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Effects of age and diet on glucose and insulin dynamics in the horse

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Summary

Background: Age and diet may affect insulin sensitivity (SI) but these factors have received limited investigation in horses.
Objectives: To measure minimal model parameters during an insulin-modified frequently sampled intravenous glucose tolerance test (FSIGTT) after adaptation to a forage only diet (HAY) or forage supplemented with either starch/sugar-rich (SS) or oil/fibre-rich (FF) concentrate feeds; and to assess glucose and insulin responses to a standardised meal challenge (SMC, 4 g/kg BW of SS) after diet adaptation in adult and aged mares.
Study design: Latin square design with eight adult (5–12 years) and nine aged (>19 years) healthy mares.
Methods: Diets were fed for 6 weeks, and the FSIGTT and SMC were performed after 31–32 and 41 days on each diet respectively. Data were analysed by a mixed ANOVA for repeated measures.
Results: Acute insulin response to glucose (AIRg) was greater and SI was lower in aged horses, compared with adults, regardless of diet. Both AIRg and SI were greater in aged mares after adaptation to SS, as compared with HAY. Similar trends, although not statistically significant, were observed after adaptation to SS in adult mares. Peak insulin concentration and area under the insulin vs. time curve during the SMC were greater in aged than adult mares with all diets. Furthermore, area under the glucose vs. time curve was lower after adaptation to SS, when compared with other diets, in both groups.
Main limitations: Transient weight loss occurred at the beginning of the study and only one sex was included. Incomplete ingestion of the SMC by four mares was another limitation.
Conclusions: Insulin responses to i.v. and enteral nonstructural carbohydrate challenge increase with age in healthy horses, regardless of diet fed.

Keywords: horse; laminitis; nonstructural carbohydrate; fat; pasture

Introduction

Factors affecting insulin sensitivity (SI) in equids include breed, body condition, pregnancy and lactation, physical activity, disease conditions, age and diet [1–5]. With respect to the latter two, ageing is associated with development of glucose intolerance and insulin resistance (IR) in people, manifested by exaggerated glucose and insulin responses to carbohydrate (CHO) challenge [6–8]. Greater insulin responses to enteral glucose challenge [9], as well as to cereal grain meals rich in hydrolysable CHO [10], have also been documented in mature horses, as compared with young horses. These findings support an age-related decrease in SI in this species as well. More recently, the term insulin dysregulation (ID) has been introduced in the equine literature to describe multiple factors that may increase serum insulin concentration, including exaggerated pancreatic response to CHO ingestion, decreased liver insulin clearance, and alterations in insulin effects in peripheral tissues (IR) [11]. Importantly, ID, especially when coupled with genetic predisposition and obesity, is considered a risk factor for development of laminitis in equids [2,5,12–14].

Diets high in nonstructural CHOs (NSC) have also been implicated in development of IR and type 2 diabetes in human subjects, even after controlling for other risk factors [15]. When horses ingest large amounts of NSCs, either in lush pasture grass or when forage is supplemented with cereal grains, greater post-prandial glucose and insulin responses, along with decreased SI have been documented [10,16–18]. Ingestion of diets high in NSC may further affect glucose and insulin dynamics by altering the enteroinsular axis and pancreatic insulin release [4,19]. As a consequence, when equids require greater caloric intake to meet demands of exercise or lactation, use of oil-fortified feeds has been recommended to decrease the risk of inducing or exacerbating ID [20].

To date, there has been limited investigation of the interaction of age and adaptation to NSC-rich or oil-fortified feeds on glucose and insulin dynamics in healthy, nonobese horses. Bamford and colleagues [21] recently reported an increase in acute insulin response to glucose administration (AIRg) and a decrease in SI after 20 weeks of feeding a NSC-rich feed; however, horses and ponies in that study also became obese with increased caloric intake. Similar changes in glucose and insulin dynamics were not found in horses and ponies adapted to an oil-fortified feed, which induced a similar degree of obesity over 20 weeks, leading these authors to speculate that adaptation to the NSC-rich feed was a more important factor altering glucose and insulin dynamics than short-term induction of obesity. However, their study did not examine potential confounding effects of age. The objectives of this study were: 1) to measure minimal model parameters during an insulin-modified frequently sampled intravenous glucose tolerance test (FSIGTT) after adaptation to a forage only diet or a forage diet supplemented with either a cereal grain-based, starch- and sugar- (hydrolysable CHO) rich or an oil and fibre-rich concentrate feed; and 2) to assess glucose and insulin responses to a standardised hydrolysable CHO-rich meal challenge (SMC) after diet adaptation in groups of healthy adult and aged horses, without development of obesity. It was hypothesised that aged horses, as compared with adult horses, would have a greater AIRg and lower SI during the FSIGTT, regardless of diet. Similarly, glucose and insulin responses to the SMC were hypothesised to be greater in aged than adult horses, again regardless of diet. Finally, in both age groups, adaptation to a cereal grain-supplemented diet was hypothesised to produce a greater AIRg and a lower SI during the FSIGTT and greater glucose and insulin responses to the SMC, as compared with the other diets.

