.

Plasma analysis

Blood samples were centrifuged at 1000 g for 10 min at 4°C and 1 mL aliquots of separated plasma were stored at -80°C until analysis. In all samples, plasma glucose concentrations were measured using a hexokinase colorimetric assay^k and insulin concentrations were measured using a radioimmunoassay^l previously validated for equine plasma [22]. In the Week 0 and Week 20 samples, concentrations of leptin^l, high-molecular weight adiponectin^m, tumour necrosis factor- α (TNF- α)ⁿ and serum amyloid A (SAA)^o were determined using previously validated assays [7,23,24].

Data Analysis

Glucose and insulin curves from the FSIGT were interpreted using MINMOD Millenium software^p as previously described [9]. Results included values for insulin sensitivity (SI), acute insulin response to glucose (AIRg), disposition index (DI), and glucose effectiveness (Sg). Data for outcome variables (Week 20 values) were analysed using the general linear model function in SPSS software^q. Diet,

breed, and the interaction term were included as fixed effects, with age and sex also evaluated during the screening process. Week 0 values were included in the model as a covariate, with the exception of Δ BW. Significant main effects were compared using Fisher's protected least significant difference test. Residual values were tested for normality using the Shapiro-Wilk test, homogeneity of variance was assessed using Levene's test, and linearity of the covariate was assessed by the visual inspection of scatter plots. All data were reported as sample mean $\pm$ s.d., except where indicated, with $P < 0.05$ considered significant.

Results

Animals and diets

All animals remained healthy throughout the study and no signs of laminitis were observed. The supplementary meals were well tolerated and meal refusals were negligible throughout the study period. Daily hay consumption (on a dry matter basis) in the FAT and GLU groups was measured to be $2.0 \pm 0.2\%$ bwt on all 3 occasions. Hay intake in the CON group was measured to be $2.4 \pm 0.2\%$ bwt per day on all 3 occasions. Estimates of group hay intake throughout the study were consistent with these results. There was no influence of breed on individual hay consumption.

Peak postprandial glucose concentrations measured during the pilot study were 5.4 ± 0.3 and 7.4 ± 0.8 mmol/L for the FAT and GLU meals, respectively ($P < 0.001$). Peak insulin concentrations measured during the pilot study were 11.6 ± 5.7 and 71.6 ± 30.8 mU/L for the FAT and GLU meals, respectively ($P < 0.001$). When these responses were assessed in the GLU group during Week 12 there was no evidence of adaptation, with equivalent responses to Week 0 values (7.3 ± 0.8 mmol/L and 71.8 ± 32.6 mU/L for glucose and insulin, respectively).

For all outcome variables, the final statistical model included the fixed effects of diet, breed, and the interaction term, with Week 0 values included as a covariate (except Δ BW). Age and sex did not influence any outcome variable and were omitted from the final model. For all outcome variables,

there was no effect of breed or the interaction term (all $P \geq 0.1$; Tables 2 and 3); however, it was considered (*a priori*) important that these effects remain in the model. Therefore, P values reported in the text represent the main effect of diet group. Further detail of the output from the statistical model is included in Supplementary Item 3.

Adiposity

Weekly measurements of BCS and ΔBW are presented in Figure 1. The FAT and GLU groups were obese ($BCS \geq 7$) at Week 20, whilst the CON group remained in moderate body condition ($BCS \leq 6$). Measures of adiposity at Week 0 and Week 20 are presented in Table 2. Body condition score, TBFM and CNS were significantly increased in the FAT and GLU groups compared with the CON group at Week 20 (all $P < 0.001$). Similar increases in ΔBW were observed between diet groups ($P = 0.2$).

Minimal model analysis

Insulin sensitivity was increased in the GLU group compared with the CON and FAT groups ($P = 0.006$). All individuals within the GLU group (regardless of breed) exhibited higher SI values at Week 20 than at Week 0. Glucose effectiveness was also increased in the GLU group compared with CON and FAT groups ($P = 0.03$). There was no difference in AIRg between groups ($P = 0.8$), whilst DI was lower in the FAT group compared with CON and GLU groups ($P = 0.005$).

