GLUCOSE AND INSULIN DYNAMICS IN LATE GESTATION MARES AND NEONATAL FOALS

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ABSTRACT

Insulin sensitivity decreases during pregnancy, presumably an adaptation ensuring sufficient glucose supply to feto-placental tissues. Feeds high in non-structural carbohydrates are also linked to diminished insulin sensitivity in horses. Because the equine fetus is highly glucose reliant, maternal glucose and insulin dynamics during pregnancy may have implications for optimal fetal development in horses. Mismanagement of maternal nutrition during gestation could predispose the offspring to metabolic disorders (e.g. insulin resistance) later in life. In horses, insulin resistance is associated with increased risk for development of laminitis. These studies measured insulin sensitivity and glucose dynamics in pregnant and non-pregnant mares fed high sugar and starch (**SS**) or high fat and fiber (**FF**) feeds, as well as neonatal foals born from pregnant mares fed SS and FF feed. Insulin modified frequently sampled intravenous glucose tolerance tests (FSIGT) were applied to pregnant Thoroughbred mares (n = 22) at 28 ± 3 wks (Period 1) and 47 wks (Period 2) gestation, as well as non-pregnant mares (n=10) measured simultaneously. Following the first FSIGT mares were fed SS or FF feed for the remainder of the study. After 11 wks adaptation to feeding, a subset of mares were evaluated with hourly blood samples for 24 h to assess glycemic and insulinemic response to three times daily feeding while on pasture. Neonatal foal FSIGTs (n=20) were conducted at 5 ± 1 d of age. The minimal model of glucose and insulin dynamics was used to determine insulin sensitivity (**SI**), glucose effectiveness (**Sg**), acute insulin response to glucose (**AIRg**) and disposition index (**DI**). Pregnant mares during Period 1 exhibited lowered SI, Sg and elevated AIRg relative to non-pregnant mares. Pregnant mares demonstrated greater glycemic and insulinemic responses to feeding of both SS and FF meals than non-pregnant mares consuming the same feeds. Also, SS feed elicited greater glycemic and insulinemic areas under the curve following feeding than FF feed in pregnant mares. These data support that pregnancy in mares in associated with lowered SI by 28 wks gestation and that altered SI, Sg and AIRg are associated with different responses to consuming SS and FF feeds. Foals exhibited high basal glucose, basal

insulin, SI and Sg relative to mature horses, indicating a large capacity for glucose uptake with or without insulin. Basal glucose concentrations were higher and basal insulin concentrations tended to be higher in SS than FF foals ($P = 0.016$ and $P = 0.071$, respectively). Glucose and insulin dynamics in late gestation mares and neonatal foals exemplify the adaptive nature of energy metabolism in horses. Furthermore, dietary energy composition affects glucose and insulin responses to feeding in late gestation mares, which in turn was associated with different basal blood concentrations of these variables in the resulting neonatal foals.

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While finishing this thesis, the tragic events of April 16th occurred on Virginia Tech's Blacksburg campus. I feel it is only appropriate to acknowledge the lives lost on this day and offer this work as a tribute to those students' lives which were cut short and their academic and personal goals that will not be met. Also I acknowledge the faculty members that lost their lives while participating in the academic process as teachers and mentors. I mourn these losses to our Hokie family and hope that this work can honor their lives as a product of the proud and resilient community that is Virginia Tech.

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CHAPTER I

Introduction

Insulin is the primary regulator of blood glucose concentrations. Among other functions, insulin stimulates clearance of glucose from the bloodstream into insulin-sensitive cells, decreases cellular glucose production and increases glucose storage or glycogen synthesis. The cellular effects of insulin rely on insulin receptors that activate intracellular signaling mechanisms. Of particular interest is the translocation of insulin-dependent glucose transporter (**GLUT**) 4 to the cellular membrane, through which glucose is transported into the cell from blood. The ability of insulin to bind to its receptors on insulin-sensitive tissues (e.g. skeletal muscle, liver), stimulate translocation of GLUT-4, and ultimately accelerate blood glucose clearance (i.e. insulin sensitivity) varies considerably depending on multiple factors, suggesting this system is highly adaptive. Changes in insulin sensitivity in response to different nutritional and physiologic conditions allows an animal to appropriately respond to fluctuating blood glucose concentrations and to partition energy substrate to those tissues having the greatest glucose requirement, such as the brain or exercising muscle. A high non-structural carbohydrate (**NSC**) diet leads to decreased insulin sensitivity in horses (Pratt et al., 2006; Treiber et al., 2005a). Thus, the effects of grazing pasture, which fluctuates in NSC concentration, is also of interest in relation to nutritional effects on insulin sensitivity and glucose dynamics (Cubitt et al., 2006; Hoffman et al., 2001). Physical conditioning has been shown to ameliorate the effect of a high NSC diet on insulin sensitivity (Pratt et al., 2006). During exercise, horses demonstrate heightened insulin sensitivity (Treiber et al., 2006a). Insulin sensitivity also changes with pregnancy and lactation (Fowden et al., 1984; Hoffman et al., 2003b). Low insulin sensitivity or insulin resistance is a hallmark of obesity and metabolic syndrome, potentially leading to laminitis in horses and to diabetes in humans (Hoffman et al., 2003a; Kronfeld et al., 2006; Rader, 2007; Treiber et al., 2006b). Clarification of maladaptive changes in glucose and insulin dynamics associated with development of obesity and laminitis could help reduce the occurrence of health problems detrimental to the horse and the equine

industry (Treiber et al., 2006b). Neonates demonstrate unique metabolic characteristics relative to mature animals as well (Forhead et al., 2004; Fowden et al., 1982). Characterization of neonatal glucose and insulin dynamics in foals and evaluation of the impact of maternal nutrition during gestation may help prevent such maladaptive changes that predispose animals to disease.

Literature Review

Assessment of Glucose and Insulin Dynamics

A complicating factor for research in glucose and insulin dynamics in horses lies in effectively evaluating a complex and dynamic physiologic system. Many methods utilizing single blood samples for evaluation of basal insulin and/or glucose concentration have been implemented to simply, inexpensively, and non-invasively screen for insulin resistance in human clinical and research settings (Monzillo and Hamdy, 2003). In horses, proxies for insulin sensitivity and beta cell responsiveness have also been established utilizing a single basal blood sample (Treiber et al., 2005b). However, while useful in large populations or situations where more invasive measures are not possible, evaluation of single basal blood samples provides a static picture of a dynamic system, leaving information regarding hepatic glucose production, insulin-mediated glucose clearance, glucose-mediated glucose clearance and beta cell dysfunction inaccessible. Improvements on these simple tests have utilized stimulation by glucose either by oral or intravenous administration followed by blood samples taken 30 min and 2 h after glucose administration. A number of indices have been developed to mathematically evaluate insulin sensitivity and metabolic clearance rates using area under the curve of glucose and insulin in combination with these basal, 30 min and 2 h glucose and insulin concentrations following glucose administration (Monzillo and Hamdy, 2003). The hyperinsulinemic, euglycemic clamp has been favored as a more ideal method for accurately evaluating insulin sensitivity and glucose clearance utilizing a known input of insulin to maintain a steady-state glucose concentration. This method has long been considered the gold standard for evaluation of insulin sensitivity, but is intensive and

expensive, limiting its usefulness in research. The minimal model approach utilizing a frequently sampled intravenous glucose tolerance test describes insulin sensitivity, insulin response to glucose, glucose-mediated glucose clearance and an index of insulin compensation for varying levels of insulin sensitivity. The frequently sampled intravenous glucose tolerance test requires only one venous catheter, single bolus doses of glucose and insulin and is accomplished with 31 timed blood samples collected over a 4 h period following glucose administration (Figure 1).

