Evaluation of the effects of pregnancy on insulin sensitivity, insulin secretion, and glucose dynamics in Thoroughbred mares

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Objective—To characterize the effects of pregnancy on insulin sensitivity (SI) and glucose dynamics in pasture-maintained mares fed supplemental feeds of differing energy composition.

Animals—Pregnant (n = 22) and nonpregnant (10) healthy Thoroughbred mares.

Procedures—Pregnant and nonpregnant mares underwent frequently sampled intravenous glucose tolerance tests at 2 times (period 1, 25 to 31 weeks of gestation; period 2, 47 weeks of gestation). Following period 1 measurements, mares were provided a high-starch (HS; 39% starch) or high-fat and -fiber (14% fat and 70% fiber) supplemental feed. From a subset of mares (n = 12), blood samples were collected hourly for 24 hours to assess glycemic and insulinemic response to feeding while pastured. The minimal model of glucose and insulin dynamics was used to estimate SI, glucose effectiveness, and acute insulin response to glucose from tolerance testing data.

Results—Pregnant mares during period 1 had a lower SI and glucose effectiveness and higher acute insulin response to glucose than did nonpregnant mares. The SI value decreased in nonpregnant but not pregnant mares from periods 1 to 2. Pregnant mares fed HS feed had a greater glycemic and insulinemic response to feeding than did any other group.

Conclusions and Clinical Relevance—Pregnant mares had slower glucose clearance and greater insulin secretion at 28 weeks of gestation than did nonpregnant mares. Glucose and insulin responses to meal feeding, particularly with HS feed, were greater in pregnant mares, indicating that pregnancy enhanced the postprandial glycemic and insulinemic effects of starch-rich feed supplements. (*Am J Vet Res* 2011;72:666–674)

Pregnancy in humans and many other animal species is characterized by adaptive changes in metabolism that initially prepare the body for the pending energy demands of placental and fetal growth and, later in gestation, enhance mobilization of substrates for fetal use.[1] In early pregnancy, SI (the ability of insulin to accelerate glucose clearance into body cells) may stay the same or, in subjects that are already insulin resistant, may slightly increase, as occurs in women.[1] As pregnancy progresses, the insulin response to glucose increases whereas whole-body insulin sensitivity decreases.[2,3] The increase in insulin response to glucose is mediated by an increase in pancreatic insulin synthesis and secretion in response to insulin -resistant peripheral tissues and by placental hormones that upregulate pancreatic β-cell function.[4,5] Low peripheral SI during pregnancy is proposed to be an adaptive mechanism that slows clearance of glucose to maternal tissues in order to ensure sufficient glucose supply for the glucose-reliant fetoplacental tissues.[2,3] However, low insulin sensitivity can

Abbreviations

AIRg	Acute insulin response to glucose
AUC	Area under the curve
BCS	Body condition score
CI	Confidence interval
DI	Disposition index
FF	High fat and fiber
FSIGTT	Frequently sampled intravenous glucose tolerance test
HS	High starch
MAX Δ	Maximum change in concentration
Sg	Glucose effectiveness
SI	Insulin sensitivity

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enhance postprandial glucose and insulin responses in pregnancy, which may affect energy balance.[2,5,6]

Pregnant women consuming high-glycemic diets during pregnancy reportedly gain more weight during pregnancy than do women consuming low-glycemic diets, suggesting that increased postprandial glycemia and insulinemia may lead to greater pregnancy weight gain.[6] Furthermore, because glucose from maternal blood is the main energy substrate used by fetoplacental tissues, alterations in maternal glucose and insulin dynamics during pregnancy may impact fetal development.[7–9] For example, maternal hyperglycemia in sheep may induce changes in placental glucose transport and fetal insulin stimulation, with implications for fetoplacental use and partitioning of energy substrate for healthy growth.[10] Additionally, acute nutrient restriction of mares during mid gestation is associated with a decrease in maternal glucose and insulin concentrations and enhanced insulin secretion in response to glucose in their neonatal foals.[11]

Insulin sensitivity and glucose dynamics are also affected by dietary energy source. Studies[12,13] involving horses have provided evidence that adaptation to concentrate feeds with HS content is associated with a decrease in SI when compared with feeds comprised of fat and fiber as the primary energy sources. Furthermore, insulin resistance and accompanying hyperinsulinemia are associated with HS diets and an increase in the risk of laminitis in horses.[5,14–16] Considering the numerous physiologic adaptations affecting glucose metabolism that occur during pregnancy, it is important to consider the additional potential effects of supplemental feeding on insulin and glucose dynamics in pregnant mares. A study[17] of grazing pregnant mares revealed that adaptation to a supplemental feed rich in starch and sugar is associated with enhanced glucose clearance during an orally administered glucose tolerance test, compared with adaptation to a supplement with fat and fiber as the primary energy sources.[17] However, to the authors' knowledge, no study has been conducted to evaluate the effects of supplemental feed energy sources on specific measures of SI during pregnancy in mares.

The purpose of the study reported here was to evaluate glucose and insulin dynamics in pregnant mares at mid and late gestation and compare them with those in nonpregnant mares grazing pasture while fed a supplemental feed with starch or with fat and fiber as the primary energy source. We hypothesized that SI would be lower and insulin secretion would be higher in pregnant mares than in nonpregnant mares and that the composition of the supplemental feed might further influence these differences.

Materials and Methods

Animals—Twenty-two pregnant (25 to 31 weeks of gestation) and 10 nonpregnant Thoroughbred mares were used in the study. Mares were maintained on pastures at the Virginia Tech Middleburg Agricultural Research and Extension Center. The experimental protocol was approved by the Virginia Tech Institutional Animal Care and Use Committee.

