Chapter 7. Carbohydrate Status in Fat-Adapted Mares During Pregnancy and Lactation

ABSTRACT

The assessment of carbohydrate status in the horse may be used to determine metabolic responses to diet or disease. Carbohydrate status is commonly assessed by means of a glucose tolerance test. The objective of this study was to determine if energy source, gestational or lactational stage influenced the responses of plasma glucose, insulin and cortisol to an oral glucose challenge in mares consuming pasture and supplements rich in starch and sugar (SS) or fat and fiber (FF). Twelve mares, six on each supplement, were used in three trials, one in the third trimester of pregnancy, one in early lactation and one in late lactation. Venous samples were taken at -30, 0, 30, 60, 90, 120, 150, 180, 240 and 300 min after an oral dose of .2 g/kg BW of glucose. Plasma was assayed for glucose, insulin and cortisol. The areas under the curve were larger during the third trimester for glucose and insulin, and during late lactation for glucose, in the FF mares, compared to the SS mares. The responses of glucose and insulin to the glucose challenge were consistent with decreased glucose clearance, an adaptation to dietary fat and fiber.

Key Words: Glucose Tolerance, Mares, Pregnancy, Lactation, Fat

Introduction

The supplementation of pasture with concentrates rich in starch and sugar or fat and fiber influenced growth of yearling Thoroughbreds (Hoffman et al., 1996). The slump in growth noted in the yearlings supplemented with the starch and sugar concentrate corresponded with rapid growth of spring pasture. It was hypothesized that the growth differences were due to an excess of dietary hydrolyzable carbohydrate, which may have affected carbohydrate status of the animals.

Carbohydrate status is commonly assessed by means of a glucose tolerance test, the temporal response of blood glucose concentration to an oral or intravenous dose of glucose (Roberts and Hill, 1973; Jacobs and Bolton, 1982). The glycemic index of a feed is an *in vivo* estimate of the digestible carbohydrates in the feed (Stull and Rodiek, 1988; Englyst et al., 1996). Although similar in procedure, the glycemic index provides an assessment of the carbohydrate in a feed, while glucose tolerance is a function of the carbohydrate status of the animal.

Glucose tolerance tests been used to assess glucose status in hyperinsulinemic ponies (Freestone et al., 1992) and to aid in diagnosis of pancreatic and small intestinal dysfunction (Roberts and Hill, 1973). Blood glucose responses and corresponding insulin concentrations may be influenced by differing energy source in diets formulated to be isoenergetic (Stull and Rodiek, 1988).

The objective of this study was to determine if energy source, gestational or lactational stage influenced the response of plasma glucose, insulin and cortisol to an

oral glucose challenge in mares consuming pasture and supplements rich in starch and sugar or fat and fiber.

Materials and Methods

Pilot study

Glucose response tests reported in the literature have been conducted on animals fasted overnight or for 24 h. To confirm that the response test could provide useful results in mares consuming forage, a pilot study was completed in March 1994 at the Virginia Tech MARE Center. The secondary objective of the pilot study was to confirm if a standardized oat dose could be used in place of an oral glucose challenge, and whether the oat dose could be applied to a large number of mares on pasture for future work.

Four pregnant mares on baseline diets of pasture supplemented with Purina Pure Pride 200 (Purina Mills, St. Louis, MO) were weighed and brought into stalls 18 h before the experiment began. They were allowed ad libitum access to mixed grass legume hay and water. Intravenous catheters were inserted and secured in the left jugular, and after an adjustment period of approximately one hour, baseline blood samples were taken. Glucose challenges were administered either using a glucose dose of 50% dextrose solution via nasogastric tube amounting to .2 g glucose/kg BW, or a dose of oats (48 lb/bu, triple washed and sifted, LaCrosse Classic) amounting to .2 g NSC/kg BW. Blood samples were taken every 30 min for a duration of 7 h, and

plasma was analyzed for glucose using a colorimetric method (Glucose Procedure #16-UV, Sigma Diagnostics, St. Louis, MO).

Results indicated that the plasma glucose response was more consistent following glucose administration than following a meal of oats (Figure 7.1). Plasma glucose returned to baseline concentrations between 4 and 5 h after the challenge.