Materials and methods

Horses and housing

Seventeen mares, including eight adult (8.1 ± 1.6 [s.d.], range 5–12 years) and nine aged (21.9 ± 1.6 [s.d.], range 19–24 years) horses, were studied.

Breeds included 12 stock-type horses (five adult and seven aged), four Thoroughbreds (two adult and two aged) and one Standardbred (adult). Aged mares weighed less than adult horses (455 ± 12 vs. 500 ± 13 kg, P = 0.02) throughout the study but body condition score (BCS scale 1–9 [22]) was not different between age groups (median [range] 5 [4–7] for adults and 5 [3–6] for aged, P = 0.20). Prior to the study start, all horses were deemed healthy on basis of physical examination. None had phenotypic characteristics of Equine Metabolic Syndrome [2], including regional adiposity or a history of or hoof changes consistent with laminitis, although one adult mare with a BCS of 7 could be considered obese. All horses were administered a dose of ivermectin paste and had a complete oral examination to ensure presence of all premolars/molars and minor dental abnormalities were corrected. All aged mares had normal overnight dexamethasone suppression test results (ODST, cortisol suppression [<1.0 ug/dL] 19 h after dexamethasone administration, 40 μg/kg, i.m. [23]) and lacked clinical signs of pituitary pars intermedia dysfunction (PPID) [24]. An ODST was not performed in adult mares due to the absence of clinical signs and low likelihood of PPID in horses <12 years [24]. Horses were grouped as pairs, one adult and one aged mare (excepting one aged mare), and two or three pairs were maintained in snow covered paddocks or dry lots to minimise pasture access (January–June). Horses were group fed while housed in paddocks, although individual feeding bins were used for the complementary feeds.

Study design

Mares were studied in a Latin square design and fed three diets for 42 days: a grass hay forage only diet (HAY); forage supplemented with a high starch and sugar (SS, rich in hydrolysable CHO) cereal grain-based complementary feed (Pleasure Sweet, Buckeye® Nutrition)[a] or forage supplemented with a low starch and sugar, oil- and fibre-rich complementary feed (Equilibrium Growth, Winergy®)[a] (FF). After 21 days on each diet, pairs were moved indoors and housed in adjacent stalls (ambient temperature, 15 ± 0.7°C) in order to be fed individually for an additional 20 days. While housed in stalls, horse pairs were turned out for exercise in a dry lot for 2 h, 3 days each week. The start of each diet period was staggered by either 2 or 4 weeks due to the limited number of stalls available; consequently, testing was performed with groups of 5–6 mares at one time. Mares were initially fed 1.6% of body weight (BW), as fed, divided into two equal feedings (07.00 and 17.00 h): HAY = 1.6% BW hay; SS or FF = 1.0% BW hay and 0.6% BW SS or FF. On day 22 and 41 of each period, horses were weighed and BCS was assessed by three trained individuals. By day 41 of the first period for the initial cohort of mares studied (February), BW had decreased (~16 [3.5%] and ~27 kg [5.4%] for aged and adult horses, respectively). Consequently, for the remainder of the study, the amount fed was increased to 1.84% BW, as fed: HAY = 1.84% BW hay; SS or FF = 1.15% BW hay and 0.69% BW SS or FF.

Insulin-modified frequently sampled intravenous glucose tolerance test (FSIGTT)

An insulin-modified FSIGTT [16,25] was performed on day 31 or 32 of each feeding period (two or three horses each day), allowing a minimum of 30 days for adaptation to each diet. During the afternoon prior to testing, 13.3 cm, 14 G polyurethane catheters were aseptically inserted into both jugular veins and patency was maintained by injection of 1 mL of Na heparin (1000 IU/mL). Mares were fasted overnight (~12 h) prior to testing. Starting at 09:00, two baseline blood samples were collected 15 min apart prior to glucose (Dextrose 50% solution)[b] administration (0.1 g/kg, i.v. bolus), followed 20 min later with insulin (Novolin R)[c] administration (20 mU/kg, i.v. bolus). Blood samples were collected from the opposite i.v. catheter at −15, −1, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150 and 180 min after glucose administration. At each sampling time, a 15 mL blood sample was collected with 4 mL transferred to a plastic tube containing lithium heparin, placed on ice and centrifuged (1500 ***g*** for 15 min at 5°C) within 30 min of collection, and 10 mL transferred to a tube without anticoagulant that was allowed to clot at room temperature for 2 h prior to centrifugation. Plasma and serum were harvested and stored at −80°C until analysis.

