Changes in glutamine metabolism indicate a mild catabolic state in the transition mare$^{1,3}$

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ABSTRACT: Glutamine is the most abundant free α-AA in the mammalian body, and large amounts of glutamine are extracted by both the fetus during pregnancy and the mammary gland during lactation. The work presented here addressed the hypothesis that there would be major changes in glutamine metabolism in the mare during the transition period, the time between late gestation, parturition, and early lactation. Eight foals were born to Standardbred mares provided with energy and protein at 10% above NRC recommendations, and foals remained with mares for 6 mo. During lactation, lean body mass decreased by 1.5% (P < 0.05), whereas fat mass was unchanged throughout gestation and lactation. There was a sharp increase in the concentration of most plasma metabolites and hormones after birth, which was due in part to hemoconcentration because of fluid shifts at parturition. Plasma glutamine concentration, however, was maintained at greater concentrations for up to 2 wk postpartum but then began to decrease, reaching a nadir at approximately 6 wk of lactation. Skeletal muscle glutamine content did not change, but glutamine synthetase expression was decreased at the end of lactation (P < 0.05). Free glutamine was highly abundant in milk early in lactation, but the concentration decreased by more than 50% after 3 mo of lactation and paralleled the decrease in plasma glutamine concentration. Thus, lactation represents a mild catabolic state for the mare in which decreased glutamine concentrations may compromise the availability of glutamine to other tissues such as the intestines and the immune system.

Key words: body composition, equine, glutamine, glutamine synthetase, lactation, pregnancy

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INTRODUCTION

Pregnancy and lactation place major demands on the mother that are accompanied by biochemical and physiological adaptations, which have been studied extensively in many species. In contrast, the transition period, defined in the dairy cow as the last weeks of gestation, parturition, and the first weeks of lactation (Grummer, 1995), has received little attention in the horse. Glucose is the major fuel for the growing fetus, but during late gestation, the supply of glucose plateaus and the fetus begins to derive energy from other substrates, such as lactate and AA. This can be problematic, because if AA are oxidized, they are not available for growth and thus could compromise fetal development. Thus, late gestation may represent a mild catabolic state in which maternal muscle protein is mobilized to meet the demands of the fetus. Similarly, lactation is recognized, in several species (Clowes et al., 2003a,b), as a mild catabolic state accompanied by loss of lean body mass.

Glutamine is the most abundant free α-AA in most mammals, being concentrated within skeletal muscle, where it may play a role in maintaining protein homeostasis (Curthoys and Watford, 1995; Watford and Wu, 2005). During gestation, there is a large uptake of glutamine by the placenta with transfer, together with glutamine synthesized within the placenta, to the fetus (Chung et al., 1998; Battaglia, 2000; Cetin, 2001; Paolini et al., 2001). Similarly, large amounts of glutamine are taken up by the lactating mammary gland, and glutamine and glutamate are the major free AA in milk (Davis et al., 1994; Sarwar et al., 1998; Matsui et al., 2003). Because of extensive intestinal me-
tabolism, little dietary glutamine enters the circulation; thus, body glutamine is synthesized de novo, primarily in skeletal muscle. The work presented here addressed the hypothesis that there would be major changes in glutamine metabolism in skeletal muscle in the mare during the transition period.

**MATERIALS AND METHODS**

All procedures were approved by the Institutional Animal Care and Use Board of Rutgers University.

**Animals and Management**

Eight clinically normal, pregnant Standardbred mares were used in this study. All parturitions occurred between February and April 2003 inside stalls, with assistance but without complications. Foals stayed with their mothers from birth until weaning at 6 mo of age. Mares and foals were housed on pasture with free access to grass and hay, and the mares received supplementation with a commercially available pellet (15% CP, 3.00 Mcal/kg of DM) provided twice daily in individual stalls. The ration was adjusted weekly, providing energy and protein supplementation at 10% above the NRC (1989) recommendations for pregnant and lactating mares. Approximately 55% of the energy and protein came from the pellets, with the remainder from the hay and grass. All animals had free access to salt-mineral blocks and water at all times. Mares were not bred back after parturition and an additional 4 healthy, nonpregnant, nonlactating Standardbred mares (8 yr old, 474.5 ± 11.5 kg) were used as a control group. Mares and foals were given appropriate periodic anthelmintics, vaccinations, and hoof trimming, according to the standard practice at the Rutgers University Equine Science Center.