Diet—Pastures, analyzed for nutrient content monthly, were a mix of grass and legume and had similar nutrient composition.[18] Mixed grass and legume hay was provided in winter months as needed on the basis of pasture conditions. During the study, after initial measurements were obtained, isocaloric (3.0 Mcal/kg on dry matter basis), isonitrogenous concentrate feeds formulated to provide adequate vitamins and minerals were offered to provide two-thirds of a horse's digestible energy requirements, with the remainder available from forage upon which horses were permitted to graze ad libitum.[19] Concentrate feeds were specially formulated for use at the Middleburg Research and Extension Center, and each batch of feed produced was analyzed for nutrient content (**Appendix**). Rations were determined on the basis of mean mare body weight and, in the pregnant mares, were adjusted in accordance with National Research Council[19] recommendations for the 9th, 10th, and 11th month of gestation.

Study design—Within each group (pregnant and nonpregnant), mares were paired by age and BCS (scale of 1 to 9, with 1 = poor, 5 = moderate, and 9 = extremely fat).[20] One mare from each pair was randomly assigned (coin toss) to receive an HS feed (11 pregnant and 5 nonpregnant mares) or an FF feed (11 pregnant and 5 nonpregnant mares). Treatment groups (pregnant HS-fed, pregnant FF-fed, nonpregnant HS-fed, and nonpregnant FF-fed) were allocated to separate but nutritionally equivalent pastures.[18] Daily concentrate feedings were divided into 3 equal portions that were fed at approximately 7 AM, 11:30 AM, and 2:30 PM via individual pans arranged in a large circle in each pasture. Feeding from pans arranged in a circle had been the typical feeding arrangement for these mares for many years and had historically facilitated consumption of 1 ration/mare. Samples of pasture (approx weekly; n = 46), hay (each batch; 2), and experimental feeds (each batch; 20 each for HS and FF) were collected throughout the study period, and nutrient concentrations were measured.[a]

With horses in their pastures, blood samples were collected, and body weight and BCS were measured monthly between 7 AM and 9 AM prior to feeding. An electronic scale was transported to each pasture and placed on a solid, level surface for weighing mares. Two trained evaluators assessed the BCS of each horse in accordance with established methods,[20] and the mean of the 2 BCSs was recorded.

FSIGTT—All pregnant and nonpregnant mares underwent an FSIGTT during late November and early December prior to feed treatments (period 1). On the morning of the FSIGTT, mares were brought in from pasture between 7 AM and 8 AM, and body weight was measured on an electronic scale. A catheter[b] was inserted into a jugular vein after aseptic preparation and local analgesia of the overlying skin, after which mares were allowed to rest for approximately 30 minutes prior to collection of initial (basal) blood samples. Mares had ad libitum access to hay (< 2% starch) and water when kept in stalls before and during the test but were not provided a morning feed ration.

Basal blood samples were obtained 15 minutes and immediately prior to IV glucose[c] administration (300

mg/kg) for analysis of plasma glucose, insulin, leptin, and triglycerides. Blood samples were then collected at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 minutes after glucose injection. At 20 minutes after glucose administration, insulin[d] (20 mU/kg, IV) was administered, and additional blood samples were obtained at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 100, 120, 150, 180, 210, and 240 minutes after glucose injection for analysis of plasma glucose and insulin.

At 46 to 47 weeks of gestation, 2 weeks prior to the predicted parturition date, a second FSIGTT (period 2) was administered to the pregnant mares, with the same nonpregnant mares used during period 1 tested concurrently. Testing dates spanned an approximately 10-week period from mid March to mid May because of the range of breeding dates among the pregnant mares.

Circadian measurement of glycemic and insulinemic feed response—To evaluate glycemic and insulinemic patterns throughout an entire day in response to feeding, a subset of mares (3 from each of the 4 feed groups) was used. All pregnant mares selected for this portion of the study were at 37 to 39 weeks of gestation. All mares had been adapted to their respective feeds for 11 weeks. A jugular catheter was placed in each horse as described at 4 PM, and blood samples were collected hourly for 24 hours beginning at 6 PM for the measurement of plasma glucose and insulin. Throughout the 24-hour period, mares behaved naturally at pasture and had no apparent signs of stress. Feeds were offered at the same times and in the same manner to which the mares were typically accustomed, and the approximate time taken to complete each meal was recorded.

Sample handling and analysis—All blood samples for the study (including samples collected by venipuncture and those drawn from the intravenous catheter during the FSIGTT) were collected into evacuated blood collection tubes containing sodium heparin as anticoagulant[e] and placed immediately in ice water. Within 30 minutes after sample collection, tubes were centrifuged for 10 minutes at 3,000 × *g* and 4°C. Plasma was collected and stored frozen at –20°C. Plasma glucose and triglyceride concentrations were measured by use of an enzymatic assay,[f] and plasma insulin concentrations were measured by use of a radioimmunoassay kit[g] previously validated for use with equine plasma.[21] Leptin was measured with the aid of a commercially available, multispecies radioimmunoassay kit[h] previously validated for use in horses.[22] Intra-assay coefficients of variation were < 1% for glucose, < 5% for triglycerides, and < 10% for insulin and leptin.

FSIGTT and minimal model analysis—Parameters (SI, Sg, AIRg, and DI) of the minimal model of insulin and glucose dynamics were determined by simultaneous fitting of glucose and insulin curves resulting from the FSIGTT according to the following established equations by use of minimal model software[i]:

$$G'(t) = -(Sg + X)\bullet G(t) + Sg\bullet Gb$$

where G(t) = glucose at minute (t), X = insulin action, and Gb = baseline glucose concentration.

$$X'(t) = -P_2\bullet X(t) + P_3\bullet (I(t) - Ib)$$

where X(t) = insulin action at minute (t), I(t) = insulin concentration at minute (t), Ib = baseline insulin concentration, P_2 = loss rate of insulin action (X), and P_3 = action of 1 unit of insulin on glucose disposal/min.