Standardised meal challenge (SMC)

On the final day of each feeding period (day 42) while still housed indoors, horses were fed a standardised amount of the cereal based feed (4 g/kg BW of SS) without hay for their morning feeding. As with the FSIGTT, a single catheter was inserted into the jugular vein during the afternoon prior to the SMC and mares were fasted overnight (~12 h) prior to testing. Three baseline blood samples were collected during the 30 min prior to the SMC (offered at 09.00) to determine baseline serum glucose and insulin concentrations. Feed remaining 1 h after offering the SMC was removed and weighed. Blood samples were collected 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240 and 360 min after the meal was offered. At each time point, a 15 mL blood sample was collected and processed as described for the FSIGTT.

Due to the staggered start of diet period one, six FSIGTT and SMC tests were performed when mares were on the lower plane of nutrition (1.6% BW, two mares on each diet) while all other tests were performed after at least 1 week on the higher plane of nutrition (1.84% BW). The study protocol and all procedures performed were approved by the Michigan State University Institutional Animal Care and Use Committee (approval #11/09-174-00).

Sample analysis

Hay and complementary feed samples, all from a single source, were collected several times during the course of the study and proximate analysis of pooled, well-mixed samples was performed [26]. Additional aliquots of pooled feed samples were submitted for analysis of starch, water-soluble CHO and ethanol-soluble CHO (Dairy One)[d]. Plasma glucose concentrations were measured in duplicate using a membrane based-glucose oxidase system to catalyse oxidation of glucose to gluconic acid and hydrogen peroxide (YSI 2300 STAT Plus Glucose and Lactate Analyser)[e]. Serum insulin concentrations were determined in duplicate using a radioimmunoassay (Coat-a-Count Insulin)[f] previously validated for equine samples [27]. The interassay CV was 9.3% and intraassay CVs were 5.2 and 8.6% for high and low control samples, respectively.

Calculations and data analysis

A sample size of nine horses of each age group was selected based on a calculated estimate of eight horses per group required to demonstrate statistical significance for a 25% difference in outcome measures, with a variance of up to 80% off the difference, a P value of 0.05, and a power of 0.8 estimated (GraphPad Prism, version 6.0)[g]. SI, AIRg, glucose effectiveness (Sg) and disposition index (DI = SI × AIRg) were calculated by minimal model analysis of glucose and insulin data from the FSIGTT [28]. For the SMC, peak glucose and insulin concentrations and time to peak concentrations were determined, and area under the 360 min curves for glucose (AUCg) and insulin (AUCi) were calculated by the trapezoidal method, using time 0 values as baseline, with commercially available computer software[g]. Outlier data points were identified by use of the Grubbs test, with a critical value of P≤0.05. One outlier (adult) for the FF diet was removed from analysis of insulin response during the SMC. Differences between age groups and diets for body weight, BCS, minimal model variables and SMC were determined via unpaired *t* tests and mixed ANOVA for repeated measures with Tukey–Kramer post hoc adjustment (SAS version 9.4)[h]. Pearson correlation analysis was also performed to compare AUCg and AUCi[g]. Data in the text, Tables and Figures are presented as means ± s.e.m., unless otherwise indicated, and significance was set at P≤0.05.

Results

Nutrient composition of the three feedstuffs is presented in Table 1. The SS complementary feed contained 43% NSC, ~3 and 4 times more than FF and HAY, respectively, while the FF feed contained 11% fat, ~1.5 and 2.3 times more than SS and HAY respectively. When either supplemental feed was combined with hay, digestible energy provided was ~12% (FF) to ~19% (SS) greater when compared with HAY alone: 40.6 ± 0.9, 45.6 ± 0.7 and 48.3 ± 0.9 kcal/kg per day for HAY, FF and SS, respectively, (P = 0.002,

TABLE 1: Nutrient composition of the three feedstuffs (HAY = grass hay; SS = cereal grain, sugar- and starch-rich feed; and FF = oil- and fibre-rich concentrate feed) and the composite diets (HAY+SS and HAY+FF), expressed on a dry matter basis, determined by proximate analysis of well-mixed composite samples of several aliquots of each feedstuff collected multiple times during the study

Component	SS	FF	HAY	HAY+SS	HAY+FF
NDF (%)	25.0	42.3	61.4	38.4	54.2
CP (%)	13.2	14.9	7.9	9.6	10.3
Fat (%)	5.3	8.3	3.6	4.2	5.3
Calcium (g/kg)	11.4	15.9	7.9	9.2	10.9
Phosphorus (g/kg)	7.5	5.4	1.6	3.8	3.0
Lignin (%)	2.8	4.1	6.9	5.4	5.9
WSC (%)	7.6	8.6	10.6	9.5	9.9
ESC (%)	7.0	6.9	5.7	6.2	6.2
Starch (%)	35.2	5.4	0.5	13.5	2.3
NSC (%, starch + WSC)	42.8	14	11.1	23.0	12.2