**Body Composition**

Body composition was determined by BW and rump fat thickness weekly during the last 8 wk of gestation, at parturition (within 2 h of birth), and weekly during the first 12 wk of lactation. Body weight was measured by using an electronic scale, and measurements of subcutaneous fat at the rump were made by ultrasound at the anatomical site described by Westervelt et al. (1976). The relationship between rump fat as determined by ultrasound (F, cm) and percentage of body fat (Y) was calculated by using the equation, \( Y = 8.64 + (4.7 \times F) \).

**Blood Collection**

With the exception of the samples collected 30 min and 24 h after parturition, samples were obtained weekly at 0700 h after overnight food deprivation. Blood samples were collected into 10-mL Vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ) containing 143 US Pharmacopeia units of sodium heparin for metabolite determinations and without anticoagulant for hormone analysis. Plasma and serum were isolated by centrifugation at 1,500 \( \times g \) for 15 min, and aliquots were stored at −80°C for subsequent analysis of lactate and hormones. Additional aliquots were added to equal volumes of cold 10% (wt/vol) perchloric acid to precipitate proteins. Supernatants were isolated by centrifugation at 1,500 \( \times g \) for 15 min, neutralized with potassium hydroxide, and stored at −80°C until analyzed for glucose, glutamate, and glutamine.

**Muscle Biopsy**

Muscle biopsies were obtained from the middle gluteus muscle, at a fixed location near the first hind part of an imaginary line between the tuber coxae and the head of the tail. Samples were obtained at 50% of the total depth of muscle, as determined by ultrasound, for each horse. Biopsies were obtained, from alternate sides, at 3 and 6 mo after parturition, and on one occasion from the control animals. Samples were immediately frozen in liquid nitrogen and stored at −80°C until analysis. For metabolite analysis, samples of muscle were homogenized (Tissue-Tearor, Biospec Products, Bartlesville, OK) directly in 10 vol of ice-cold 10% (wt/vol) perchloric acid. Deproteinized supernatants were isolated and neutralized as described for plasma. For Western blot analysis, additional muscle samples were extracted by homogenization in 5 to 10 vol of Tris (100 mM), EDTA (1 mM), dithiothreitol (1 mM), pH 8.0, containing 0.05% (vol/vol) Protease Inhibitor Cocktail III (Calbiochem, San Diego, CA).

**Milk Collection**

Approximately 50 mL of milk was collected from 4 mares at 7 d and at 3 and 6 mo after parturition. Milk was deproteinized with an equal volume of ice-cold 10% (wt/vol) perchloric acid, and the samples were treated as for the plasma and muscle samples described above.

**Hormone and Metabolite Analyses**

Plasma leptin concentrations were determined by using a multispecies leptin kit (Linco Research, St. Louis, MO) previously validated for horses (McManus and Fitzgerald, 2000; Gordon and McKeever, 2006), demonstrating a within-assay CV of 8.5%. Plasma insulin and cortisol concentrations were determined by using solid-phase RIA kits (Coat-a-Count, Diagnostic Products, Los Angeles, CA) previously validated for horses (Freestone et al., 1991), with a within-assay CV of <3%. Plasma lactate content was determined by using a YSI Sport 1500 lactate analyzer (YSI Inc., Yellow Springs, OH), plasma glucose was measured in the neutralized deproteinized extracts by using a hexokinase-based method (Gordon and McKeever, 2006), and plasma protein concentration was determined with a handheld
refractometer (Leica Optical Products, Buffalo, NY). Free glutamate and glutamine concentrations were determined in neutralized deproteinized extracts of plasma, milk, and skeletal muscle after first converting the glutamine to glutamate by using glutaminase, followed by enzymatic detection of glutamate (Lund, 1974). The within-assay CV for all metabolite assays were <5%.