Insulin sensitivity represents the acceleration of glucose clearance from the circulation by the insulin present (SI = P_3/P_2). The Sg is the basal (unstimulated) glucose clearance rate. Acute insulin response to glucose is the initial insulin response available to act on glucose clearance (via SI) measured in the first 10 minutes following glucose injection, but prior to exogenous insulin administration, and DI, calculated as SI × AIRg, is an index of glucose clearance from the circulation attributable to the combined effects of the initial AIRg and the tissue SI.

Statistical analysis—Results are reported as mean ± SD. Differences between feed and pregnancy groups for metabolic and minimal model parameters were determined via mixed ANOVA for repeated measures and Tukey-Kramer post hoc adjustment.[j] The AUCs for circadian plasma glucose and insulin concentrations were determined by trapezoidal approximation, with the mean value of 5 AM through 7 AM samples (approx 15 hours after the last meal) used as a basal value for each mare.[k] Differences in AUCs, MAX Δ's (maximum concentration – basal concentration), and basal concentrations among the 4 groups were determined via ANOVA.[l] Values of $P \leq 0.05$ were considered significant for these analyses.

Extreme data points (outliers) were identified by use of the Grubbs test, with a critical value of $P \leq 0.05$. One outlier in the nonpregnant FF-fed mares during period 1 was removed from analysis of SI because this value was > 2.5 times as great as the maximum value indicated for a 95% CI for SI in horses.[23] Outliers for AIRg for a different mare (which developed laminitis 2 months after study completion) in the nonpregnant FF-fed group from both periods were also removed from analysis because both values were > 1.5 times as great as the maximum value indicated for a 95% CI for AIRg in horses.[23]

Results

Animals—The mean ± SD age of the 22 pregnant mares was 9.6 ± 2.8 years, and that of n = 10 nonpregnant mares was 11.7 ± 4.4 years. At the start of the study, the mean BCS of pregnant mares was 6.2 ± 0.6, and that of nonpregnant mares was 5.8 ± 0.7. Body weight in both pregnant mare groups (fed HS or FF feeds), as well as in the nonpregnant HS-fed group, increased from late fall (mid gestation) to spring (late gestation). Mare BCS did not change in pregnant HS-, pregnant FF-, or nonpregnant HS-fed groups during the 4- to 5-month study period. However, BCS decreased in FF-fed nonpregnant mares (**Table 1**).

Diets—On average, throughout the study period, each mare received between 4.5 and 6 kg of feed/d, depending on pregnancy status and stage of gestation. Starch content of HS feed was approximately 9 times as high as that in FF feed, whereas fat content and to-

Table 1—Mean ± SD body weight and BCS before (period 1) and after (period 2) pregnant and nonpregnant mares were fed a diet supplemented with HS or FF feed (n = 11 pregnant and 5 nonpregnant mares/group).

		Pregnant		Nonpregnant	
Variable	**Period**	**HS**	**FF**	**HS**	**FF**
Body weight (kg)	1	601 ± 35	595 ± 44	572 ± 44	595 ± 39
	2	662 ± 45*	644 ± 61*	625 ± 58*	612 ± 60
BCS	1	6.2 ± 0.7	6.4 ± 0.7	6.6 ± 0.5	6.6 ± 0.9
	2	6.5 ± 0.3	5.8 ± 0.7	6.8 ± 0.5	5.3 ± 0.7*

*Values differ significantly ($P < 0.05$) between periods within the indicated treatment group.

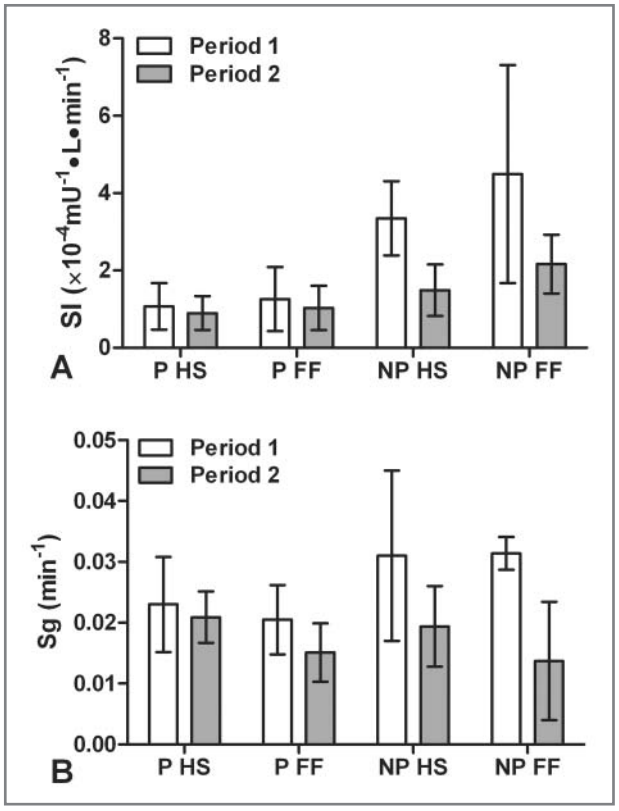

Figure 1—Mean ± SD SI (A) and Sg (B) before (period 1) and after (period 2) pregnant (P) and nonpregnant (NP) mares were fed a diet supplemented with HS or FF feed (n = 11 pregnant and 5 nonpregnant mares/feed group). Values of both variables (SI and Sg) differed significantly ($P < 0.001$) between pregnant and nonpregnant mares during period 1. Between periods, the SI value decreased in nonpregnant HS- and FF-fed mares ($P = 0.003$ and $P = 0.001$, respectively), and the Sg value decreased only in nonpregnant FF-fed mares ($P = 0.03$). No other differences were detected.