Plasma measurements

Leptin concentrations were significantly increased in the FAT and GLU groups compared with the CON group (Table 3; $P = 0.003$). Resting insulin concentrations were higher ($P = 0.04$) in the GLU group, whilst there were no differences in Week 20 glucose, adiponectin, TNF- α or SAA concentrations between diet groups (all $P > 0.4$).

Discussion

In the present study, horses and ponies that became obese whilst consuming a high-fat (low-glycaemic) diet did not demonstrate reduced insulin sensitivity when compared with control animals. Furthermore, the SI values of horses and ponies which consumed a ration containing a once daily glycaemic load were increased despite the induction of obesity. These findings contradict our original hypothesis, which predicted that increased adiposity would be associated with a reduction in SI values. Therefore, obesity *per se* does not appear to be essential in the short-term induction of insulin dysregulation in horses and ponies.

Insulin sensitivity was assessed with a FSIGT, considered to be one of the most accurate quantitative methods used in the research setting [25]. Insulin sensitivity (SI), the measure of insulin-dependent glucose disposal, was increased in the GLU group, meaning that insulin was more effective at promoting glucose uptake from the bloodstream into the tissues. Insulin-independent glucose disposal (Sg) was also increased in the GLU group, meaning that glucose was more effective in promoting its own disposal. The acute insulin response to glucose (AIRg) quantifies endogenous insulin release in response to the intravenous glucose infusion, and was similar between groups. The disposition index (DI) is the multiplication product of SI x AIRg, and indicates whether AIRg is adequate relative to SI values [26]. In the present study, DI was lower in the FAT group, although the biological significance of this finding is not clear since SI values were not considered to be low.

The animals in this study were only obese for a short period of time. It therefore cannot be discounted that long-standing obesity might play a role in the pathogenesis of insulin dysregulation. Our timeframe was similar to a previous study that induced obesity alongside significant decreases in SI in mature Arabian horses [8]. The Arabian horses demonstrated slightly higher mean BCS (8.0 ± 0.7) than those reported in the FAT and GLU groups, whilst the TBFM of the Arabian horses was also higher than those reported in the present study. A comparison of specific TBFM values is difficult due to differences in TBFM assessment methodology (ultrasonographic rump fat depth vs. D₂O dilution). Although higher Week 20 BCS values might have been preferable, animal ethics approval prevented animals in the present study going beyond BCS 8.

Body condition scoring was used throughout the study as a practical, non-invasive means to assess progress. Horses and ponies in the FAT and GLU diet groups achieved a final BCS in the ‘obese’ range (≥ 7 out of 9). However, this method is only semi-objective and does not correlate with TBFM in a linear manner, especially at BCS greater than 6 out of 9 [27]. Total body fat mass percentage was determined using a D₂O dilution technique that has been validated against post mortem dissection, and is considered to be the ‘gold standard’ measurement of TBFM in the live horse [21]. The values for TBFM reported in the present study lie within the 95% confidence interval of the correlation with BCS reported by Dugdale et al. [27].

Studies which have induced IR in horses (with or without concurrent obesity) have utilised diets rich in sugar and starch, provided as multiple meals each day [8-10]. Glucose was chosen as the NSC source in this study, as it induces higher post-prandial glucose and insulin responses compared with fructose and inulin [28]. A study which quantified the glycaemic and insulinaemic responses of horses and ponies to a meal containing 1.5 g/kg bwt glucose has been previously reported by our group [14]. Glucose was provided in one meal per day to the GLU group to maximise peak postprandial glycaemic and insulinaemic responses. We aimed to make this peak as high as possible, and therefore chose a single meal, as a potential ‘second-meal effect’ [19] might have reduced insulin responses in the evening meal. The fat supplement was not added to the morning glucose-containing meal to avoid modification of the pancreatic insulin response. When the postprandial glycaemic and insulinaemic responses to the GLU meal were assessed in Week 12, there was no evidence of adaptation (reduced peak glucose or insulin concentrations) to the added glucose. The once daily nature of the GLU diet might have meant that insulin concentrations were not sustained for long enough to cause insulin receptor down-regulation. In fact, the GLU diet appeared to improve glucose and insulin dynamics, as evidenced by increased values for SI and Sg. Chronic stimulation of the pancreas by multiple ‘sweet feed’ meals per day might therefore be required to induce IR.