**Western Blotting**

Muscle homogenates were centrifuged (8,000 × g for 15 min) and the supernatants were used for Western blotting to estimate the abundance of glutamine synthetase (Huang et al., 2007; Wang and Watford, 2007; Manso Filho et al., 2008). The protein content of the supernatants was determined by using the Bradford dye-binding method (Bio-Rad Laboratories, Hercules, CA) with BSA as the standard. Equal amounts (30 μg) of sample protein were solubilized and submitted to electrophoresis in 4 to 12% gradient-resolving SDS gels (Invitrogen, Carlsbad, CA), followed by electrotransfer to pure nitrocellulose membranes. Evenness of transfer was assessed by Ponceau staining. Membranes were blocked overnight with 5% (wt/vol) nonfat dried milk in 20 mM Tris, 120 mM NaCl, 0.1% (vol/vol) Tween 20, pH 8.0, followed by washing and incubation with primary antibody (mouse antishell glutamine synthetase, BD Laboratories, San Jose, CA) for 60 min. Glutamine synthetase bands were visualized by using horseradish peroxidase conjugated goat antimouse secondary antibody (mouse antisheep glutamine synthetase, BD Laboratories, San Jose, CA) for 60 min. Glutamine synthetase protein abundance was expressed as arbitrary densitometry units.

**Statistical Analyses**

Data were analyzed (SigmaStat, version 3, Jandel Scientific, San Rafael, CA) by 1-way ANOVA for repeated measures. Post hoc differences were identified by using Tukey’s test, with a significance value set at \( P < 0.05 \). Correlations were performed by using the Pearson product moment correlation. All data are reported as means ± SEM.

**RESULTS**

**Body Composition**

Large increases in fetal mass during the last 3 wk of gestation and intense milk production during lactation produced significant changes in body composition in transition mares. Because of the presence of the fetus during pregnancy, data were analyzed separately for the gestation and lactation phases. Body mass (Figure 1A) changed during gestation \( (P < 0.05) \), but not during lactation. Maximal BW during gestation was observed between 2 and 3 wk before parturition. In contrast, lean mass (Figure 1A) changed during both periods \( (P < 0.05) \), with maximal lean body mass occurring 3 wk before parturition. During the progression of lactation, lean body mass decreased by a mean of 1.5% \( (P < 0.05) \), but fat mass and percentage of fat (Figure 1B) were unchanged during both gestation and lactation.

**Plasma Hormones and Metabolites**

Plasma insulin (Figure 2A) concentrations were slightly greater throughout gestation than during lactation \( (P < 0.05) \) and there was a transient increase in insulin concentration immediately after birth \( (P < 0.05) \). A similar brief increase in plasma cortisol (Figure 2B) concentration was observed \( (P < 0.05) \), but otherwise cortisol concentrations were stable throughout gestation and the first few weeks of lactation. Eight weeks after parturition, however, plasma cortisol concentration began to increase and continued to increase throughout the next 4 wk \( (P < 0.05) \). Plasma leptin (Figure 2C) concentrations were somewhat variable, but did show a spike immediately after birth \( (P < 0.05) \) and then fell slightly during the first few weeks of lactation before increasing slowly as lactation progressed \( (P < 0.05) \).

Total plasma protein (Figure 3A) concentration did not change throughout the transition period, with the exception of a transient increase immediately after birth \( (P < 0.05) \). The concentration of glucose (Figure 3B) in the plasma was stable throughout gestation, then showed a transient increase immediately after birth \( (P < 0.05) \), which was followed by a decrease to below preparturition concentrations 1 wk after parturition \( (P < 0.05) \). Plasma glucose concentrations remained low for 7 to 8 wk and then increased to new steady-state concentrations slightly greater than those seen before parturition \( (P < 0.05) \). Plasma lactate (Figure 3B) concentrations were also constant throughout gestation and increased immediately after birth \( (P < 0.05) \). In this case, however, the elevated concentrations were maintained for the first 2 wk of lactation before returning to preparturition values by 5 wk after birth.