NDF, neutral detergent fibre; CP, crude protein; WSC, water-soluble carbohydrates; ESC, ethanol-soluble carbohydrates; NSC, nonstructural carbohydrate.

with SS and FF significantly greater than HAY). As mentioned, BW decreased (P = 0.03) in both age groups from barn entry on day 21 to day 41 during period 1, regardless of diet (Table 2). BW loss was attributed to time of year (winter) and a transient period of decreased feed intake when horses were initially placed into stalls during period 1, despite having been previously housed in the same facility. Once the amount of feed provided was increased, BW and BCS tended to increase a small amount over the remainder of the study in both adult and aged mares (Table 2).

Minimal model parameters

AIRg was significantly affected by age (P = 0.02), with mean AIRg greater in aged compared with adult mares across all diets. In aged mares AIRg was lower (P = 0.03) after adaptation to the HAY diet when compared with SS; AIRg was also numerically lowest after adaptation to HAY in adult mares but the difference did not reach statistical significance. SI was significantly greater (P = 0.003) in adult when compared with aged mares, regardless of diet (Table 3). Furthermore, a significant age × diet interaction (P = 0.002) was also detected. For adult mares, there was no difference in SI among the three diets but in aged mares SI was lower with HAY and FF when compared with SS (Table 3). Although there was no statistically significant difference (P = 0.08) between age groups, DI was greater (P = 0.004) after adaptation to the SS diet, as compared with the HAY diet. In adult mares, mean DI was also greater (P = 0.005) with FF than HAY. There were no significant differences in Sg with either age or diet (Table 3).

Insulin and glucose dynamics during the SMC

Glucose (Fig 1) and insulin (Fig 2) concentrations reached peak values within 90–120 min after the meal was offered. There was no effect of age (P = 0.32) or diet (P = 0.10) on peak glucose concentration after eating a meal rich in hydrolysable CHO. Similarly, time to peak glucose concentration was not affected by age (P = 0.13) or diet (P = 0.10). In contrast, peak insulin concentration, but not time to peak concentration, was greater (P = 0.03) in aged when compared with adult mares. There was no effect of diet on peak (P = 0.80) or time to peak (P = 0.18) insulin concentration (Table 4).

AUCg was not different (P = 0.66) between age groups, regardless of diet (Table 4). However, AUCg was lower (P = 0.04) after adaptation to SS, in comparison to HAY, in both age groups. AUCi was lower (P = 0.03) in adult mares, as compared with aged mares, regardless of diet (Table 4). Although AUCi in adult horses was 40% lower after adaptation to SS, as compared with the other diets fed to this age group, this was not a statistically significant finding (P = 0.29). Not surprisingly, AUCg and AUCi were positively correlated (r = 0.44, P = 0.003).

A limitation of the SMC was incomplete ingestion, defined as leaving more than 0.4 kg (~20%) of the meal after 60 min, by four mares (two adult and two aged mares) in eight trials (two, four and two of these instances occurring after adaptation to HAY, FF and SS, respectively). When data for these eight studies were compared with data for the remaining 36 studies during which >90% of the meal was ingested, AUCg was lower (P = 0.03) for the eight studies with partial meal consumption but there was no difference in AUCi (P = 0.78) (Fig 3).

Discussion

In the study reported here, glucose and insulin dynamics were affected by age and diet in healthy horses. Consistent with findings of Liburt and coworkers [29], AIRg was greater and SI was lower in aged mares when compared with adult mares. Notably, diet did not significantly affect AIRg or SI in adult mares; whereas, both SI and AIRg were greater in aged mares after adaptation to SS when compared with HAY (and FF for SI). These age- and diet-associated differences in glucose and insulin dynamics in response to i.v. glucose challenge were, for the most part, corroborated by findings during the SMC. Specifically, peak insulin concentration and AUCi were greater in aged than in adult mares with all diets. In contrast to our hypothesis, however, AUCg in aged mares was lower after adaptation to SS when compared with the other diets, although this finding was consistent with the greater SI observed in aged mares adapted to this diet.