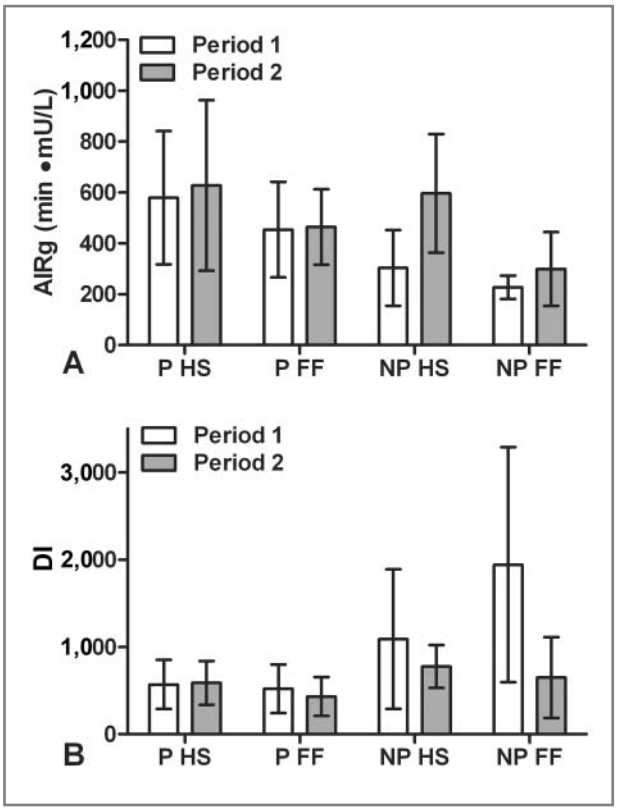

Figure 2—Mean ± SD AIRg (A) and DI (B) before (period 1) and after (period 2) pregnant (P) and nonpregnant (NP) mares were fed a diet supplemented with HS or FF feed (n = 11 pregnant and 5 nonpregnant mares/feed group). During period 1, pregnant mares had a higher AIRg and lower DI than did nonpregnant mares ($P = 0.04$ and $P < 0.001$, respectively). Between periods, AIRg increased in nonpregnant HS-fed mares only ($P = 0.03$) and DI decreased in nonpregnant FF-fed mares only ($P = 0.01$). No other differences were detected.

tal fiber were approximately 4 and 2.5 times as great, respectively, in FF versus HS feed. The mean percentages of crude protein, water-soluble carbohydrates, and starch in pasture forage increased from late fall to spring, whereas mean percentages of neutral detergent fiber and acid detergent fiber decreased ($P < 0.001$ for all). Daily observation at feeding time revealed that the rate of feed intake began to slow in late March, and the mares' actual feed intake, particularly during the afternoon meal, decreased throughout April, concurrent with the increase in nonstructural carbohydrates and protein in pasture forage. This occurred first in the FF-fed groups and then in all groups intermittently throughout April.

Minimal model parameters—Insulin sensitivity was more than 3-fold as low in pregnant mares as in nonpregnant mares during period 1 ($P < 0.001$; **Figure 1**). Insulin sensitivity did not change from period 1 to 2 in pregnant mares regardless of feed treatment ($P = 0.75$), but decreased in nonpregnant HS- and FF-fed mares between periods ($P = 0.003$ and $P = 0.001$, respectively). In period 2, SI

in pregnant and nonpregnant mares did not differ ($P = 0.14$).

Glucose effectiveness was lower in pregnant mares than in nonpregnant mares during period 1 ($P = 0.006$; Figure 1). In pregnant mares, Sg did not change from period 1 to 2 ($P = 0.39$); however, in nonpregnant mares, it decreased between periods ($P = 0.001$), attributable to a decrease in Sg for FF-fed but not HS-fed nonpregnant mares ($P = 0.03$ and $P = 0.27$, respectively). There was no difference in Sg between pregnant and nonpregnant mares in period 2 ($P = 0.95$).

During period 1, pregnant mares had a higher AIRg than did nonpregnant mares ($P = 0.04$; **Figure 2**). Between periods, AIRg increased in nonpregnant HS-fed mares ($P = 0.03$), but did not change in any other group. The AIRg did not differ between pregnant and nonpregnant mares in period 2 ($P = 0.70$).

Pregnant mares had a lower DI than did nonpregnant mares during period 1 ($P < 0.001$), but the DI did not differ between these 2 groups in period 2 ($P = 0.71$; Figure 2). Only nonpregnant FF-fed mares had a significant ($P = 0.01$) decrease in DI between periods attributable to a decrease in SI without a compensatory increase in AIRg ($P = 0.01$).

Plasma biochemical analysis—During period 1, the basal glucose concentrations did not differ between

Table 2—Mean ± SD basal plasma concentrations of glucose, insulin, triglycerides, and leptin before (period 1) and after (period 2) pregnant and nonpregnant mares were fed a diet supplemented with HS or FF feed (n = 11 pregnant and 5 nonpregnant mares/group).