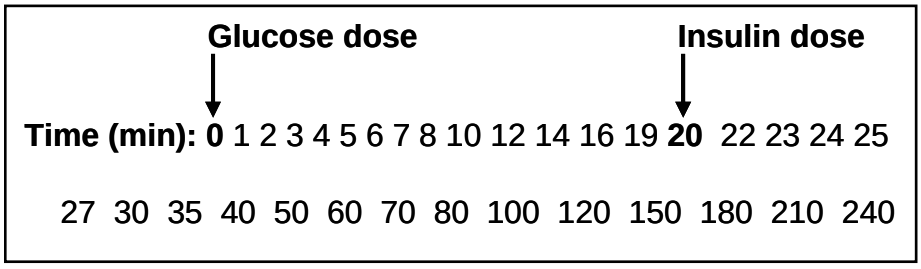

Figure 1. Time protocol (min) of blood samples relative to glucose administration for application of the insulin modified frequently sampled intravenous glucose tolerance test. This protocol is utilized for the minimal model approach of assessing glucose and insulin dynamics.

A computer model (MinMod Millenium 5.10) is utilized which measures changes in glucose concentration relative to changes in insulin concentration providing a more appropriately dynamic and descriptive model of the glucose-insulin system.

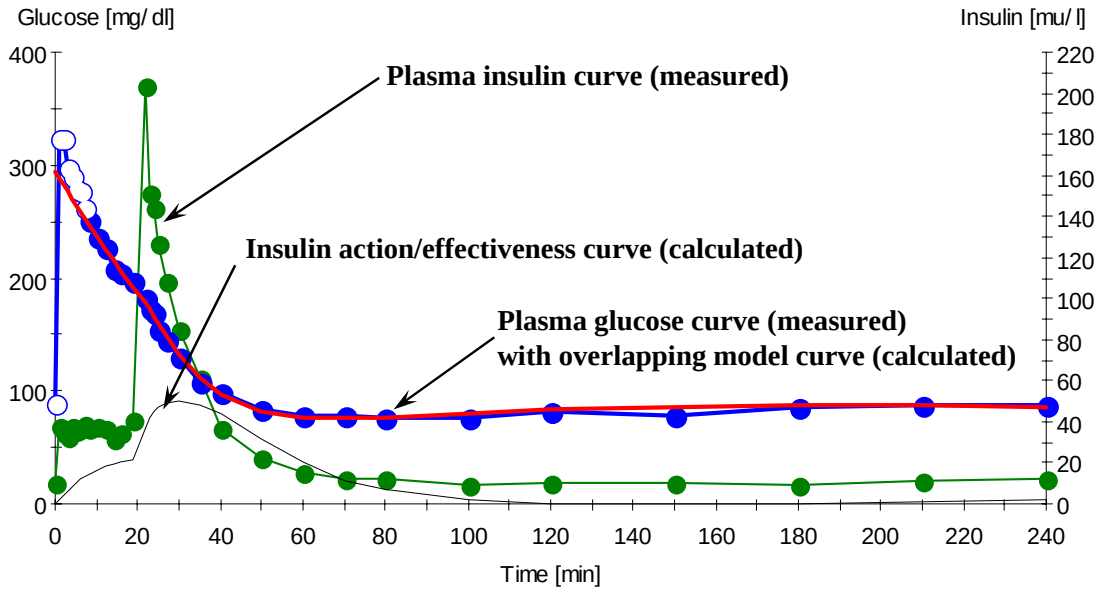

Figure 2. Results of minimal model analysis from MinMod Millenium 5.10 include calculated curves for insulin action and glucose clearance.

Special Considerations for Glucose and Insulin Dynamics in Horses

Supplemental feeding. Non-structural carbohydrates (**NSC**), particularly starch which is high in cereal grains, are primarily digested and absorbed in the foregut of horses. Structural carbohydrates, the fiber component of forages which form the foundation of most horse diets, are digested primarily by fermentation in the hindgut. Horses are grazing animals, meaning they succeed consuming large quantities of structural carbohydrates throughout the day and can depend less on starch. The modern horse, however, often receives supplemental feeding of higher energy density feeds to meet the demands of athletic performance, breeding, pregnancy, lactation and growth under conditions of confinement. Typically equine diets include cereal grains (e.g. corn, oats) which have high starch concentrations. It is well established that feeding grains elicits a glycemic and insulinemic response in horses though this response varies among different horses and feeds (de Fombelle et al., 2004; Hoffman et al., 2003b; Jose-Cunilleras et al., 2004; Stull and Rodiek, 1988; Vervuert et al., 2004, 2003; Williams et al., 2001). Corn and oats have been shown to elicit a glycemic response approximately

60% of that observed with direct intragastric glucose administration indicating a substantial impact on glucose and insulin dynamics following meal ingestion (Jose-Cunilleras et al., 2004). Feeding high NSC (starch and sugar) feeds has been linked to diminished insulin sensitivity in horses (Hoffman et al., 2003a; Treiber et al., 2005a). In lactating mares, peak glucose and insulin concentrations, as well as glucose and insulin area under the curve, were greater following consumption of a meal high in NSC versus a fat and fiber based feed (Williams et al., 2001).

Pasture. Pasture forages can also accumulate substantial amounts of NSC (Longland and Byrd, 2006). However, little work has been published evaluating the effects of grazing pasture on glucose and insulin dynamics in horses. Temporal and environmental factors affect NSC content of pasture grasses; thus grazing horses consume varying amounts of NSC depending on time of day, season, temperature and forage species (Cubitt et al., 2006; McIntosh, 2007). Grazing pastures high in NSC is associated with development of insulin resistance and subsequent laminitis in predisposed ponies suggesting a nutritional relationship between pasture or season and the glucose-insulin system (Treiber et al., 2006c). Indeed, pasture associated laminitis is the greatest owner reported cause of laminitis in horses (USDA, 2000).

Pregnancy. Because the equine feto-placental unit is highly glucose reliant, maternal glucose and insulin dynamics during pregnancy may have implications for optimal fetal development in horses (Fowden et al., 2000b). In most mammalian species, insulin sensitivity decreases during pregnancy, presumably an adaptation ensuring sufficient glucose supply to feto-placental tissues (Bell and Bauman, 1997). Therefore, pregnancy represents a unique metabolic state which should be considered when determining appropriate nutritional management of pregnant animals. Women consuming high-glycemic diets during pregnancy have been shown to gain more weight during pregnancy than women consuming low-glycemic diets (Clapp, 2002). Chronic hyperglycemia (2-3 wks) in ewes has been associated with a decrease in GLUT concentrations in placental epithelial cell membranes dividing maternal and fetal bloodstreams (Das et al., 2000). However, changes in expression of GLUT in the placenta may not be a limiting factor for glucose transfer from maternal to fetal blood since these transporters normally operate far below levels of saturation (Bell et al., 1999). Hyperglycemia may induce changes in

placental blood flow, affecting oxygen and global nutrient delivery, having implications for utilization of all nutrients and partitioning of energy substrate into oxidative and nonoxidative pathways for the developing feto-placental unit (Aldoretta and Hay, 1999).

Neonates. The neonatal period marks a transition from continual parenteral to intermittent enteral nutrient supply. In order to successfully make this transition, the insulin secretory capacity of the pancreas develops throughout the fetal period (Fowden et al., 1980; Fowden et al., 2005). Early metabolic development of horses is of interest since the diet (i.e. mare's milk) of the developing foal differs markedly from mature horses and the gastrointestinal tract is still transforming into the mature simple stomach, hind-gut fermentation system utilized by horses. Little work has characterized glucose and insulin dynamics in the neonatal foal. Insulin response to intravenous glucose challenge has been demonstrated in 1, 5, and 9 d old pony foals and was similar at all three ages (Holdstock et al., 2004). Fetal overgrowth induced by transferring pony embryos into Thoroughbred mares resulted in higher basal insulin and greater insulin response to glucose in 2 d old foals than foals not subject to fetal overgrowth by embryo transfer (Forhead et al., 2004). In 14-30 d old llama crias, glucose was cleared more rapidly than rates normally observed in adults in response to glucose challenge and rate of clearance was significantly increased following insulin challenge (Cebra and Tornquist, 2005). Evaluation of specific parameters of glucose and insulin dynamics estimated by the minimal model has never been conducted in foals.