A greater increase in circulating insulin concentration, in response to both i.v. glucose (AIRg) and ingestion of the SMC, in aged mares could be a consequence of increased pancreatic insulin release, decreased insulin clearance or a combination of the two. A growing body of evidence in other species suggests that insulin action on target tissues decreases with age, due to decreased responsiveness of insulin signalling pathways that lead to translocation of GLUT-4 vesicles to the plasma membrane [8,30–33]. Waller and colleagues [34] demonstrated decreased basal and insulin-stimulated skeletal muscle cell surface GLUT-4 expression in IR mares, as compared with insulin sensitive mares, despite similar skeletal muscle cell GLUT-4 content. This finding suggests that insulin signalling pathways are also dysfunctional in IR horses, but effects of age were not evaluated in that study. Additional factors that also contribute to development of IR with ageing in human subjects include reduced physical activity, decreased lean muscle mass, increased visceral adiposity, and inflammation and

TABLE 2: Body weights (BW) and body condition scores (BCS: 1-9/9) at barn entry (day 22) and at the end (day 41) of each feeding period in eight adult horses and nine aged horses. Values reported as means ± s.e.m.

		Period 1		Period 2		Period 3	
	Group	Day 22	Day 41	Day 22	Day 41	Day 22	Day 41
BW (kg)	Adult*	509 ± 10^{a}	482 ± 12^{x}	517 ± 12^{a}	522 ± 12^{y}	518 ± 11^{a}	520 ± 7^{y}
	Aged	466 ± 13^{ab}	449 ± 13^{x}	456 ± 15^{a}	453 ± 17^{x}	464 ± 15^{b}	464 ± 16^{y}
BCS (1–9)	Adult	5.1 ± 0.1^{a}	4.9 ± 0.2^{x}	5.2 ± 0.1^{ab}	5.3 ± 0.1^{xy}	5.4 ± 0.2^{b}	5.6 ± 0.2^{y}
	Aged	4.8 ± 0.3^{ab}	4.6 ± 0.2^{x}	4.5 ± 0.3^{a}	4.7 ± 0.3^{x}	5.0 ± 0.3^{b}	5.1 ± 0.3^{x}

*Indicates significant (P<0.05) difference between adult and aged horses for all times; within age groups, means with different superscript letters across horizontal rows differ at P<0.05 (^{a,b} for day 22 [barn entry] and ^{x,y} for day 41 [period end]).

TABLE 3: Acute insulin response to glucose administration (AIRg), insulin sensitivity (SI), glucose effectiveness (Sg) and disposition index (DI) determined by minimal model analysis of glucose and insulin data obtained from an insulin-modified frequently sampled i.v. glucose tolerance test performed in eight adult horses and nine aged horses that had been adapted to three diets for 4 weeks: HAY (grass hay only); SS (hay plus a cereal grain feed rich in hydrolysable CHO [sugar and starch feed]); and FF (hay plus a fat- and fibre-rich concentrate feed). Values reported as means ± s.e.m.

	Group	HAY	SS	FF
AIRg ([mU/L] per min)	Adult*	113.1 ± 20.4	157.9 ± 24.7[a]	140.4 ± 19.2[a]
	Aged	209.7 ± 29.5[a]	286.0 ± 35.5[b]	238.1 ± 28.5[ab]
SI (L/min/mU)/10^4	Adult*	2.52 ± 0.53	3.64 ± 0.55[a]	3.39 ± 0.52[a]
	Aged	0.77 ± 0.27[a]	2.19 ± 0.35[b]	0.99 ± 0.24[a]
Sg (per min/10^2)	Adult	1.87 ± 0.25	2.73 ± 0.32	2.34 ± 0.27
	Aged	1.81 ± 0.23	1.90 ± 0.17	1.80 ± 0.21
DI/(10^2)	Adult	2.41 ± 0.76[a]	6.22 ± 1.1[b]	5.11 ± 0.78[b]
	Aged	1.56 ± 0.65[a]	5.13 ± 0.89[b]	2.45 ± 0.67[a]

*Indicates significant ($P<0.05$) difference between adult and aged horses for all dietary treatments. Within age group, means with different superscript letters across horizontal rows differ at $P<0.05$.

oxidative stress leading to mitochondrial dysfunction [8]. As a consequence, a greater pancreatic β-cell insulin release is required to re-establish euglycemia following a meal. Similar to human subjects, aged horses tend to have less structured physical activity and decreased muscle mass (supported by a lower BW despite similar size in aged mares in this study) and these factors likely contribute to ageing-associated ID in this species as well. In support, exercise training of aged mares has been shown to decrease AIRg and improve SI, although the former was not a significant finding [29]. Next, age-associated decreases in hepatic and renal function could result in decreased insulin clearance, prolonging half-life and duration of an increased circulating insulin concentration. Finally, although all aged mares had normal ODST results, this test is insensitive for detection of earlier stages of PPID [35]. Thus, although differences in function of the hypothalamic–pituitary–adrenal axis between adult and aged mares cannot be excluded as an additional factor contributing to ID in aged horses, a recent study found no difference in glucose infusion rate during an isoglycaemic hyperinsulinaemic clamp in aged horses with and without PPID [36].