		Pregnant		Nonpregnant	
Variable	**Period**	**HS**	**FF**	**HS**	**FF**
Glucose (mg/dL)	1	93.3 ± 3.9	93.9 ± 3.5	97.6 ± 7.3	92.5 ± 4.3
	2	95.7 ± 5.2	99.0 ± 5.4	103 ± 6.6	101 ± 4.9
Insulin (mU/L)	1	11 ± 6.1	11 ± 3.3	9.3 ± 1.7	8.9 ± 6.4
	2	10 ± 8.8	9.8 ± 3.6	20 ± 9.3*	17 ± 17
Triglycerides (mg/dL)	1	28.3 ± 5.6	33.4 ± 10	24.2 ± 4.3	24.3 ± 12
	2	29.3 ± 7.6	39.8 ± 15	25.8 ± 5.6	22.0 ± 16
Leptin (ng/mL)	1	4.7 ± 2.7	3.7 ± 1.0	5.1 ± 3.1	3.7 ± 1.8
	2	5.5 ± 3.8	3.4 ± 1.8	9.3 ± 3.9*	5.9 ± 4.5

See Table 1 for key.

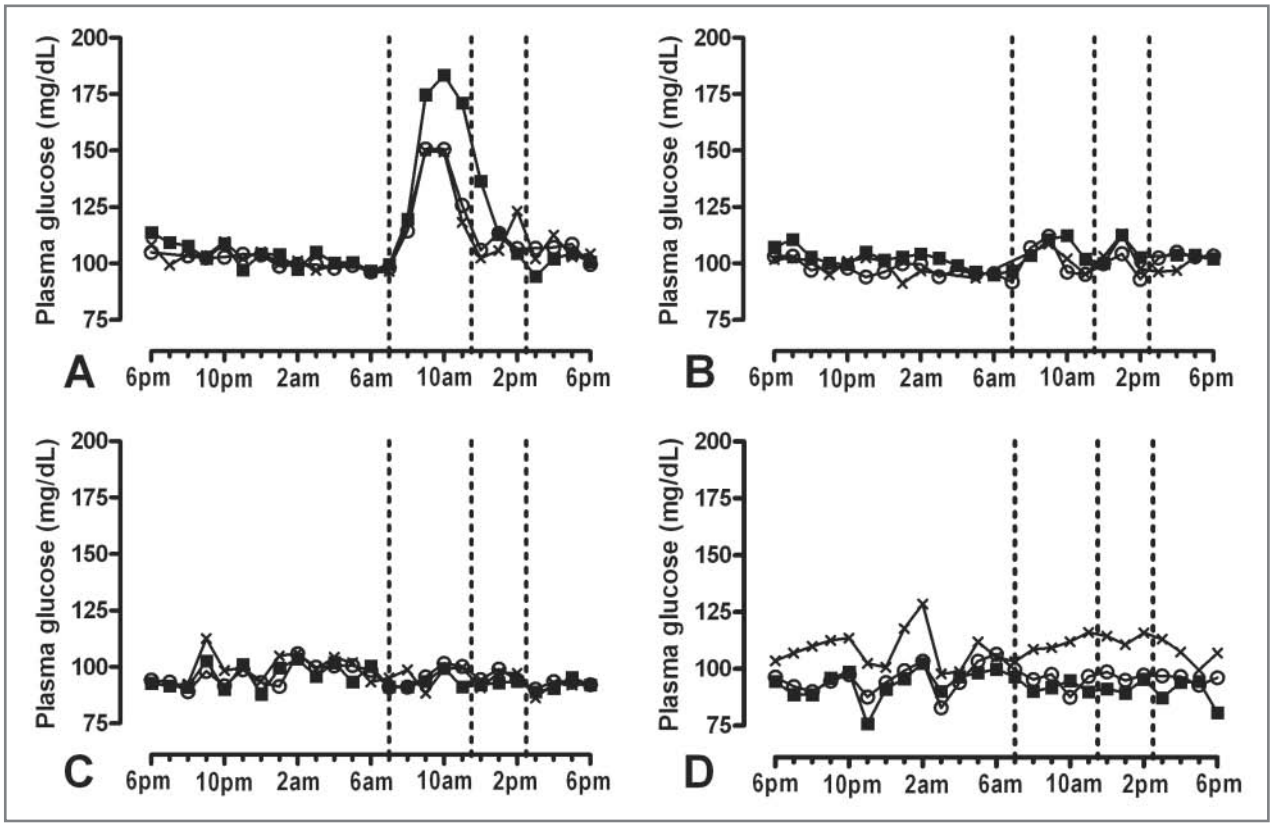

Figure 3—Plasma glucose concentrations over a 24-hour period in response to feeding (vertical dashed lines) in grazing pregnant mares provided an HS (A) or FF (B) feed and nonpregnant mares provided an HS (C) or FF (D) feed (n = 3 horses/group). Different symbols represent individual horses in each group.

pregnant and nonpregnant mares ($P = 0.87$), but it increased from period 1 to 2 in pregnant ($P = 0.05$) and nonpregnant ($P = 0.008$) mares, with no feed effects detected. Basal plasma insulin concentrations did not differ between pregnant and nonpregnant mares during period 1 ($P = 0.92$) but increased from period 1 to 2 in nonpregnant HS-fed mares ($P = 0.02$), contributing to higher basal insulin concentrations in nonpregnant versus pregnant mares in period 2 ($P = 0.02$). Plasma triglyceride concentrations were higher in pregnant than in nonpregnant mares ($P = 0.01$) overall, but no effects of period or feed treatment were detected. Plasma leptin concentrations did not differ between pregnant and nonpregnant mares in period 1 ($P = 0.99$). The leptin concentrations increased, however, in nonpregnant HS-fed mares between periods 1 and 2 ($P < 0.001$), contributing to greater concentrations in nonpregnant versus pregnant mares in period 2 ($P = 0.02$; **Table 2**).