The FAT group consumed approximately 25% of daily DE content as fat, supporting previous findings that supplemental fat is well tolerated in horses [29]. High-fat diets are commonly used to induce IR in laboratory animal models of human type II diabetes mellitus [30]. These diets are often much higher in fat content (over 50% daily DE) than those reported in equine studies, and certainly SI was not found to be reduced in the FAT group. The similar rate of increase in BCS, and similar final TBFM in the FAT and GLU groups suggests that total ration digestibility was comparable between groups. There was no difference in daily hay intake between the FAT and GLU groups, meaning that the FAT diet did not suppress dry matter intake relative to the GLU diet.

A strength of the present study was the inclusion of a control population which maintained a moderate body condition throughout the study period. This allowed us to verify that any changes detected in the FAT and GLU groups were due to the effects of adiposity and diet, and not due to seasonal or management influences. Although measures of adiposity (TBFM, BCS, CNS) did not change in the CON group over the study period, there was an increase in body weight similar to that of the FAT and GLU groups. As total fat mass was not increased in the CON group, this interesting finding could therefore be due to an increase in gut fill (or less likely lean muscle mass). This point emphasises the need to monitor multiple morphometric measurements when assessing weight gain and loss in horses and ponies.

There are breed-related differences in the prevalence of obesity and laminitis among horses and ponies [31,32]. Standardbreds, ponies and Andalusians were enrolled to examine the effect of the study diets across a range of starting insulin sensitivities [14]. The study was underpowered to detect specific differences between breeds within each diet group, but the inclusion of these breeds was considered important in the study design to allow data to be extrapolated to the wider equine population. Although the numbers of horses in this study were small, we expected to see large decreases in SI values after the induction of obesity. It is important to note that all animals demonstrated increased SI and Sg values in the GLU group, regardless of breed.

Horses and ponies in the FAT and GLU groups demonstrated increases in plasma leptin at Week 20, at concentrations similar to those reported in previous studies of obese horses [23,33]. Leptin is secreted constitutively by adipocytes and plasma concentrations therefore reflect the adiposity of an individual. It has not been established whether a state of leptin resistance occurs in obese horses with insulin dysregulation, as has been ascribed to obese human patients with the metabolic syndrome [34,35]. Adiponectin concentrations were not different between diet groups in this study. Adiponectin has an inverse relationship with insulin concentrations in obese horses [7], and was decreased in ponies with a history of pasture-associated laminitis [36]. There were no changes in circulating TNF- α or SAA concentrations detected in this study. This finding supports recent work, in which cytokine-mediated inflammation was not associated with obesity or insulin dysregulation in horses [37]. Further work remains to fully elucidate the role of adipokines and proinflammatory cytokines in obese and IR horses.

This study demonstrates that although obesity is frequently associated with IR, increased adiposity *per se* may not directly impair insulin sensitivity. Increased adiposity was not associated with decreased SI when a diet containing only small amounts of NSC was used. Further studies are required to ascertain whether chronic obesity represents a different subset of risk factors for the development of insulin dysregulation. If we are to improve the management of horses and ponies at risk of laminitis, then a better understanding of the influence of diet on glucose and insulin dynamics is crucial.

Authors' declaration of interests

P.A.H. is both a collaborating author and an employee of WALTHAM, who part-funded this work. No competing interests have been declared for the other authors.

Ethical animal research

The use of animals in this study was approved by the University of Melbourne Animal Ethics Committee (ID 1011918).

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Authorship

N.J.B. contributed to study design, study execution, data analysis and interpretation, and drafted the manuscript. S.J.P. contributed to study design and study execution. P.A.H. contributed to study design, data interpretation, and revised the manuscript. S.R.B. contributed to study design, study execution, data interpretation, and revised the manuscript. All authors approved the final manuscript.

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^eStart to Finish, Eden Prairie, Minnesota, USA.

^fValue Plus Animal Health Care, Horsley Park, New South Wales, Australia.

^gCambridge Isotope Laboratories, Tewksbury, Massachusetts, USA.

^hBD Vacutainer, Plymouth, UK.

ⁱIso-Analytical Ltd., Crewe, UK.

^jActrapid, Novo Nordisk A/S, Bagsvaerd, Denmark.