With regard to diet, our hypothesis that AIRg would be greater after adaptation to SS, when compared with HAY or FF, was only supported in aged horses (AIRg was numerically highest in adult horses after adaptation to SS, but this was not a statistically significant finding). In contrast, our hypothesis that SI would be lower after adaptation to SS, as compared with HAY or FF, was refuted. In fact, SI was greatest in aged horses after adaptation to SS (again, although not a statistically significant finding, SI was also numerically highest after adaptation to SS in adult horses). Variable changes in AIRg and SI have also been reported in recent studies of Andalusian and Standardbred horses and ponies (aged 5–19 years) fed NSC-rich or oil-fortified diets for 20 weeks, with a goal of inducing obesity-associated IR [21,37]. Specifically, feeding supplemental glucose (1.5 g/kg BW) added to a concentrate meal once daily resulted in a nonsignificant decrease in AIRg while, similar to our findings in aged mares, SI nearly doubled [37]. However, when a progressively increasing amount of micronised maize (ultimately providing 3.34 g/kg BW of additional NSC daily) was fed twice daily, AIRg nearly doubled and SI decreased by more than 50% [21]. The latter findings were consistent with the increase in AIRg and decrease in SI reported when even greater obesity was induced in Arabian geldings (aged 8–20 years) overfed with a commercial sweet feed for 16 weeks [18]. In contrast, when obesity was induced with overfeeding oil-fortified feeds, changes in AIRg and SI were not observed [21,37]. These disparate findings led to the conclusion that development of obesity and IR may be functionally uncoupled, at least during short-term induction of obesity [21]. These authors further speculated that once daily intake of glucose may not produce an insulin response lasting long enough to downregulate insulin receptors and induce IR; rather more frequent exposure to a high NSC diet (as either multiple NSC-rich meals or pasture access) may be necessary to alter SI. Finally, unlike our study, age range of horses used in these studies was wide and potential confounding effects of ageing-associated changes in glucose and insulin dynamics were not considered. It also warrants emphasis that the mares in our study did not gain weight during the feeding periods and that SI increased after adaptation to the SS diet (fed twice daily), at least for the aged mares. All in all, these apparently conflicting results of effects of feeding NSC-rich meals on AIRg and SI warrant further study and emphasise the importance of providing details of animal age, BW and BCS in study methods.

Disposition index, the product of AIRg and SI, was nearly threefold greater in both age groups after adaptation to SS, supporting greater β-cell responsiveness to i.v. glucose administration [38]. In contrast, the ability of glucose to drive its own disposal (tissue uptake) without the influence of insulin (Sg) was not different between diets or age groups. This finding

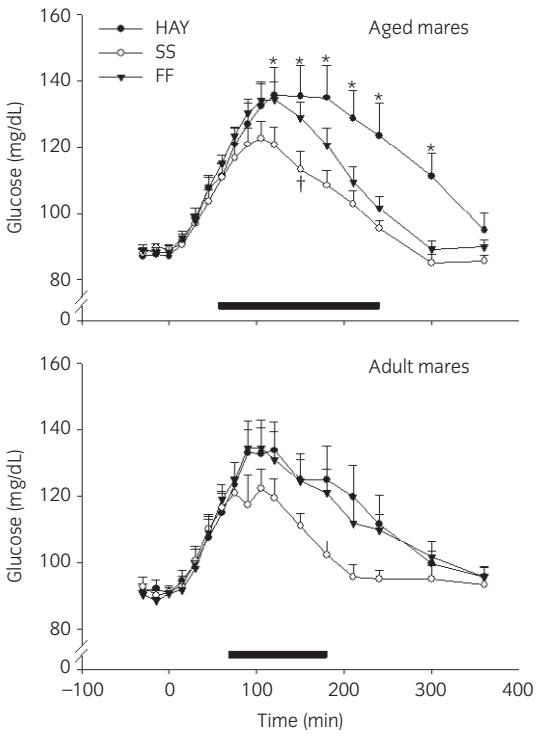

Fig 1: Mean ± s.e.m. glucose concentrations in response to the standardised meal challenge (4 g/kg SS concentrate feed offered for 60 min) in aged (upper panel) and adult (bottom panel) mares. The filled bars above the x-axes indicate time points that were different ($P<0.05$) from baseline; asterisks indicate time points at which SS<HAY ($P<0.05$); cross indicates a time point at which SS<FF ($P<0.05$).