Circadian glycemic and insulinemic response— The 12 mares (3 from each feed group) were each generally observed to remain at 1 feed pan containing 1 ration and, at each feeding, consumed their meals within

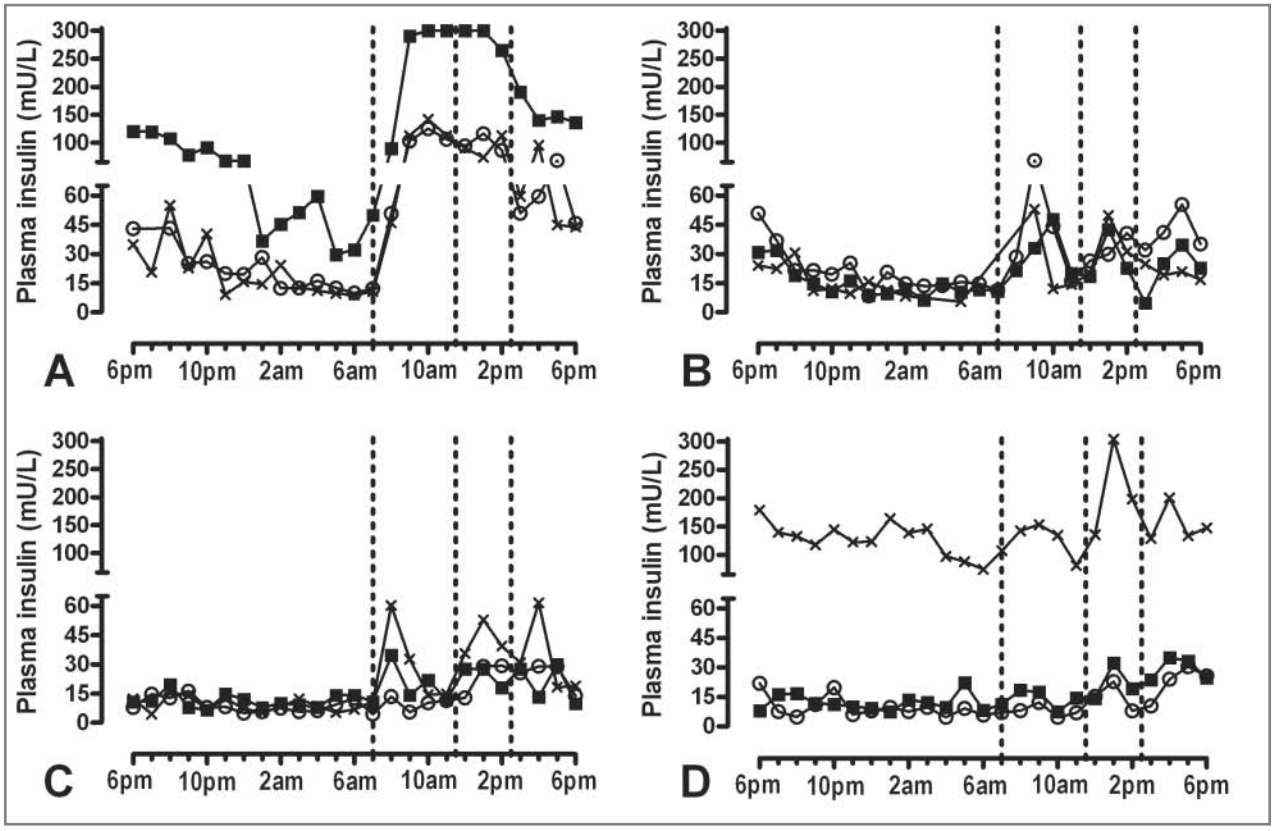

Figure 4—Plasma insulin concentrations over a 24-hour period in response to feeding (vertical dashed lines) in grazing pregnant mares provided an HS (A) or FF (B) feed and nonpregnant mares provided an HS (C) or FF (D) feed (n = 3 horses/group). Different symbols represent individual horses in each group.

Table 3—Mean ± SD (range) basal concentrations and total AUC for plasma glucose and insulin concentrations over a 24-hour period in pregnant and nonpregnant mares fed a diet supplemented with HS or FF feed (n = 3 horses/group) 3 times daily.

	Pregnant		Nonpregnant	
Variable	**HS**	**FF**	**HS**	**FF***
Basal glucose (mg/dL)	98 ± 0.7 (98–99)	95 ± 1.0 (94–96)	92 ± 0.3 (92–92)	97 ± 9.0 (91–107)
MAX Δ glucose (mg/dL)	64 ± 18[a] (53–84)	18 ± 2.0[b] (17–20)	15 ± 5.0[b] (12–21)	15 ± 5.0[b] (12–21)
AUC glucose (h•mg/dL)	303 ± 67[a] (260–380)	155 ± 26[b] (128–180)	94 ± 23[b,c] (74–119)	81 ± 10[c] (70–91)
Basal insulin (mU/L)	20 ± 15 (10–37)	10 ± 4 (6–14)	9.8 ± 3.0 (8–13)	37 ± 46 (7–90)
MAX Δ insulin (mU/L)	166 ± 76[a] (113–253)	46 ± 9[a,b] (37–54)	32 ± 19[b] (21–54)	86 ± 111[a,b] (21–214)
AUC insulin (h•mU/L)	1,467 ± 879[a] (941–2,482)	320 ± 81[b] (227–372)	182 ± 121[b] (95–320)	507 ± 690[a,b] (93–1,302)

*Includes data from 1 hyperinsulinemic mare that developed laminitis 2 months later.
[a–c]Different superscript letters indicate significant ($P < 0.05$) differences between treatment groups.

approximately 30 to 45 minutes, with HS meals usually consumed 10 to 15 minutes faster than FF meals. During the initial delivery of the meals (approx first 5 minutes of feeding), some mares would switch feed pans or otherwise compete for food, which may have resulted in some variation in amount consumed at any particular feeding. However, after all feed pans were filled (within 5 minutes), there was little disruption of meal consumption, and for the remainder of feeding, all mares were observed to consume a single ration.