Link between Maternal Nutrition and Neonatal Health: Fetal Programming

A number of hypotheses have emerged over recent decades to describe the connection between environmental conditions during gestation and incidence of metabolic and cardiovascular disease in adult life in humans. The "thrifty genotype" hypothesis was first raised to suggest evolutionary adaptations for survival in times of famine, a relevant historical threat to human survival (Neel, 1999). Such adaptations are now detrimental to health in modern developed countries with abundant food supply. The "thrifty phenotype" hypothesis suggests that early undernutrition causes a reallocation of energy and nutrients to favor development of critical organs (e.g. the brain) at the expense of less

critical organs and systems (e.g. the pancreas) contributing to the eventual failure of those systems, or disease onset, in adulthood (Hales and Barker, 2001). More recently, the term "predictive adaptive responses" has been applied to changes in development made in response to the environment in utero which are assumed to be adaptive and predictive of the environment into which the offspring will enter following parturition (Gluckman and Hanson, 2004). The "developmental origins hypothesis" proposes adult onset disease originates due to alterations made during impressionable periods of development, due to specific nutritional conditions experienced during gestation and infancy and includes ideas from all of the preceding hypotheses (Barker, 2004). All of these hypotheses describe different aspects of what is probably a combination of long term evolutionary and short term phenotypic adaptations that occur during early development to ensure the best possible chance of survival in a particular environment.

These ideas have developed from an abundance of epidemiological data in humans linking maternal nutrition during pregnancy to various measures of the resulting neonates. Higher postprandial blood glucose concentrations in pregnant women have been associated with fetal macrosomia, suggesting changes in fetal growth induced by maternal nutrition (Combs et al., 1992). Also, higher post-prandial insulin response and larger infant birthweights have been observed in pregnant women consuming high compared to low-glycemic diets (Clapp, 2002). Besides stimulating peripheral glucose uptake, insulin also stimulates peripheral and hepatic production of insulin-like growth factors (**IGF**). Maternal IGF promotes fetoplacental growth and development indicating a potential role of altered maternal IGFs in fetoplacental growth restriction or macrosomia (Bell et al., 1999; Sferruzzi-Perri et al., 2006). Thus, IGFs could contribute to the associations observed between elevated postprandial glucose and insulin concentrations and infant birthweight.

While epidemiological evidence in humans is substantial, a lack of controlled studies represents the greatest limitation to addressing the unanswered questions regarding developmental nutrition, namely maternal nutrition during gestation and its link to disease in mature animals and humans. Altered growth of key organs during fetal development could contribute to permanent structural differences which predispose an individual to disease later in life (Nathanielsz, 2006). Rats born and reared by

nutritionally restricted (50% of control diets) dams, compared to rats born and reared by control dams, demonstrated a decline in glucose tolerance due to pancreatic insufficiency (decreased beta cell mass, decreased insulin secretion) between three and twelve months of age (Garofano et al., 1999). Additionally, lambs from undernourished ewes exhibited altered adiposity and reduced glucose tolerance compared to controls (Ford et al., 2007). In rats born from protein restricted mothers, cross fostering onto non-restricted rat dams led to maintenance of higher BW in adulthood than controls implying gestational conditions contributed to a thrifty phenotype later causing an overweight condition (Ozanne et al., 2004).

Glucose and the Feto-Placental Unit

Glucose is the primary energy substrate for the maintenance and growth of both the placenta and fetus in late gestation (Bell and Ehrhardt, 2002; Fowden et al., 2000b). For example, in the pony fetus, absolute glucose utilization doubles from mid (< 220 d) to late (300+ d) gestation (Fowden et al., 2000b). However, fetal growth is balanced with the ability of the placenta to supply ample glucose for this phase of rapid growth and development, and is marked by a decrease in glucose delivery per unit weight of the fetus. A 50 to 60% fall in umbilical glucose delivery, per unit weight of fetus, was observed in pony fetuses in late gestation (Fowden et al., 2000b). A probable alternative energy substrate for the equine fetus is lipid which is likely produced by a combination of placental and fetal tissues (Fowden et al., 2000b; Stammers et al., 1995). However, maternal glucose still represents the majority of energy substrate utilized by the horse fetus (about 85%) in late gestation (Fowden et al., 2000b).

Placental glucose transport. A number of factors contribute to the functional ability of the placenta: surface area for exchange, metabolic activity, and glucose transport capacity. Glucose transport across the placenta is controlled by two primary factors: a concentration gradient between maternal and fetal compartments and the expression of glucose transporter (**GLUT**) proteins that facilitate transport of glucose from maternal to fetal blood (Bell and Ehrhardt, 2002). In horses, maternal blood glucose concentration (~100 mg/dL) is approximately twice that of fetal blood (~50 mg/dL) (Fowden et al.,

2000b). The placenta of the mare requires glucose to cross multiple epithelial layers all sealed with tight junctions: maternal endothelium, uterine epithelium, fetal trophoblast epithelium, and fetal endothelium to cross from maternal to fetal blood. Differential localization of insulin independent GLUT 1 and GLUT 3 isoforms, with GLUT 3 having a higher affinity for glucose, across these tissue layers facilitates the concentrations gradient across each tissue layer (Wooding and Fowden, 2006) (Figure 3).

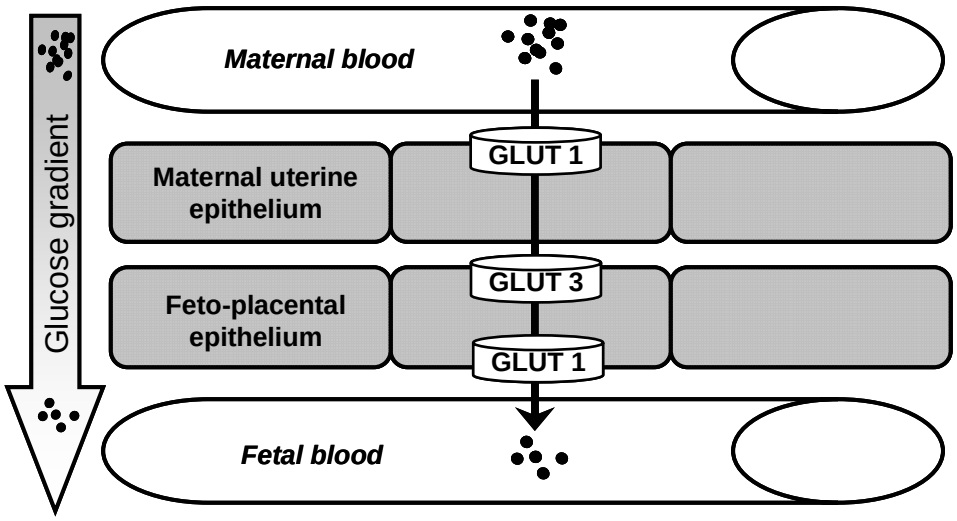

Figure 3. Diagrammatic representation of glucose transport driven by the maternal-fetal blood glucose concentration gradient, facilitated by GLUT 1 and GLUT 3 localization between maternal and fetal compartments in the epitheliochorial placenta of horses.