Plasma glutamate (Figure 4) concentrations were stable throughout the transition period. In contrast, plasma glutamine (Figure 4) concentrations remained stable throughout gestation, but almost doubled immediately after birth and remained elevated 1 wk later \( (P < 0.05) \). The concentration then began to decline to below preparturition concentrations, reaching a nadir by wk 6 \( (P < 0.05) \), after which they increased and stabilized by wk 8 to preparturition concentrations. The concentrations of glutamine in plasma during most of gestation and lactation were consistently below those detected in control nonpregnant, nonlactating mares \( (329 ± 29 \text{nmol/mL}, n = 4, P < 0.05) \).
Figure 1. Body mass, lean mass, fat mass, and percentage of body fat for transition mares. (A) Body mass (filled symbols) was unchanged in pregnancy and lactation, whereas lean body mass (open symbols) did not change during pregnancy but did decrease ($P < 0.05$) during lactation. (B) Fat mass (kg, filled symbols) and percentage of body fat (open symbols) were unchanged throughout the study period. Results are presented as means ± SEM for 8 animals.
Muscle Glutamine Content and Glutamine Synthetase Expression

Glutamine was highly abundant in skeletal muscle and the concentration did not change during lactation; similarly, muscle glutamate content was stable throughout lactation (Figure 5). Glutamine synthetase protein was readily detectable in skeletal muscle (Figure 6), with the abundance in samples taken 3 mo into lactation being similar to that obtained from control (nonpregnant, nonlactating) mares. Six months after parturition, however, muscle glutamine synthetase abundance was less ($P < 0.05$).

Milk Glutamine

Milk obtained 7 d after parturition contained large amounts of free glutamine and glutamate, with glutamate concentrations approximately 40% those of glutamine (Figure 7). At 3 and 6 mo after parturition, however, milk glutamine content had declined by more than half ($P < 0.05$) to be equal to the concentration of glutamate, which remained unchanged throughout lactation.

DISCUSSION

This is the first report of changes in body composition in the transition mare, and the finding that during lactation the mares lost lean body mass while maintaining fat mass is somewhat surprising. A loss of fat mass during lactation has been reported for several species, and loss of lean body mass is usually associated with insufficient protein nutrition (Meijer et al., 1993; Pine et al., 1994; Clowes et al., 2003a,b, 2005; Guan et al., 2004). Loss of lean body mass may have detrimental consequences for the future performance of the mare, and it should be noted that such losses occurred in the present study despite provision of supplemental concentrates. In the current work, the loss of lean body mass indicates that skeletal muscle protein was being mobilized early in lactation, presumably to provide AA precursors for the milk directly and for the gluconeogenesis required for the synthesis of lactose. The findings of high glutamine synthetase expression in skeletal muscle together with a reduction in circulating glutamine concentrations early in lactation also support this interpretation. Similar decreases in the concentration of circulating glutamine have been reported for the lactating dairy cow (Meijer et al., 1995; Plaizier et al., 2001; Doepele et al., 2006). In addition, lactation is accompanied by a decrease in intramuscular glutamine content in the dairy cow (Meijer et al., 1995) and increased expression of skeletal muscle glutamine synthetase in rats (Xiao et al., 2004), although such changes were not seen in the mares used in this study.

The sharp increase in the concentrations of most plasma hormones and metabolites at parturition is due, in part, to the hemoconcentration caused by fluid shifts and losses during the foaling process. For example, plasma protein concentrations increased by approximately 25%, and there was an approximately 40% increase in the mean concentrations of glucose, cortisol, and leptin, whereas the insulin concentration increased to more than 3 times the preparturition value. However, these increases were transient and were not seen