^kCayman Chemical Co., Ann Arbor, Michigan, USA.

^lCoat-A-Count, Siemens Diagnostics, Los Angeles, California, USA.

^mMillipore, Billerica, Massachusetts, USA.

363 ⁿThermo Fisher Scientific, Scoresby, Victoria, Australia.

364 ^oTridelta Development, Maynooth, County Kildare, Ireland.

365 ^pVersion 6.02, University of Pennsylvania, Kennett Square, Pennsylvania, USA.

366 ^qVersion 22, IBM Corp., New York, New York, USA.

Tables

TABLE 1: Nutrient and ingredient composition of the study diets (Week 20). All feeds were sourced from single batches and multiple samples of each ingredient were combined for proximate analysis. Horses and ponies were fed either control (CON; n=6), high-fat (FAT; n=6) or high-glycaemic (GLU; n=6) supplementary feeds divided into two daily meals. The GLU group received dextrose powder in the morning meal only, and the fat supplement in the afternoon meal only (providing a once daily glycaemic stimulus).

	Hay	Supplementary feeds		
		CON	FAT	GLU
Energy				
DE (MJ/kg feed, DM basis)	7.1	9.4	16.4	14.8
DE (as fed; MJ/100 kg bwt)		3.8	13.1	13.1
Nutrient (%)				
CP	7.7	11.9	14.7	13.3
ADF	46.0	37.9	27.3	24.7
NDF	75.8	58.6	38.7	34.9
NSC	9.2	18.4	5.9	22.3
WSC	7.3	11.4	5.5	21.8
Starch	1.8	7.0	0.4	0.4
Fat	1.8	3.8	27.8	17.9
Ash	5.5	5.7	5.9	5.3
Ingredient (g/100 kg bwt)				
Soyahull pellets		200	300	300
Chaff		200	300	300
Fat supplement		0	200	137
Dextrose powder		0	0	150
Vitamin/mineral supplement		6	6	6

DE = digestible energy, DM = dry matter, CP = crude protein, ADF = acid detergent fibre, NDF = neutral detergent fibre, NSC = non-structural carbohydrates, WSC = water-soluble carbohydrates.

TABLE 2: Morphometric measurements (sample mean $\pm$ s.d.) of horses and ponies fed a control (CON; n=6), high-fat (FAT; n=6) or once daily high-glycaemic (GLU; n=6) diet. The CON group maintained moderate body condition whilst FAT and GLU groups became obese over a 20-week period. P values represent the effects on Week 20 values.

Variable	Week	Diet Group			P value		
		CON	FAT	GLU	Diet	Breed	Diet x Breed
BCS (1-9 scale)	0	5.0 $\pm$ 0.4	4.8 $\pm$ 0.4	4.9 $\pm$ 0.4			
	20	5.3 $\pm$ 0.6 ^a	7.3 $\pm$ 0.4 ^b	7.6 $\pm$ 0.4 ^b	<0.001	0.9	0.3
TBFM (%)	0	7.6 $\pm$ 6.5	8.8 $\pm$ 3.3	8.7 $\pm$ 4.5			
	20	6.2 $\pm$ 3.3 ^a	15.1 $\pm$ 3.3 ^b	15.0 $\pm$ 4.1 ^b	<0.001	0.5	0.2
CNS (1-5 scale)	0	1.6 $\pm$ 0.6	1.8 $\pm$ 0.9	1.5 $\pm$ 0.6			
	20	1.7 $\pm$ 0.4 ^a	3.0 $\pm$ 0.9 ^b	2.5 $\pm$ 0.6 ^b	<0.001	0.5	0.2
Δ BW (%)	0	-	-	-			
	20	9.2 $\pm$ 3.1	10.8 $\pm$ 3.6	12.3 $\pm$ 2.9	0.2	0.2	0.2

BCS = body condition score [15,16], TBFM = total body fat mass, CNS = cresty neck score [20],

Δ BW = change in bodyweight from Week 0. ^{a,b}Different superscript letters indicate significant

difference between diet groups at Week 20 (P<0.05).