In a discussion of placental glucose transport, it is important to note that the placenta itself is a significant consumer of glucose from maternal blood. Uteroplacental tissues utilize more glucose and oxygen than the fetus per unit of weight (Aldoretta and Hay, 1999; Fowden et al., 2000a).

Effects of maternal glycemic state. Maternal glycemic and/or insulinemic state influences the glucose concentration gradient between cell layers, but may also impact expression of GLUT 1 and GLUT 3. In late gestation ewes, hyperglycemia induced by chronic glucose infusion resulted in decreased concentrations of GLUT 1 and GLUT 3 in

maternal and fetal epithelial layers and hypoglycemia induced by chronic insulin infusion resulted in decreased concentrations of GLUT 1 (Das et al., 2000; Das et al., 1998). Thus, hyper- and hypoglycemia in ewes decrease GLUT 1 expression in the basolateral portions of the maternal and fetal epithelial layers, whereas GLUT 3 expression in the apical fetal epithelium is only modified by hyperglycemia. The impact of these alterations in GLUT 1 and GLUT 3 expression on the fetus are not entirely clear. Decreased concentrations of GLUT 1 and GLUT 3 in response to hyperglycemia may have a protective effect on the glycemic state of the fetus, while decreases in GLUT 1 in response to chronic hypoglycemia may or may not impact glucose delivery to the fetus. Perhaps down-regulation of GLUT 1 in response to hypoglycemia does not further limit glucose availability to the fetus, but is merely a response to a decrease in utilization of the transporter. Instead of supporting expression of GLUT 1, in a state when GLUT 1 proteins may be far from saturated, cellular resources and membrane space can be reserved for the transport of alternative fuels such as amino acids, lactate and lipid without limiting the ability of the placenta to transport available glucose. Furthermore, down-regulation of GLUT 1 increases the relative importance of GLUT 3, a transporter with a higher glucose affinity, possibly helping compensate for lower substrate availability and driving the diminished concentration gradient between maternal and fetal compartments.

Together with changes in GLUT transporter expression, changes in glucose metabolism by uteroplacental tissues play a role in buffering changes in maternal glucose supply. In late gestation ewes of a low glycemic state (about 70% normal), a smaller fraction of the uteroplacental oxygen consumption was utilized for glucose oxidation, while absolute oxygen consumption remained constant, when compared to the same ewes in a high glycemic state (Aldoretta and Hay, 1999). This suggests that a decreased maternal glucose supply contributes to a shift in the type of substrate utilized in oxidative pathways, implying greater oxidation of alternative fuels such as amino acids, lactate and lipid when glucose supply is low. In horses, fetal blood glucose decreased and fetal blood urea increased significantly in response to maternal fasting, indicating fetal amino acid catabolism in response to reduced glucose availability (Fowden et al., 2000b).

Maternal glucose supply has implications for glucose transport to the fetus by impacting the maternal-fetal glucose concentration gradient, GLUT transporter expression and metabolic pathways utilized by the placenta. However, studies examining these effects in sheep, and especially horses, are largely limited to short term blood glucose manipulation by infusions and fasting. Examination of nutritional effects, specifically dietary energy composition, on maternal glucose and insulin dynamics in relation to feto-placental glucose dynamics and neonatal health has yet to be conducted in horses.

Objectives

The objectives of this study are:

1. to evaluate the effect of dietary energy composition in combination with stage of gestation on aspects of maternal glucose and insulin dynamics by application of the minimal model to grazing late gestation versus non-pregnant mares
2. to examine the glycemic and insulinemic response to feeding of test diets in these late gestation and non-pregnant mares while grazing pasture
3. to evaluate the effect of maternal dietary energy composition during late gestation on glucose and insulin dynamics in the resulting neonatal foals
4. to characterize glucose and insulin dynamics by the minimal model approach in healthy neonatal foals

Hypotheses

Our hypotheses as related to the above stated objectives are:

1. Insulin sensitivity will be lower in pregnant mares compared to non-pregnant mares and will decrease with advancing gestation. Consumption of a high sugar and starch feed will further exacerbate this decline in insulin sensitivity associated with gestation.
2. High sugar and starch feed will elicit a greater glycemic and insulinemic response to feeding in pregnant and non-pregnant mares than fat and fiber based feed.
3. Foals born to dams consuming sugar and starch feed throughout late gestation will exhibit different glucose and insulin dynamics including lowered insulin sensitivity compared to foals born fto dams consuming fat and fiber feed.
4. Neonatal foals will exhibit different glucose and insulin dynamics, including heightened insulin sensitivity, than observed in mature horses.

CHAPTER II

Insulin sensitivity and glucose dynamics in pregnant and non-pregnant mares

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Abstract

Pregnancy is associated with a decline in maternal insulin sensitivity. Feeding high sugar and starch feeds is also associated with diminished insulin sensitivity. Providing horses such feeds during pregnancy could impact maternal glucose and insulin dynamics, potentially influencing feto-placental glucose utilization and fetal development. This study examined insulin sensitivity and glucose dynamics in pregnant and non-pregnant mares fed high sugar and starch (SS) or high fat and fiber (FF) feeds. Insulin modified frequently sampled intravenous glucose tolerance tests (FSIGT) were applied to pregnant Thoroughbred mares (n = 22) at 28 ± 3 wks (Period 1) and 47 wks (Period 2) gestation, as well as non-pregnant mares (n=10) measured simultaneously. Following the first FSIGT mares were fed SS or FF feed for the remainder of the study. After 11 wks adaptation to feeding, a subset of mares were evaluated with hourly blood samples for 24 h to assess glycemic and insulinemic response to three times daily feeding while on pasture. The minimal model of glucose and insulin dynamics was used to estimate insulin sensitivity (**SI**), glucose effectiveness (**Sg**), acute insulin response to glucose (**AIRg**) and disposition index (**DI**) from glucose and insulin concentrations throughout the FSIGT. Pregnant mares during Period 1 exhibited lower SI, Sg and elevated AIRg relative to non-pregnant mares. Insulin sensitivity decreased in non-pregnant, but not pregnant, mares from Period 1 to 2. Pregnant mares demonstrated greater glycemic and insulinemic responses to feeding of both SS and FF meals than non-pregnant mares consuming the

same feeds. Also, SS feed elicited greater glycemic and insulinemic areas under the curve following feeding than FF feed in pregnant mares. These data support that pregnancy in mares in associated with lowered SI by 28 wks gestation and that altered SI, Sg and AIRg are associated with different postprandial responses to consuming SS and FF feeds. These differences in maternal glucose and insulin dynamics associated with different dietary carbohydrate composition could have implications for feto-placental glucose transport and metabolism, potentially impacting fetal development.

***Keywords**: glucose dynamics, insulin sensitivity, maternal nutrition, minimal model, pregnant mare*

Introduction

Decreased insulin sensitivity associated with progression of pregnancy in women has been demonstrated extensively. This change in carbohydrate metabolism is presumably an adaptation of pregnancy which encourages glucose sparing by maternal tissues in order to ensure sufficient glucose supply to feto-placental tissues (Butte, 2000; Fowden et al., 1984). However, data specifically demonstrating differences in insulin sensitivity between pregnant and non-pregnant mares is lacking. Feeds high in non-structural carbohydrates (**NSC**) are also linked to diminished insulin sensitivity in horses (Treiber et al., 2005a). Because the majority of energy substrate utilized by feto-placental tissues is glucose from maternal blood, maternal glucose and insulin dynamics during pregnancy has implications for optimal fetal development in horses (Fowden et al., 2000b). Our objective was to evaluate glucose and insulin dynamics in pregnant mares at mid- and late gestation compared to non-pregnant mares supplemented a concentrate utilizing primarily NSC (49% NSC, 4% fat) or fat (14% NSC, 13% fat) as an energy source. We hypothesized that insulin sensitivity would decrease from mid- to late gestation in pregnant mares and that a high NSC diet would further exacerbate a decline in insulin sensitivity related to gestation.