Meal response patterns of plasma glucose and insulin in individual mares over a 24-hour period were graphically displayed (**Figures 3 and 4**). Pregnant mares consuming HS feed had markedly greater circadian glucose and insulin perturbations than pregnant FF-fed mares or nonpregnant HS- and FF-fed mares as indicated by several variables (**Table 3**). Basal glucose concentrations were also greater in pregnant than in nonpregnant HS-fed mares ($P < 0.01$) and tended to be greater in pregnant than in nonpregnant FF-fed mares ($P = 0.10$). The MAX Δ for glucose was greater in pregnant versus nonpregnant HS-fed mares ($P < 0.01$), but did not differ between pregnant and nonpregnant FF-fed mares. The AUC for glucose was greater in pregnant versus nonpregnant HS-fed mares ($P < 0.01$) and in pregnant versus nonpregnant FF-fed mares ($P = 0.05$).

Basal insulin concentrations were not affected by pregnancy status in mares receiving either type of feed. The MAX Δ and AUC for insulin were greater in pregnant versus nonpregnant HS-fed mares ($P = 0.04$ and $P = 0.02$, respectively), but not in pregnant versus nonpregnant FF-fed mares. Lack of differences in insulin-related variables among these FF-fed mares was likely attributable to a nonpregnant FF-fed mare that had a basal insulin concentration approximately 10 times as high as the mean value reported for healthy Thoroughbred horses[23] (Figure 4). Although this mare had no known history of laminitis, clinical signs of laminitis developed approximately 2 months after completion of the study. The body weight and BCS (mean BCS of 6) of the mare did not change during the study.

Discussion

In the study reported here, lower SI, lower Sg, and AIRg were detected in pregnant mares entering the last third of the gestation period, compared with nonpregnant mares evaluated simultaneously. Pregnant mares consuming HS feed in the last third of gestation had greater glycemic and insulinemic responses to feeding than did nonpregnant mares or pregnant mares of similar age and body condition consuming an FF feed. A decrease in SI between late fall and spring in pasture-maintained nonpregnant mares was also evident. Feeding management of the pastured broodmares in this study is typical of broodmare management worldwide, considering that it is common to provide supplemental feeds during the last third of gestation, which is a time of substantial fetal growth and of less seasonal availability of high-quality pasture.[19]

The data in the present study suggested the last third of the gestation period is associated with a lowering of SI (ie, insulin-mediated glucose clearance) in mares. A temporal decrease in SI during the last third of gestation was not detected, but lower SI was detected in pregnant versus nonpregnant mares at approximately 28 weeks of gestation. Because this study did not involve evaluation of mares prior to the final third of gestation or of the same mares prior to their pregnancy, it is unclear when a temporal decrease in SI associated with pregnancy occurs in mares. We hypothesize that this pregnancy-associated decrease in SI occurs prior to 28 weeks of gestation, which is a possibility that should be examined in future studies.

Reference values have been developed on the basis of data collected from an assortment of clinically normal horses aged 6 months to 19 years, including Thoroughbred and Arabian geldings, Thoroughbred weanlings, and pregnant Thoroughbred mares, by use of the same FSIGTT and minimal model procedures as were used in the present study.[23] In comparison with those values, the mean SI in the pregnant mares at 28 weeks of gestation in our study was within the second-lowest reference quintile, whereas the mean SI in the nonpregnant mares was within the highest reference quintile (95% CI for SI, 0.16×10^{-4} $mU^{-1}/L/min^{-1}$ to 5.88×10^{-4} $mU^{-1}/L/min^{-1}$).[23] Thus, although pregnant and nonpregnant mares in the present study differed in SI, all SI values were within the reference range established for horses.

Pregnant mares in the present study also had lower Sg, indicating slower glucose clearance with or without insulin during pregnancy. The higher AIRg in pregnant versus nonpregnant mares at the beginning of the study may have represented a compensatory response to the lower rate of overall glucose clearance and may have been caused by a pregnancy-associated upregulation of β-cell function, which has been reported for humans and rodents.[4,5] However, DI, an index of total insulin action that reflects insulin secretion and tissue SI, was also lower in pregnant versus nonpregnant mares, suggesting an increase in insulin secretion did not entirely compensate for the lower SI in pregnant mares. These changes in glucose and insulin dynamics associated with pregnancy were not associated with differences in basal glucose and insulin concentrations between pregnant and nonpregnant mares at either measurement period. Therefore, basal glucose homeostasis was not altered by pregnancy in this study.

On the other hand, glycemic and insulinemic responses to meal feeding were significantly altered in pregnant mares, with prolonged hyperglycemia and hyperinsulinemia throughout a 24-hour period in mares fed the HS but not the FF feed. The 95% CIs for plasma glucose and insulin concentrations in the aforementioned reference values[23] are, respectively, 74 to 125 mg/dL and 1.2 to 40.4 mU/L. However, the postfeeding excursions in plasma glucose (Figure 3) and insulin (Figure 4) concentrations in pregnant mares fed the HS feed extended well beyond these concentrations. Differences between glycemic and insulinemic responses to HS versus FF feeds in the nonpregnant mares were not apparent in our sample of mares, perhaps because of variation in glycemic and insulinemic responses among individual horses, the use of only 3 mares/group, or the division of rations into 3 small portions/d. However, the difference in glycemic and insulinemic response to feeding between pregnant and nonpregnant mares suggested that mares with altered glucose metabolism (low

SI, Sg, and DI and high AIRg) due to pregnancy represent a population of horses particularly sensitive to the dietary energy composition of feed.