TABLE 3: Frequently-sampled intravenous glucose tolerance test (FSIGT) results and basal plasma concentrations in groups of horses and ponies fed a control (CON; n=6), high-fat (FAT; n=6) or once daily high-glycaemic (GLU; n=6) diet. The CON group maintained moderate body condition whilst FAT and GLU groups became obese over a 20-week period. Data are presented as sample mean $\pm$ s.d. P values represent the effects on Week 20 values.

Variable	Week	Diet group			P value		
		CON	FAT	GLU	Diet	Breed	Diet x Breed
SI ($\times 10^{-4}$ /[mU·min])	0	2.6 $\pm$ 2.4	2.6 $\pm$ 1.3	2.6 $\pm$ 2.0			
	20	2.7 $\pm$ 0.9 ^a	2.6 $\pm$ 1.3 ^a	4.7 $\pm$ 1.7 ^b	0.006	0.6	0.7
AIRg ([mU·min]/L)	0	165 $\pm$ 82	267 $\pm$ 141	289 $\pm$ 122			
	20	182 $\pm$ 58	179 $\pm$ 121	197 $\pm$ 95	0.8	0.2	0.1
DI ($\times 10^{-2}$)	0	3.0 $\pm$ 2.0	6.1 $\pm$ 4.0	5.5 $\pm$ 3.7			
	20	5.7 $\pm$ 3.3 ^a	3.2 $\pm$ 1.4 ^b	8.7 $\pm$ 4.8 ^a	0.005	0.3	0.3
Sg ($\times 10^{-2}$ /min)	0	0.8 $\pm$ 0.5	0.9 $\pm$ 0.5	0.9 $\pm$ 0.5			
	20	1.4 $\pm$ 0.7 ^a	1.5 $\pm$ 0.8 ^a	2.6 $\pm$ 0.8 ^b	0.03	0.3	0.3
Glucose (mmol/L)	0	4.8 $\pm$ 0.4	4.8 $\pm$ 0.4	4.8 $\pm$ 0.6			
	20	5.1 $\pm$ 0.4	5.0 $\pm$ 0.3	5.1 $\pm$ 0.4	0.9	0.1	0.5
Insulin (mU/L)	0	4.0 $\pm$ 1.6	2.8 $\pm$ 1.3	4.1 $\pm$ 0.9			
	20	4.0 $\pm$ 2.1 ^a	4.6 $\pm$ 1.3 ^a	8.5 $\pm$ 3.6 ^b	0.04	0.7	0.9
Leptin (ng/mL)	0	1.5 $\pm$ 1.2	1.6 $\pm$ 1.0	1.3 $\pm$ 0.9			
	20	2.0 $\pm$ 0.8 ^a	6.5 $\pm$ 2.5 ^b	6.7 $\pm$ 3.2 ^b	0.003	0.7	0.4
Adiponectin (μ g/mL)	0	4.3 $\pm$ 4.1	3.2 $\pm$ 2.8	3.6 $\pm$ 1.7			
	20	4.4 $\pm$ 3.9	3.1 $\pm$ 2.5	3.5 $\pm$ 1.6	0.9	0.2	0.5
TNF- α (ng/mL)	0	2.1 $\pm$ 1.6	1.2 $\pm$ 1.1	0.7 $\pm$ 0.5			
	20	2.0 $\pm$ 1.5	0.7 $\pm$ 0.5	0.7 $\pm$ 0.5	0.4	0.9	0.3
SAA (μ g/mL)	0	1.9 $\pm$ 0.9	0.9 $\pm$ 0.6	1.7 $\pm$ 1.0			
	20	4.3 $\pm$ 2.1	5.5 $\pm$ 3.2	6.7 $\pm$ 2.2	0.7	0.5	0.8

SI = insulin sensitivity, AIRg = acute insulin response to glucose, DI = disposition index, Sg = glucose effectiveness, TNF- α = tumour necrosis factor- α , SAA = serum amyloid A. ^{a,b}Different superscript letters indicate significant difference between diet groups at Week 20 (P<0.05).

392 **Figure legends**

393

394 **FIGURE 1:** Body condition score (BCS; A) and percentage change in body weight (Δ BW; B)
395 measured weekly in the control (CON; n=6), high-fat (FAT; n=6) and high-glycaemic (GLU; n=6)
396 diet groups over a 20-week period. Points represent mean $\pm$ s.d.

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