Materials and Methods

Animals and Management

Pregnant (28 ± 3 wks gestation, n=22) and non-pregnant (n=10) Thoroughbred mares were maintained on pastures at the Virginia Tech Middleburg Agricultural Research and Extension Center. Pastures were a mix of grass and legume forage species and mixed grass and legume hay was provided in winter months as needed based on pasture conditions. Following initial measurement (described below), isocaloric, isonitrogenous feeds balanced to provide adequate vitamins and minerals were offered to provide two-thirds of DE requirements, with the remainder available from forage. Mares were assigned to either high sugar and starch (SS) or high fat and fiber (FF) feed according to age (pregnant: 9.6 ± 2.8 y, non-pregnant: 11.7 ± 4.4 y) and current body condition score (**BCS**) (pregnant 6.2 ± 0.6, non-pregnant 5.8 ± 0.7). Daily rations were divided into three equal portions that were fed at approximately 0700, 1130, and 1430 into individual pans arranged in a large circle in each pasture, with groups separated into different pastures according to feed and pregnancy status. Mares were rotated among pastures with similar botanical composition to ensure equivalent pasture conditions for all groups. Pastures, hay, and experimental feeds were sampled throughout the study period and analyzed for nutrient content (Dairy One Forage Laboratory, Ithaca, NY).

Frequently Sampled Intravenous Glucose Tolerance Test Protocol

Insulin modified frequently sampled intravenous glucose tolerance tests (**FSIGT**) were applied to all pregnant and non-pregnant mares during late November and early December prior to feed treatments (Period 1). On the morning of the FSIGT mares were brought in from pasture between 0700 and 0800 and BW measured on an electronic scale. Jugular catheters were placed with aseptic preparation and local analgesia of the overlying skin, after which mares were allowed to rest for approximately 30 min prior to baseline blood samples. Body condition was also evaluated by the average of two BCS

assigned by two trained evaluators (Henneke et al., 1983). Mares had ad libitum access to hay and water when stalled before and during the test, but were not provided a morning feed ration. Baseline blood samples were taken 15 min and immediately prior to intravenous glucose administration (300 mg/kg BW 50% dextrose, Vedco Inc., St. Joseph, MO). Blood samples were taken at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 min following glucose administration. At 20 min post-glucose administration, an insulin dose (20 mIU/kg BW Humulin R, Lilly, Lake Forest, IL) was administered with additional blood samples obtained at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 100, 120, 150, 180, 210, and 240 min post-glucose injection.

At 46.5 ± 0.5 wks of gestation, approximately 2 wks prior to predicted foaling date, a second FSIGT (Period 2) was applied with non-pregnant mares tested concurrently. Testing dates spanned across an approximately 10 wk period from mid-March to mid-May because of the range of breeding dates for the pregnant mares.

Monthly Evaluation

Monthly basal blood samples were collected between 0700 and 0800 in the pasture between Periods 1 and 2. Mares were not provided their 0700 meal until after all animals had been sampled. Following blood samples, BW was measured on an electronic scale and BCS evaluated by the same two evaluators as Period 1 and 2.

Circadian Measurement of Glycemic and Insulinemic Feed Response

To evaluate daily glycemic and insulinemic patterns in response to feeding, a subset of mares (n=3 from each group: pregnant SS, pregnant FF, non-pregnant SS, non-pregnant FF) were monitored hourly for a 24 h period. Pregnant mares selected for this portion of the study were at 38 ± 1.4 wks gestation, which was not different between SS and FF fed mares. All mares had been adapted to their respective feeds 11 wks. Mares were catheterized in the pasture at 1600 and blood samples taken hourly for 24 h beginning at 1800. Mares and their environment were managed to avoid stress and undue interference with their natural behavior at pasture throughout the 24 h blood sampling period. Feeds

were delivered at the same times and in the same manner as mares were normally accustomed and approximate time taken to complete meals recorded.

Sample Handling and Analysis

All blood samples were collected into blood collection tubes containing sodium heparin as anticoagulant (BD Vacutainer, Fisher Scientific Company, Newark, DE) and placed immediately in ice water. Within 30 min tubes were centrifuged for 10 min at 3000 g and 4 ºC. Plasma was collected and stored frozen at -20° C.

Glucose and insulin concentrations were measured in all plasma samples. Triglyceride concentration was measured in FSIGT baselines and monthly samples between periods. Leptin was measured in FSIGT baseline samples. Plasma glucose concentrations were measured by the glucose oxidase method using a chemical autoanalyzer (Kit # 442640, Beckman Synchron CX5CE, Brea, CA). Plasma insulin was assayed by a commercially available radioimmunoassay kit (Coat-A-Count Insulin, Diagnostic Products Corp., Los Angeles, CA). Intra-assay CV was 6.4% for this insulin assay. Leptin was analyzed by radioimmunoassay (Multi-species Leptin RIA, Linco Research, Inc.). Triglyceride concentrations were measured by enzymatic assay (Beckman Instruments, CX5 Chemistry Analyzer).

Parameters of the minimal model of insulin and glucose dynamics; insulin sensitivity (**SI**), glucose effectiveness (**Sg**), acute insulin response to glucose (**AIRg**), and disposition index (**DI**); were determined using MinMod Millenium 5.10. Differences between feed and pregnancy groups for metabolic and minimal model parameters were determined by mixed analysis of variance with repeated measures (SAS Institute Inc., Cary, NC). Areas under the curve (AUC) above baseline for circadian glucose and insulin were determined by trapezoidal approximation using the mean of 0500 – 0700 samples as a baseline for each mare (Graphpad Prism 4.0). Differences in AUC, peak concentration, and basal concentration were determined by analysis of variance (StataCorp, College Station, TX). One outlying observation for SI was removed from analysis in the non-pregnant FF fed mares during Period 1. Outlying values for AIRg for a different mare in the non-pregnant FF group from both periods were also removed from

analysis. Outliers were determined by Grubbs' test with a critical value of $P < 0.05$. The mare excluded from AIRg analysis was also excluded from analysis of the 24 h feed response portion of this study due to severe hyperinsulinemia. This mare was later observed to become progressively insulin resistant, hyperinsulinemic and develop pasture-based laminitis.

Results

Animals and Feed Treatments

Body weight in both pregnant mare groups increased from mid- to late gestation ($P < 0.001$). A small (0.6 score units) but statistically significant decrease in BCS was detected in FF pregnant mares across periods ($P = 0.031$), but BCS did not change in SS pregnant mares. In non-pregnant mares, BW increased in SS mares ($P = 0.004$), but did not change in FF mares ($P = 0.84$). Mean BCS also increased by about one score in the non-pregnant SS group ($P = 0.007$), but did not change in FF mares ($P = 0.41$). Results of mare BW and BCS are summarized in Table 1. Results of nutrient analysis, including crude protein, crude fat, NSC, ADF and NDF, for pasture, hay, and SS and FF feeds are summarized in Table 2. Monthly pasture NSC content is depicted in Figure 1.

Minimal Model Parameters

Insulin sensitivity (SI) was lower in pregnant than in non-pregnant mares during Period 1 ($P < 0.001$). Insulin sensitivity did not change from Period 1 to 2 in pregnant mares regardless of feed treatment ($P = 0.75$), but decreased in non-pregnant SS and FF fed mares between FSIGT periods ($P = 0.003$ and $P = 0.001$, respectively). Insulin sensitivity in pregnant and non-pregnant mares was not different in Period 2 ($P = 0.14$) (Figure 2A).