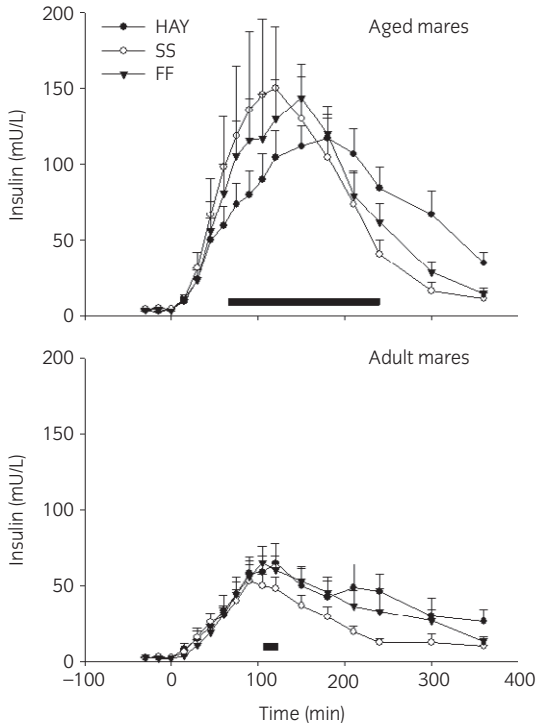

Fig 2: Mean ± s.e.m. insulin concentrations in response to the standardised meal challenge (4 g/kg SS concentrate feed offered for 60 min) in aged (upper panel) and adult (bottom panel) mares. The filled bars above the x-axes indicate time points that were different (P<0.05) from baseline.

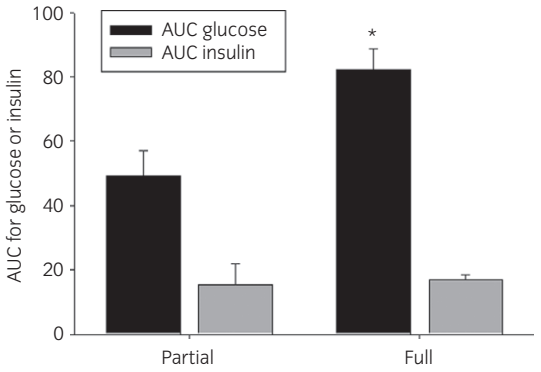

Fig 3: Mean ± s.e.m. area under the curves for glucose (AUCg ([mg/L] per min)/10^3, black fill) and insulin (AUCi ([mU/L] per min)/10^3, grey fill) in mares that consumed >90% (full) of the SS meal in the standardised meal challenge (n = 36 studies) as compared with mares that left more the 20% (partial) of the SS meal after 60 min (*denotes P<0.05).

suggests that neither age nor diet had a significant effect on peripheral tissue glucose transporters that are not regulated by insulin (i.e. GLUT-1 and GLUT-12) [34,39].

The finding of a lower AUCg response to the SMC after adaptation to SS in both age groups was also contrary to our hypothesis. However, as reported in other species, adaptation to SS may have increased small intestinal capacity for glucose uptake by upregulation of sodium/glucose cotransporters (SGLT1) on enterocytes [40,41]. Increased amounts of luminal sugars can also lead to greater secretion of incretins from enteroendocrine cells that are dispersed within the intestinal mucosa [40]. Both upregulation of SGLT1 transporters and increased activity of the enteroinsular axis after adaptation to SS should increase the rate of glucose absorption. Thus, the lower AUCg response to the SMC after adaptation to SS in both age groups could potentially be explained by increased intestinal glucose uptake in combination with enhanced hepatic and peripheral tissue glucose disposition, blunting the rise in circulating glucose concentration.

There were several limitations of this study. First, transient weight loss occurred in the first cohort of horses studied in period 1. However, this problem occurred with both age groups and across diets. Second, only one sex was studied. Third, not all horses ate the full SMC. Fortunately, incomplete ingestion of the SMC was similar across age groups and diets, minimising any confounding effect. It warrants emphasis that the SMC was designed to be an integrated assessment of intestinal CHO absorption, the enteroinsular axis and tissue glucose disposition, rather than an attempt to develop a novel diagnostic test for IR. Incomplete meal ingestion would represent a substantial limitation as a diagnostic test, as compared with

TABLE 4: Measures of glucose and insulin responses before, during and after the standardised CHO-rich meal challenge (4 g/kg of the SS cereal grain feed) administered in eight adult horses and nine aged horses that were adapted to three diets for 6 weeks: HAY (grass hay only); SS (hay plus a cereal grain feed rich in nonstructural CHO [sugar and starch feed]); and FF (hay plus a fat- and fibre-rich concentrate feed). Values are reported as mean ± s.e.m.