Dietary experiment

Twenty mares were maintained on adjacent bluegrass clover pastures. In mid gestation, the mares were paired by age, breeding date and sire of their foal, and then randomly assigned into two groups. Ten mares and their foals were fed a corn grain and molasses supplement (SS) and ten a corn oil and fiber (beet pulp, soy hulls and oat straw) supplement (FF). The supplements were formulated to be isocaloric and isonitrogenous, with mineral contents balanced to complement the pasture and to meet or exceed current recommendations (NRC, 1989). Samples of supplements and pastures were submitted for proximate and mineral analysis (Northeast DHIA Forage Testing Laboratory, Ithaca, NY). Non-structural carbohydrate of the supplements and pasture samples was calculated by difference from the proximate analysis. Feed samples were also analyzed for hydrolyzable carbohydrate using direct methods (Smith, 1981).

Twelve mares, six on each diet, were chosen for use in this study based on their acceptance of standing in a stall. Three trials were completed, one during the third trimester of gestation, one in early lactation (1 to 2 mo after foaling), and the last in late lactation (5 to 6 mo after foaling). All of the mares but one (in the SS group) were

pregnant during late lactation, as determined by ultrasound examination. For each trial, the mares were weighed and brought into stalls 12 to 18 h before the onset of the experiment. The groups of mares were kept together in order to avoid social dislocative stress. Ad libitum access to hay and water was allowed, but no supplements were fed during this time. An i.v. catheter was inserted and secured in the left jugular vein, and after an adjustment period of approximately one hour, baseline blood samples were taken, at -30 and -1 min before the glucose challenge. Each mare was given a glucose challenge of 50% dextrose solution via nasogastric tube at .2 g/kg BW. Venous samples were taken at 30, 60, 90, 120, 150, 180, 240 and 300 min after the glucose challenge. The blood samples were heparinized immediately, centrifuged, and plasma aliquots were removed within 10 to 20 min of collection. Plasma was frozen pending analysis. Glucose concentrations were determined by colorimetric assay (Glucose Procedure #16-UV, Sigma Diagnostics, St. Louis, MO), insulin and cortisol using radioimmunoassays (Coat-A-Count Insulin, Coat-A-Count Cortisol, Diagnostic Products, Los Angeles, CA). Glucose, insulin and cortisol data were summarized as least squares means and standard errors and plotted over time. The magnitude of each response was calculated as the area under the curve (AUC) by graphical approximation.

Results

The composition of the supplements is shown in Table 7.1. Direct analysis of hydrolyzable carbohydrate indicated concentrations in the SS supplement at 64 ± 5%

DM, the FF supplement at 15 ± 2% DM. Basal concentrations of glucose, insulin and cortisol are shown in Table 7.2.

Glucose. Initial plasma glucose concentrations were about 80 mg/dL for mares fed both supplements in the third trimester and late lactation (Figures 7.2 and 7.4) and for mares fed FF in early lactation (Figure 7.3). Peak values were reached at 60 min in the third trimester and late lactation, but at 30 min in early lactation. Baseline values were resumed after 240 min in the third trimester and 180 min in early and late lactation.

The AUC (Table 7.3) was larger in the FF mares than in the SS mares during the third trimester (P = .035) and late lactation (P = .11). There were no major changes in AUC and glucose metabolism in response to gestational or lactational stage of the SS mares. The relatively smaller AUC for the FF mares during early lactation may reflect increased clearance due to adaptation and increased glucose metabolism due to a need for lactose to make milk.

Insulin. Initial insulin concentrations were about 20 µIU/mL for mares fed both supplements in the third trimester (Figure 7.2) and about 10 µIU/mL in early and late lactation (Figures 7.3 and 7.4). Peak values were reached at 30 min in the SS supplemented mares and 60 min in the FF supplemented mares in the third trimester, early and late lactation. Initial values were resumed in the SS mares after 150 min in the third trimester and late lactation, and after 90 min in early lactation. For the FF mares, initial values were resumed after 240 min in early and late lactation, and after 150 min in early lactation.