Glucose effectiveness (Sg) or insulin-independent glucose clearance was lower in pregnant than non-pregnant mares during Period 1 ($P = 0.006$). In pregnant mares, Sg did not change from Period 1 to 2 ($P = 0.39$). In non-pregnant mares, Sg decreased

across periods (P = 0.001), attributable to a decrease in Sg for FF but not SS non-pregnant mares (P = 0.025 and P = 0.265, respectively). There was no difference in Sg between pregnant and non-pregnant mares in Period 2 (P = 0.95) (Figure 2B).

During Period 1, pregnant mares had higher AIRg than non-pregnant mares (P = 0.044). Across periods, AIRg increased in non-pregnant SS mares ($P = 0.026$), but did not change in any other group (Figure 3A).

Disposition index (DI) is defined by the minimal model as SI × AIRg and estimates compensation for lowered insulin insensitivity by increased insulin output in response to glucose administration. Pregnant mares had lower DI than non-pregnant mares during Period 1 ($P < 0.001$), but were not different in Period 2 ($P = 0.71$). Only non-pregnant FF mares decreased significantly in DI between periods due to a decrease in SI without a compensatory increase in AIRg ($P = 0.010$) (Figure 3B).

Basal Plasma Markers of Energy Homeostasis

During Period 1, baseline glucose concentrations were not different between pregnant and non-pregnant mares (P = 0.873) and increased from Period 1 to 2 in pregnant ($P = 0.053$) and non-pregnant ($P = 0.008$) mares, with no feed effects observed. Basal plasma insulin concentrations were not different between pregnant and non-pregnant mares during Period 1 ($P = 0.92$), but increased from Period 1 to 2 in non-pregnant SS mares (P = 0.020) contributing to higher basal insulin concentrations in non-pregnant than pregnant mares in Period 2 (P = 0.020). Plasma leptin concentrations were not different ($P = 0.99$) between pregnant and non-pregnant mares during Period 1, but also increased ($P < 0.001$) between periods in non-pregnant SS mares. Plasma triglyceride concentrations were not affected by diet, pregnancy, or period. All basal blood variables across periods and intermediate months are reported in Table 3.

Circadian Glycemic and Insulinemic Response

Mares were observed to remain at one feed pan containing a single ration and complete their meals in approximately 30-45 min, with SS meals usually consumed 10-

15 min faster than FF meals. This pattern of feeding behavior was observed throughout the study. Meal response patterns of plasma glucose and insulin over a 24 h period are shown for each group (Figure 4). Pregnant mares consuming SS feed demonstrated markedly different circadian glucose and insulin patterns than FF pregnant mares or SS and FF non-pregnant mares.

Glucose AUC and peak glucose concentration were greater in pregnant than non-pregnant SS mares ($P = 0.007$ and $P = 0.008$, respectively) and in pregnant than non-pregnant FF mares ($P = 0.053$ and $P = 0.015$, respectively). Basal glucose concentrations were also greater in pregnant than non-pregnant SS mares ($P = 0.006$) and tended to be greater in pregnant than non-pregnant FF mares ($P = 0.096$).

Insulin AUC tended to be greater in pregnant than non-pregnant SS mares ($P = 0.066$) and was greater in pregnant than non-pregnant FF mares ($P = 0.041$). Peak insulin concentrations were greater in pregnant than non-pregnant SS mares ($P = 0.055$) and tended to be greater in pregnant than non-pregnant FF mares ($P = 0.060$). Basal insulin concentrations were not affected by pregnancy status within either feed treatment.

Pregnant SS mares had significantly greater glucose AUC ($P = 0.023$) and tended to have greater insulin AUC than pregnant FF mares ($P = 0.087$). There were no significant differences detected between non-pregnant mares fed SS and FF feed for the parameters measured.

Discussion

The results of this study indicate that pregnant mares entering the last third of gestation in late fall to early winter have lower peripheral insulin sensitivity, lower insulin independent glucose clearance, and higher insulin response to glucose than non-pregnant mares under the same management evaluated simultaneously. Pregnant mares adapted to high NSC feed in the last third of gestation have greater glycemic and insulinemic responses to feeding than non-pregnant mares or pregnant mares consuming a high fat and fiber feed. Lastly, insulin sensitivity decreases between late fall and spring in pasture maintained non-pregnant mares, potentially attributable to changing pasture conditions, an adaptation not observed in pregnant mares.

Effects of Pregnancy Status

These data support that pregnancy is associated with lowered SI (i.e. insulin-mediated glucose clearance). In mares, this change appears to occur prior to 28 wks gestation, the time when mares in the present study were first examined and observed to exhibit SI within the second lowest reference quintile previously defined for healthy horses (Treiber et al., 2005b). Mean SI in the non-pregnant mares measured simultaneously fell within the highest reference quintile. Lowered peripheral insulin sensitivity is proposed to be an adaptive mechanism during pregnancy which impedes clearance of glucose into maternal tissues in order to shunt glucose to feto-placental tissues which are highly glucose reliant (Fowden et al., 1984). Thus, maternal hepatic glucose production and fatty acid utilization increase to compensate for combined feto-placental and maternal energy needs (Bell and Bauman, 1997; Butte, 2000). Pregnant mares in this study also exhibited lower Sg or rate of insulin-independent glucose clearance implying slower glucose clearance with or without insulin during pregnancy. Higher AIRg observed in pregnant mares indicates greater compensatory insulin output presumably to help offset lower peripheral glucose clearance. However, DI, which estimates compensation for lowered SI by accounting for AIRg, was also lower in the pregnant compared to non-pregnant mares suggesting that elevated insulin secretion is not entirely compensating for the lower SI observed in these 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 non-pregnant mares at either period measured. Therefore, while pregnancy was characterized by numerous metabolic adaptations, glucose homeostasis was not altered by pregnancy in this study.

Feeding Effects in Pregnancy

Glucose and insulin concentrations in horses are frequently disrupted day to day by feeding grain meals. Grains typically included in horses' feeds (e.g. corn and oats) have been shown to elicit a glycemic response approximately 60% of that observed with direct

intragastric glucose administration of an equal quantity of available carbohydrate (Jose-Cunilleras et al., 2004). In lactating mares fed experimental feeds similar to those in the present study, peak glucose and insulin concentrations, as well as glucose and insulin AUC, were greater following SS than FF feed intake (Williams et al., 2001). Results of the 24 h portion of this study indicate that feeding high NSC feed to late gestation mares results in prolonged hyperglycemia and hyperinsulinemia throughout a 24 h period relative to feeding a fat and fiber based feed.

Glucose transport across the placenta is insulin independent. Therefore, alterations in maternal insulin action would not apply to glucose transport across this tissue. Instead, glucose transport from the maternal to the feto-placental compartment is facilitated by a concentration gradient and insulin independent glucose transporters (**GLUT**) 1 and 3 (Wooding et al., 2000). Maternal hyperglycemia could increase the blood glucose concentration gradient between maternal and fetal compartments leading to greater glucose delivery to feto-placental tissues. Expression of GLUT in placental tissues may also be affected. Chronic hyperglycemia in ewes has been linked to decreased expression of GLUT 1 and 3 in placental tissues, as well as changes in placental blood flow, and energy substrate partitioning and utilization (Aldoretta and Hay, 1999; Bell and Ehrhardt, 2002). Changes in blood flow could occur due to the periods of prolonged hyperinsulinemia associated with hyperglycemia observed in these pregnant mares. Insulin is associated with endothelial dysfunction in insulin resistant subjects through its effects on endothelin-1 and nitric oxide production (Eades et al., 2007; Kim et al., 2006). Such changes in vascular function would affect glucose delivery to feto-placental tissues as well as delivery of other essential nutrients and oxygen. Maternal hyperinsulinemia could also affect maternal insulin-like growth factor (**IGF**) production. Maternal IGF-I and IGF-II promote fetoplacental growth and development indicating a potential role for IGFs in fetal macrosomia associated with elevated postprandial insulin concentrations (Bell and Ehrhardt, 2002; Sferruzzi-Perri et al., 2006).