	Group	HAY	SS	FF
Basal glucose (mg/L)	Adult	90.9 ± 2.7	90.6 ± 1.7	90.3 ± 2.4
	Aged	87.3 ± 3.0	89.5 ± 1.9	88.4 ± 2.7
Peak glucose (mg/L)	Adult	139.2 ± 6.5	124.2 ± 4.8	144.8 ± 9.3
	Aged	142.9 ± 8.1	123.5 ± 4.4	134.3 ± 5.9
Time to peak glucose (min)	Adult	122.5 ± 12.0	105.0 ± 4.6	108.8 ± 6.8
	Aged	136.9 ± 9.8	106.9 ± 7.3	118.3 ± 7.1
AUCg ([mg/L] per min)/10^3	Adult	88.0 ± 15.9[a]	49.1 ± 12.0[b]	81.2 ± 16.0[a]
	Aged	112.8 ± 15.3[a]	52.8 ± 8.5[b]	79.2 ± 10.1[ab]
Basal insulin (mU/L)	Adult	2.7 ± 0.2	2.8 ± 0.6	3.3 ± 0.3
	Aged	4.6 ± 1.0	4.9 ± 0.8	4.0 ± 0.3
Peak insulin (mU/L)	Adult*	64.5 ± 11.0	53.4 ± 7.9	65.2 ± 8.5
	Aged	117.8 ± 16.4	151.6 ± 25.6	144.2 ± 19.9
Time to peak insulin (min)	Adult	120.0 ± 14.8	97.5 ± 3.7	113.6 ± 12.5
	Aged	185.6 ± 36.1	125.6 ± 17.7	143.3 ± 18.4
AUCi ([mU/L] per min)/10^3	Adult*	12.9 ± 2.7	8.1 ± 1.3	12.2 ± 2.2
	Aged	24.1 ± 3.0	22.6 ± 5.6	26.4 ± 3.9

*Indicates significant (P<0.05) difference between adult and aged horses for all dietary treatments. Within age group, means with different superscript letters differ at P<0.05.

oral sugar administration [42]. In an attempt to address some of these limitations and more specifically investigate the effects of different CHO-rich diets, a subsequent and recently published study from our group investigated glucose and insulin dynamics in healthy nonobese adult and aged Thoroughbred and Standardbred horses [43]. Similar to the findings of our study, aged horses were found to have a greater AIRg, regardless of diet, while adaptation to both starch and sugar-rich diets improved SI in both adult and aged horses, compared with when fed the control diet. In contrast to our findings, however, even after adaptation to these starch- and sugar-rich diets, and these changes in SI, feeding such diets resulted in a greater insulin response, especially in aged horses. These apparently contradictory results illustrate the complexity of factors, including age, breed, and diet, that may influence glucose and insulin responses to enteral CHO challenge in horses.

Conclusions

Insulin responses to i.v. or enteral NSC challenge increase with age in healthy horses, regardless of diet fed. The aged horses in this study were not overweight and did not have evidence of β-cell failure or PPID. Curiously, glucose clearance (AUCg) during the SMC improved in both age groups after adaptation to the SS. This finding could suggest that addition of some NSC to a forage diet may actually sensitise the enteroinsular axis and enhance post-prandial glucose uptake and clearance, regardless of age. However, this finding was not confirmed in a subsequent study by our group in which adaptation to starch- and sugar-rich diets produced what would be considered an undesirable greater post-prandial insulin response [43]. Thus, further studies are needed before manipulation of dietary NSC content can be made, in an attempt to limit the risk of laminitis in at risk equids.

Authors' declaration of interests

J.L. Rapson, H.C. Schott II, B.D. Nielsen, L.J. McCutcheon and R.J. Geor have no conflicts of interest. P.A. Harris is an employee of the WALTHAM Centre for Pet Nutrition.

Ethical animal research

The study protocol and all procedures performed were approved by the Michigan State University Institutional Animal Care and Use Committee (approval #11/09-174-00).

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Authorship

J.L. Rapson contributed to study design and execution, data interpretation and drafted the manuscript. H.C. Schott II contributed to study design and execution, data analysis and interpretation, and revision of the manuscript. B.D. Nielsen contributed to study design and execution, data interpretation and revision of the manuscript. L.J. McCutcheon contributed to study design and execution, data interpretation and revision of the manuscript. P.A. Harris contributed to study design, data interpretation and revision of the manuscript. R.J. Geor contributed to study design and execution, data analysis and interpretation, and revision of the manuscript. All authors approved the final manuscript.

Manufacturers' addresses

[a]MARS Horsecare US, Inc., Dalton, Ohio, USA.
[b]Vedco Inc. St. Joseph, Missouri, USA.
[c]Novo Nordisk, Inc., Plainsboro, New Jersey, USA.
[d]DHIA Forage Testing Laboratory, Ithaca, New York, USA.
[e]Yellow Springs Instruments, Yellow Springs, Ohio, USA.
[f]Siemens Medical Solutions Diagnostics, Los Angeles, California, USA.
[g]GraphPad Software Inc., San Diego, California, USA.
[h]SAS Institute Inc., Cary, North Carolina, USA.

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