The AUC (Table 7.3) was larger in the FF mares than in the SS mares during the third trimester of pregnancy (P = .035). There was a tendencey for a smaller AUC during early lactation, compared to the third trimester (P = .09) and late lactation (P = .24), in both the SS and FF mares

Cortisol. Cortisol concentrations varied throughout the response times in both groups. No response to either diet differed from the expected circadian rhythm, although cortisol was increased during early lactation in the SS mares but not in the FF mares. The AUC was not calculated for cortisol concentrations.

Discussion

In an oral glucose tolerance test, the initial change in glucose reflects absorption and clearance; blood glucose concentratration increases when the rate of glucose entry into the blood exceeds the rate of removal. Blood glucose concentrations are regulated by insulin, cortisol and other hormones (Kaneko, 1989). As blood glucose rises, insulin is released and facilitates the transfer of glucose into peripheral tissues. The subsequent fall in blood glucose is a reflection of clearance rate exceeding that of absorption.

Glucose absorption is not rate limited, therefore a larger plasma response curve as found here was a result of slower clearance. Higher plasma glucose would stimulate increased insulin response, as noted here. Glucose utilization was decreased in animals adapted to dietary fat (Kronfeld et al., 1994). The slower

clearance found in this study could be due to fat or fiber adaptation of the FF supplemented mares.

In addition, the difference in insulin responses may have been due to small intestinal release of gastric inhibitory polypeptide (GIP), which has been shown to mediate insulin response in humans (Dupre et al., 1973). Nutrients stimulating the release of GIP include fat as the most potent stimulus, followed by glucose and amino acids (Stull and Rodiek, 1988). The corn oil in the FF diet may have influenced GIP to mediate additional insulin secretion during the hyperglycemic response.

The lower plasma cortisol concentration in the FF mares during early lactation may reflect a lower level of excitability (Holland et al., 1996). Although care was taken to ensure that all horses were treated alike, slight differences may have increased stress of the SS mares and confounded cortisol at this time.

The SS supplemented mares, compared to the FF mares, had approximately 4.2 times more hydrolyzable carbohydrate in their daily diet. Adaptation to the SS supplement may have enhanced the ability of those mares to utilize glucose at a faster rate.

The increase in glucose and insulin responses during the third trimester, compared to early and late lactation may have been due to physiological state, or it may have been due to seasonal differences in pasture. Mares in the third trimester of pregnancy were consuming primarily hay with their supplement on a daily basis, as pasture quality was relatively poor. In contrast, mares in early lactation were consuming rapidly growing spring pasture. Additionally, the mares in late lactation had

the benefit of an unusually mild October with optimal rainfall, and the pasture at this time was found to be at its nutritional peak (Wilson et al., 1997).

In this study, the peaks of glucose and insulin in response to the oral glucose load were lower during early and late lactation than in the third trimester of pregnancy. Basal plasma glucose concentrations in horses were not influenced by pregnancy or lactation, but the plasma glucose response to an i.v. injection was affected by lactation (Evans, 1971). Marked changes in carbohydrate metabolism, including hyperinsulinemia, enhanced β cell sensitivity, increased degradation of insulin, and insulin resistance were demonstrated in the pregnant mare (Fowden et al., 1984). These changes resulted in exaggerated responses to feeding and fasting in pregnant, as compared to non-pregnant mares. The increased glucose and insulin response to the oral glucose load during the third trimester of pregnancy, as found here, agrees with other reports.

Implications

The response to a standard glucose challenge was influenced by adaptation to dietary fat, and possibly, hydrolyzable carbohydrate. Corn oil in the FF supplement may have influenced GIP mediation of additional insulin during the hyperglycemic response to a glucose load. Adaptation to increased dietary hydrolyzable carbohydrate to utilize glucose at a faster rate would lower the plasma glucose response to an oral load.

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Table 7.1. Nutrient profile of the supplements and pasture fed to mares during gestation and lactation as analyzed by the Northeast DHIA Forage Testing Laboratory (Ithaca, NY). Data are summarized on a dry matter basis as means ± SE for supplements and as a 90% confidence interval for pasture.