In pregnant mares, no changes in SI, Sg, AIRg, or DI were evident in either feed group during the final third of gestation. However, in nonpregnant mares during the same period, consumption of HS feed but not FF feed resulted in weight gain, an increase in basal plasma leptin and insulin concentrations, and an increase in AIRg. However, both groups of nonpregnant mares had a decrease in SI from the beginning to end of the study, suggesting that factors other than HS feed and weight gain contributed to changes in glucose and insulin dynamics over this interval. Period 2 measurements were performed in the spring, when pasture forage quantity and quality are typically higher than in other seasons.[24] The observed metabolic changes in nonpregnant mares may, therefore, have been the result of a combination of increased forage availability and altered forage nutrient content (eg, higher protein, higher nonstructural carbohydrates, and lower fiber), compared with availability and content in period 1.[25] High plasma insulin concentrations have been reported for grazing mares during spring,[26] indicating that glucose-insulin dynamics may typically change with season in nonpregnant grazing horses. Changes in SI were not detected in the pregnant mares, perhaps because their metabolism was already altered by pregnancy and lacked additional flexibility to respond to environmental changes.

Potential metabolic changes associated with the endocrine hormone status of nonpregnant mares emerging from seasonal anestrus (ie, cyclic changes in estrogen and progesterone secretion), relative to those in pregnant mares maintaining pregnancy, may also contribute to changes in SI detected in nonpregnant mares from late fall to spring that were not observed in the pregnant mares.[27] Decreases in SI have been reported for mares and women in association with cyclic increases in progesterone during the luteal phase of the estrous or menstrual cycle.[27,28] However, we acknowledge that, because of the wide range of testing dates for period 2, additional studies are needed to specifically determine the effects of environmental changes (eg, pasture and reproductive cyclicity) on glucose-insulin dynamics in nonpregnant grazing mares.

The decrease in body condition in pregnant FF-fed mares coupled with the significant increase in body weight and plasma leptin concentration in the nonpregnant HS-fed mares suggested that the energy composition of feed affected body composition in these mares. Dissimilar weight gain associated with high versus low starch feed has also been reported for Arabian horses.[29] Because all mares were provided two-thirds of their digestible energy requirements from the isocaloric feed supplements, it is unlikely that differences in digestible energy intake of the feeds resulted in the observed changes in body condition. However, differences in digestion and metabolism of the different energy sources could influence forage intake from pasture, contributing to dissimilar energy intake between feed groups. It should also be considered that the apparent lower palatability of FF feed may have resulted in reduced consumption of FF relative to that of HS feed in the present study during the month of April, when pasture nonstructural carbohydrate content (and presumably palatability) was higher than in other months, especially period 1 (winter). This was particularly evident in FF-fed groups, but both HS- and FF-fed groups refused feed intermittently through the month of April.

The results of the present study indicated that pregnant mares entering the last third of the gestation period in late fall to early winter had lower peripheral SI, lower Sg, and higher AIRg than did nonpregnant mares. These differences are associated with greater glycemic and insulinemic responses to consuming HS feed, compared with pregnant mares fed an FF feed and nonpregnant mares fed either type of feed. Therefore, consideration of dietary energy content of feed may be of particular importance when pregnant mares are fed, as the perturbations to typical glucose and insulin dynamics appear exaggerated because of the physiologic condition of pregnancy. Additional studies are warranted to evaluate the effects of feed supplement amount and composition on glycemic-insulinemic responses to feeding and on glucose metabolism throughout pregnancy in mares.

a. Dairy One Forage Laboratory, Ithaca, NY.
b. 14-gauge MILACATH, Mila International Inc, Erlanger, Ky.
c. 50% dextrose, Vedco Inc, St Joseph, Mo.
d. Humulin R, Lilly, Lake Forest, Ill.
e. BD Vacutainer, Fisher Scientific Co, Newark, Del.
f. Beckman Instruments, Sigma Diagnostics, St Louis, Mo.
g. Coat-A-Count Insulin, Siemens Healthcare Diagnostics Inc, Deerfield, Ill.
h. Millipore Inc, Billerica, Mass.
i. MinMod Millenium, version 5.10, Richard Bergman, University of Southern California, Los Angeles, Calif.
j. SAS, version 9.1, SAS Institute Inc, Cary, NC.
k. GraphPad Prism, version 4.0, GraphPad Software Inc, San Diego, Calif.
l. Stata, version 11, StataCorp LP, College Station, Tex.

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Appendix

Nutrient analysis (mean ± SD percentages) for various components of HS (n = 20 batches) and FF (20 batches) horse feeds, pasture forage (46 samples, weekly), and hay (2 batches).

Component	HS	FF	Pasture	Hay
Crude protein	14.5 ± 1.5	15.5 ± 0.7	17.3 ± 5.6	16.5 ± 0.1
Crude fat	3.4 ± 0.6	13.9 ± 2.2	2.7 ± 0.8	2.7 ± 0.1
Water-soluble carbohydrate	11.2 ± 2.3	8.2 ± 1.1	8.1 ± 3.4	6.6 ± 0.1
Starch	39.2 ± 6.3	4.4 ± 1.8	1.3 ± 0.3	1.7 ± 0.4
Acid detergent fiber	10.0 ± 1.7	28.2 ± 2.6	34.4 ± 6.4	39.9 ± 2.1
Neutral detergent fiber	17.6 ± 2.7	42.2 ± 3.0	62.3 ± 7.8	54.6 ± 0.4

Data are reported on a 100% dry-matter basis.