![Figure 2. Plasma hormone concentrations for transition mares. (A) Insulin, (B) cortisol, and (C) leptin concentrations all increased transiently after parturition. Plasma cortisol increased significantly ($P < 0.05$) 8 wk after parturition. Results are presented as means ± SEM for 8 animals.](image-url)
1 wk after parturition. In contrast, the concentrations of glutamine and lactate each increased by approximately 80% and remained elevated for more than 1 wk, indicating that factors in addition to fluid shifts were responsible. Because the fetus is known to extract large amounts of glutamine and lactate from the maternal circulation (Silver, 1984; Silver et al., 1994), it is highly likely that the increases in the circulating concentrations of these metabolites simply reflect the removal of this site of utilization. Thus, although the fetus is no longer present, maternal tissues continue to produce large amounts of glutamine and lactate that maintain the elevated circulating concentrations for the next few weeks. However, as lactation progresses there would be increasing utilization of glutamine by the lactating mammary gland. This increase in utilization apparently outstrips maternal glutamine synthesis, thereby resulting in decreased circulating glutamine concentrations. Interestingly, plasma glucose concentrations were least early in lactation at a time when plasma lactate concentrations were increased. Because the major fate of plasma lactate is utilization for gluconeogenesis, this observation may mean that gluconeogenesis, although required for lactose synthesis, is in some way limited at 1 wk after parturition. In contrast, the concentrations of glutamine and lactate each increased by approximately 80% and remained elevated for more than 1 wk, indicating that factors in addition to fluid shifts were responsible. Because the fetus is known to extract large amounts of glutamine and lactate from the maternal circulation (Silver, 1984; Silver et al., 1994), it is highly likely that the increases in the circulating concentrations of these metabolites simply reflect the removal of this site of utilization. Thus, although the fetus is no longer present, maternal tissues continue to produce large amounts of glutamine and lactate that maintain the elevated circulating concentrations for the next few weeks. However, as lactation progresses there would be increasing utilization of glutamine by the lactating mammary gland. This increase in utilization apparently outstrips maternal glutamine synthesis, thereby resulting in decreased circulating glutamine concentrations. Interestingly, plasma glucose concentrations were least early in lactation at a time when plasma lactate concentrations were increased. Because the major fate of plasma lactate is utilization for gluconeogenesis, this observation may mean that gluconeogenesis, although required for lactose synthesis, is in some way limited at
this time. Isotopic measurements of glutamine and lactate turnover in the lactating mare are required before any definitive conclusions can be drawn.

Glutamine is the most abundant free α-AA in human blood, and decreased circulating glutamine concentrations are usually associated with catabolic states and poor outcomes in a variety of patients (Tjader et al., 2007). Little information is available on glutamine metabolism in the horse (Manso Filho et al., 2008). In the present study, plasma glutamine values of approximately 300 nmol/mL were very similar to those reported by others (Rogers et al., 1984; Silver et al., 1994; Routledge et al., 1999; Young et al., 2003; Hackl et al., 2006; Harris et al., 2006). Furthermore, Silver et al. (1994) reported a decrease in glutamine concentrations in ponies during starvation, and Routledge et al. (1999) found a similar decrease after a viral challenge. The finding of a decrease in circulating glutamine concentrations during lactation, when large amounts are presumably being extracted by the mammary gland, as evidenced by the very high glutamine content of equine milk (Figure 5; see also Davis et al., 1994; Sarwar et al., 1998; Matsui et al., 2003) and comparison with other species (Meijer et al., 1993; Wu and Knabe, 1993), means that glutamine availability for maternal organs, such as the small intestine and immune cells, may be limiting as lactation proceeds.

The decrease in free glutamine content of milk as lactation progressed may indicate a relationship between maternal plasma glutamine and milk glutamine concentrations. However, because milk volume and composition change throughout lactation, firm conclusions are not possible. It is known, however, that the total AA concentration of mare milk decreases with the progression of lactation (Csapó-Kiss et al., 1995), which is in agreement with our findings for glutamine. The results of the present experiment also indicate that skeletal muscle glutamine synthetase expression was decreased late in lactation, a time when lactation demand had also decreased and the foals were almost weaned. The provision of large amounts of glutamine in the milk during early lactation is possibly related to the important role of this AA in the growth and development of the digestive tract, an organ that, in the adult horse, is known to utilize large amounts of glutamine (Duckworth et al., 1992; Salloum et al., 1993).

The results presented here indicate that lactation represents a mild catabolic state for the mare, even when supplemental nutrition is provided and when the mares have not been bred back. Loss of endogenous protein at this time is probably related to the need to provide glutamine both directly for milk nitrogen (free AA and proteins) and for the gluconeogenesis required for lactose synthesis. The resulting decrease in circulating glutamine concentrations would be expected to compromise glutamine availability to other tissues, such as the intestines and the immune system, in the lactating mare.

**LITERATURE CITED**


Equine glutamine metabolism