Nutrient	SS (n = 10)	FF (n = 15)	Pastures (n = 38)
DM, %	90.0 ± 0.36[a]	91.7 ± 0.30[b]	15.7 – 36.5
DE, MJ/kg*	14.7 ± 0.2[a]	10.8 ± 0.1[b]	9.2 – 14.1
CP, %	15.0 ± 0.6	14.6 ± 0.5	16.5 – 29.5
ADF, %	9.1 ± 0.8[a]	28.3 ± 0.64[b]	20.3 – 35.6
NDF, %	15.3 ± 1.2[a]	41.2 ± 1.0[b]	36.5 – 60.0
Fat, %	2.4 ± 0.7[a]	10.4 ± 0.6[b]	2.7 – 5.6
NSC, %	62.4 ± 0.8[a]	26.5 ± 0.7[b]	6.1 – 23.9
Ash, %	4.9 ± 0.3[a]	7.2 ± 0.3[b]	7.35 – 12.82
Ca, %	0.77 ± 0.08[c]	1.01 ± 0.07[d]	0.36 – 1.04
P, %	0.45 ± 0.03	0.48 ± 0.03	0.26 – 0.46
Mg, %	0.17 ± 0.01[a]	0.23 ± 0.01[b]	0.18 – 0.27
K, %	0.98 ± 0.04[a]	1.30 ± 0.04[b]	1.93 – 3.72
Na, %	.20 ± .02	0.20 ± 0.01	0 – 0.035
S, %	0.17 ± 0.01[a]	0.20 ± 0.01[b]	0.16 – 0.39
Fe, mg/kg	191 ± 31[a]	485 ± 26[b]	0 – 1845
Zn, mg/kg	93.7 ± 7.1	101 ± 6.0	16.8 – 60.0
Cu, mg/kg	41.4 ± 5.2[c]	65.2 ± 4.4[d]	0 – 63.2
Mn, mg/kg	35.6 ± 3.0[a]	59.2 ± 2.5[b]	51.8 – 124.6
Se, mg/kg	0.6[e]	0.6[e]	< 0.08
I, mg/kg	0.6[e]	0.6[e]	< 0.08

*Calculated by the Northeast DHIA Laboratory.

[a,b]Values with subscripts c,d are different ($P < 0.001$).

[c,d]Values with subscripts e,f are different ($P < 0.05$)

[e] Calculated using NRC (1989) tables.

Table 7.2. Basal concentrations (mean ± SE) of plasma glucose, insulin and cortisol in Thoroughbred mares, fed supplements rich in sugar and starch (SS) or fat and fiber (FF), during late gestation, early and late lactation.

Time, Supplement	Glucose, mg/dL	Insulin µIU/mL	Cortisol, µg/dL
Third Trimester			
SS	87.6 ± 5.1	22.8 ± 3.5	5.3 ± .8
FF	80.8 ± 4.3	25.4 ± 5.7	4.5 ± .4
Early Lactation			
SS	65.1 ± .7	9.1 ± 2.0	16.6 ± 1.6
FF	75.3 ± 3.8	8.0 ± 1.2	11.9 ± 1.3
Late Lactation			
SS	78.7 ± 5.3	8.2 ± 2.8	12.4 ± 1.9
FF	82.9 ± 2.4	16.7 ± 2.6	11.6 ± 1.0

Table 7.3. Magnitude of response, expressed as means ± SE of the area under the curve (AUC) of plasma glucose and insulin to a glucose challenge.

Plasma Variable	Diet	Third Trimester	Early Lactation	Late Lactation
Glucose, min*mg*dL^{-1}	SS	1930 ± 320[a]	1580 ± 400	1870 ± 370[c]
	FF	2920 ± 370[b]	1510 ± 61	2600 ± 310[d]
Insulin, min*µIU*mL^{-1}	SS	1330 ± 230[a]	770 ± 220	2550 ± 790
	FF	5350 ± 2520[b]	1490 ± 350	2430 ± 470

Means with superscripts a,b differ (*P* = .035).
Means with superscripts c,d differ (*P* = .11).