It is unknown whether hyperglycemia and hyperinsulinemia associated with feeding constrained to half or a third of each day, as observed in this study, would have different effects on GLUT expression, blood flow, and substrate utilization than observed in studies inducing hyperglycemic by chronic infusion, for example. However, elevated

postprandial blood glucose concentrations in pregnant women have been associated with fetal macrosomia, indicating hyperglycemia induced by meal consumption can induce changes in fetal growth (Combs et al., 1992). Whether these changes occur by alteration in GLUT expression, placental blood flow, and/or uteroplacental glucose metabolism remains unknown.

Differences between glycemic and insulinemic responses to these feeds in the non-pregnant mares were not as apparent in this small sample size, but this difference in feed response between pregnant and non-pregnant mares suggests that mares with altered glucose metabolism (lowered SI, Sg, DI and elevated AIRg) due to pregnancy represent a population of horses particularly sensitivity to the carbohydrate composition of feed.

Changes with Feed Treatment and Season

In pregnant mares, no changes in SI, Sg, AIRg or DI in either feed group occurred over the final third of gestation. In non-pregnant mares, adaptation to a high NSC diet but not a high fat and fiber feed resulted in weight gain, increased body condition, increased basal plasma leptin concentration, and higher insulin response to glucose. However, both groups of non-pregnant mares demonstrated decreased insulin sensitivity suggesting that factors other than high NSC feed and weight gain contributed to changes in glucose and insulin dynamics. Period 2 measurements occurred in the spring, when pasture forage quantity and quality is increased (Cubitt et al., 2006). The observed metabolic changes in non-pregnant mares may, therefore, be due to a combination of increased forage availability and altered forage nutrient content, particularly higher NSC (Longland and Byrd, 2006). Such changes in insulin sensitivity were not observed in the pregnant mares likely because their metabolism was already altered by pregnancy and lacked further flexibility to respond to changes in pasture conditions. Potential metabolic changes associated with the endocrine milieu of non-pregnant mares coming out of seasonal anestrus, relative to pregnant mares maintaining pregnancy, may also contribute to changes in SI observed in non-pregnant mares from winter to spring that were not observed in the pregnant mares (Cubitt et al., 2007).

The decrease in body condition in pregnant FF mares, though small and probably physiologically insignificant, coupled with the significant increase in condition in the SS non-pregnant mares suggests that energy composition of feed impacts body condition. Pregnant women consuming high-glycemic diets during pregnancy have been shown to gain more weight during pregnancy than women consuming low-glycemic diets (Clapp, 2002). Dissimilar weight gain associated with high NSC feed relative to high fat and fiber feed has also been reported in Arabian horses (Treiber et al., 2006a). Since all mares were fed according to DE requirements and feeds were isocaloric, it is unlikely that differences in DE intake of the feeds prompted these changes in body condition. Differences in digestion and metabolism of the different energy sources could influence forage intake from pasture due to different influences on satiety or other physiologic mechanisms controlling fat deposition. It should be noted that the apparent lower palatability of FF feed may have resulted in reduced consumption of FF relative to SS feed in this study during the month of April when pasture NSC (and presumably, palatability) was highest. During April, mares backed off of feed temporarily, first in pregnant mares and then in both pregnant and non-pregnant groups. This occurred particularly in FF fed groups, but both SS and FF groups were observed to refuse feed intermittently through the month of April.

Elevated plasma triglyceride concentrations, along with lower insulin sensitivity, has been implemented as a risk factor for development of pasture based laminitis and is incorporated into the definition of pre-laminitic metabolic syndrome defined for ponies (Treiber et al., 2006c). However, changes in SI observed in this study were not accompanied by altered triglyceride concentrations. This could highlight a difference between normal adaptive changes in metabolism associated with season in grazing horses and changes leading to a clinical condition (e.g. laminitis).

Implications

The results of this study indicate that pregnant mares entering the last third of gestation in late fall to early winter have lower peripheral insulin sensitivity, lower insulin independent glucose clearance, and higher insulin response to glucose than non-

pregnant mares. These differences are associated with greater glycemic and insulinemic responses to consuming high sugar and starch feed compared to both pregnant mares fed high fat and fiber feed and non-pregnant mares fed either type of feed. Thus, consideration of dietary energy composition is of particular importance when feeding pregnant mares. Differences in glucose and insulin dynamics associated with pregnancy in this study led to recurrent periods of hyperglycemia and hyperinsulinemia following consumption of high NSC feed. Prolonged hyperglycemia and hyperinsulinemia may affect glucose utilization by feto-placental tissues and influence fetal development. Maladaptive changes in fetal development associated with maternal nutrition could predispose the foal to insulin resistance and associated diseases such as laminitis and developmental orthopedic disease later in life. Research examining links between insulin resistance in mature horses and their nutritional environment during fetal development may elucidate the impact of pregnant mare nutrition on the health of horses.

Table 1. Mean (±SD) of body weight (BW) and body condition score (BCS) are represented before (Period 1) and after (Period 2) dietary feed treatments, as well as for each month between period measurements. Mares are divided by pregnancy status and feed treatment: high sugar and starch (SS) versus high fat and fiber (FF). Significant differences across periods (*, $P < 0.05$) are noted within treatment groups.

	Period	Month	Pregnant		Non-pregnant	
			SS	FF	SS	FF
BW, kg	1		601 ± 35	595 ± 44	572 ± 44	595 ± 39
		Jan	612 ± 35	606 ± 55	570 ± 38	600 ± 37
		Feb	602 ± 35	601 ± 52	581 ± 44	596 ± 42
		Mar	634 ± 43	627 ± 63	600 ± 48	631 ± 60
		Apr	649 ± 49	638 ± 59	628 ± 50	640 ± 52
	2		662 ± 45*	644 ± 61*	625 ± 58*	612 ± 60
BCS	1		6.1 ± 0.6	6.3 ± 0.6	6.0 ± 0.4	5.5 ± 0.9
		Jan	6.0 ± 0.4	6.3 ± 0.5	6.4 ± 0.3	6.0 ± 1.0
		Feb	6.5 ± 0.5	6.4 ± 0.6	6.9 ± 0.4	6.1 ± 0.8
		Mar	6.2 ± 0.4	5.9 ± 0.7	6.6 ± 0.6	6.7 ± 1.1
		Apr	6.2 ± 0.6	6.3 ± 0.5	6.8 ± 0.6	6.0 ± 1.4
	2		6.2 ± 0.4	5.6 ± 0.6*	7.1 ± 0.4*	6.4 ± 0.7

Table 2. Results of nutrient analysis of high sugar and starch (SS) and high fat and fiber (FF) feeds, pastures and hay. Mean and standard deviation are represented for crude protein (CP), crude fat, non-structural carbohydrates (NSC), acid detergent fiber (ADF), neutral detergent fiber (NDF), and digestible energy (DE) on a % dry matter (DM) basis.

	SS (n=20)		FF (n=20)		Pasture (n=46)		Hay (n=2)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
% CP	14.5	1.5	15.5	0.7	17.3	5.6	16.5	0.07
% Crude fat	3.4	0.6	13.9	2.2	2.7	0.8	2.7	0.1
% NSC	50.4	5.7	13.0	2.9	9.4	3.5	8.3	0.5
% ADF	10.0	1.7	28.2	2.6	34.4	6.4	39.9	2.1
% NDF	17.6	2.7	42.2	3.0	62.3	7.8	54.6	0.4
DE, Mcal/kg	3.0	0.3	3.0	0.2	1.1	0.2	0.98	0.02

Table 3. Mean (±SD) of plasma concentrations of glucose, insulin, triglyceride and leptin are represented before (Period 1) and after (Period 2) dietary feed treatments, as well as for each month of the study. Mares are divided by pregnancy status and feed treatment: high sugar and starch (SS) versus high fat and fiber (FF). Unless otherwise noted in a superscript, n=11 in pregnant SS and FF groups and n=5 in SS and FF non-pregnant groups.

Status	Period	Month	Pregnant		Non-pregnant	
			SS	FF	SS	FF
Glucose, mg/dL	1		93.3 ± 3.9	93.9 ± 3.5	97.6 ± 7.3	92.5 ± 4.3
		Jan	91.4 ± 5.4	95.3 ± 4.9	87.8 ± 3.3	88.8 ± 4.0
		Feb	92.1 ± 2.3	95.4 ± 4.4	94.7 ± 3.0	94.6 ± 5.4
		Mar	94.0 ± 2.9$^{n=9}$	94.8 ± 2.2$^{n=9}$	92.5 ± 1.8$^{n=4}$	91.7 ± 4.0$^{n=3}$
		Apr	99.6 ± 2.7$^{n=6}$	99.8 ± 3.0$^{n=5}$	93.9 ± 1.8$^{n=3}$	96.8 ± 1.7$^{n=2}$
	2		95.7 ± 5.2	99.0 ± 5.4*	103 ± 6.6	101 ± 4.9*
Insulin, mIU/L	1		11 ± 6.1	11 ± 3.3	9.3 ± 1.7	8.9 ± 6.4
		Jan	8.6 ± 6.5	8.4 ± 3.5	4.6 ± 1.4	6.1 ± 6.0
		Feb	8.2 ± 5.3	6.5 ± 2.0	17 ± 7.4	9.3 ± 5.7$^{n=4}$
		Mar	9.1 ± 6.8$^{n=9}$	5.1 ± 2.2$^{n=9}$	5.5 ± 1.4$^{n=4}$	3.1 ± 0.5$^{n=3}$
		Apr	16 ± 13$^{n=6}$	11 ± 4.0$^{n=5}$	10 ± 2.5$^{n=3}$	9.4 ± 3.0$^{n=2}$
	2		10 ± 8.8	9.8 ± 3.6	20 ± 9.3*	17 ± 17
Triglyceride, mg/dL	1		28.3 ± 5.6	33.4 ± 10	24.2 ± 4.3	24.3 ± 12$^{n=4}$
		Jan	24.4 ± 5.1	27.7 ± 6.7	21.6 ± 3.0$^{n=4}$	20.3 ± 7.2$^{n=3}$
		Feb	22.8 ± 6.6	21.5 ± 4.6$^{n=9}$	12.4 ± 1.1	19.7 ± 4.2$^{n=2}$
		Mar	22.9 ± 4.7$^{n=9}$	26.5 ± 8.1$^{n=9}$	16.9 ± 5.2$^{n=4}$	11.0 ± 0.9$^{n=2}$
		Apr	38.3 ± 9.6$^{n=6}$	39.1 ± 9.8$^{n=5}$	19.8 ± 4.1$^{n=2}$	17.4 ± 5.9$^{n=2}$
	2		29.3 ± 7.6	39.8 ± 15	25.8 ± 5.6	22.0 ± 16$^{n=4}$
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

Table 4. Mean (±SD) and ranges for total area under the curve (AUC), basal concentrations and peak concentrations for plasma glucose and insulin in response to feeding over a 24 h period in grazing pregnant and non-pregnant mares on pasture supplemented with a high sugar and starch (SS) or high fat and fiber (FF) feed. Significant differences (*) and trends (#) are noted across pregnancy status, within same feed treatment. Significant differences (α) and trends (β) are noted across feed treatment, within same pregnancy status.

		Pregnant		**Non-pregnant**	
		SS (n=3)	**FF (n=3)**	**SS (n=3)**	**FF (n=2)**
Basal glucose, mg/dL	Mean ± SD	98 ± 1.5	94 ± 2.5$^{\beta}$	91 ± 1.7*	89 ± 1.0$^{\#}$
	Range	97-100	92-97	89-92	89-90
Peak glucose, mg/dL	Mean ± SD	162 ± 19	113 ± 0.8$^{\alpha}$	108 ± 5.0*	105 ± 3.0$^{\alpha}$*
	Range	151-183	112-114	104-113	103-107
AUC glucose, h*mg/dL	Mean ± SD	303 ± 67	155 ± 26$^{\alpha}$	94 ± 23*	76 ± 9*
	Range	260-380	128-180	74-119	67-82
Basal insulin, mIU/L	Mean ± SD	24 ± 22	9.3 ± 3.3	8.7 ± 3.7	9.1 ± 3.0
	Range	11-50	5.5-11	4.5-11	7-11
Peak insulin, mIU/L	Mean ± SD	190 ± 91	56 ± 10$^{\beta}$	42 ± 17$^{\#}$	33 ± 3.0$^{\#}$
	Range	125-291	48-68	29-62	30-35
AUC insulin, h*mIU/L	Mean ± SD	1467 ± 879	320 ± 81$^{\beta}$	182 ± 121$^{\#}$	109 ± 22*
	Range	941-2482	227-372	95-320	93-125

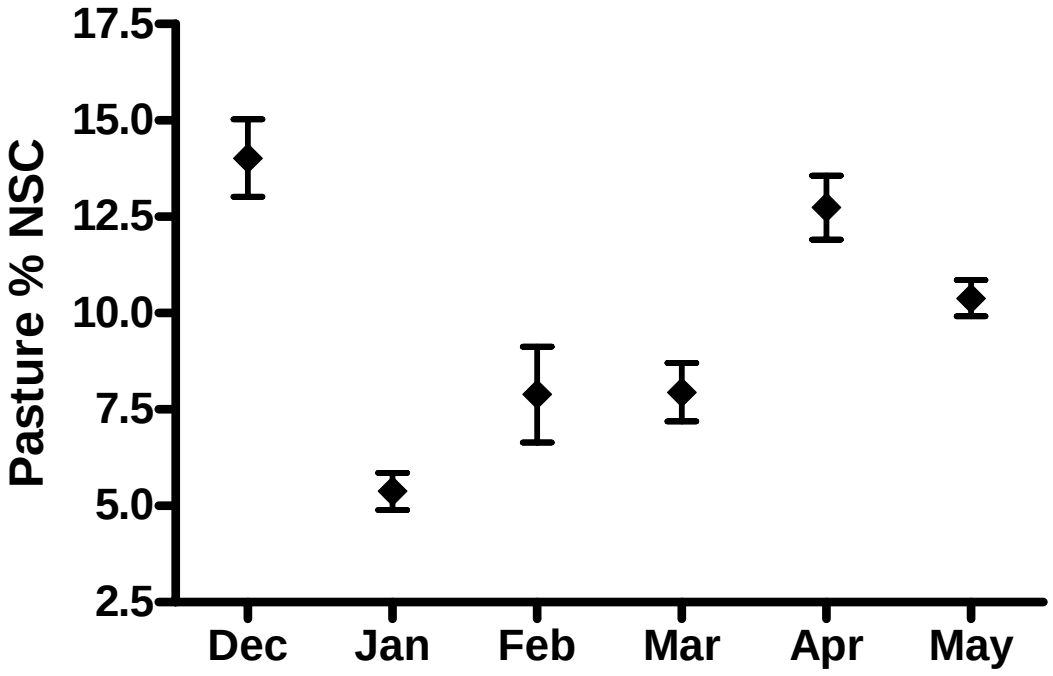

Figure 1. Non-structural carbohydrate (NSC) content on a % dry matter basis of pasture mares were grazing from Dec through May. Symbols and bars represent means and standard error.

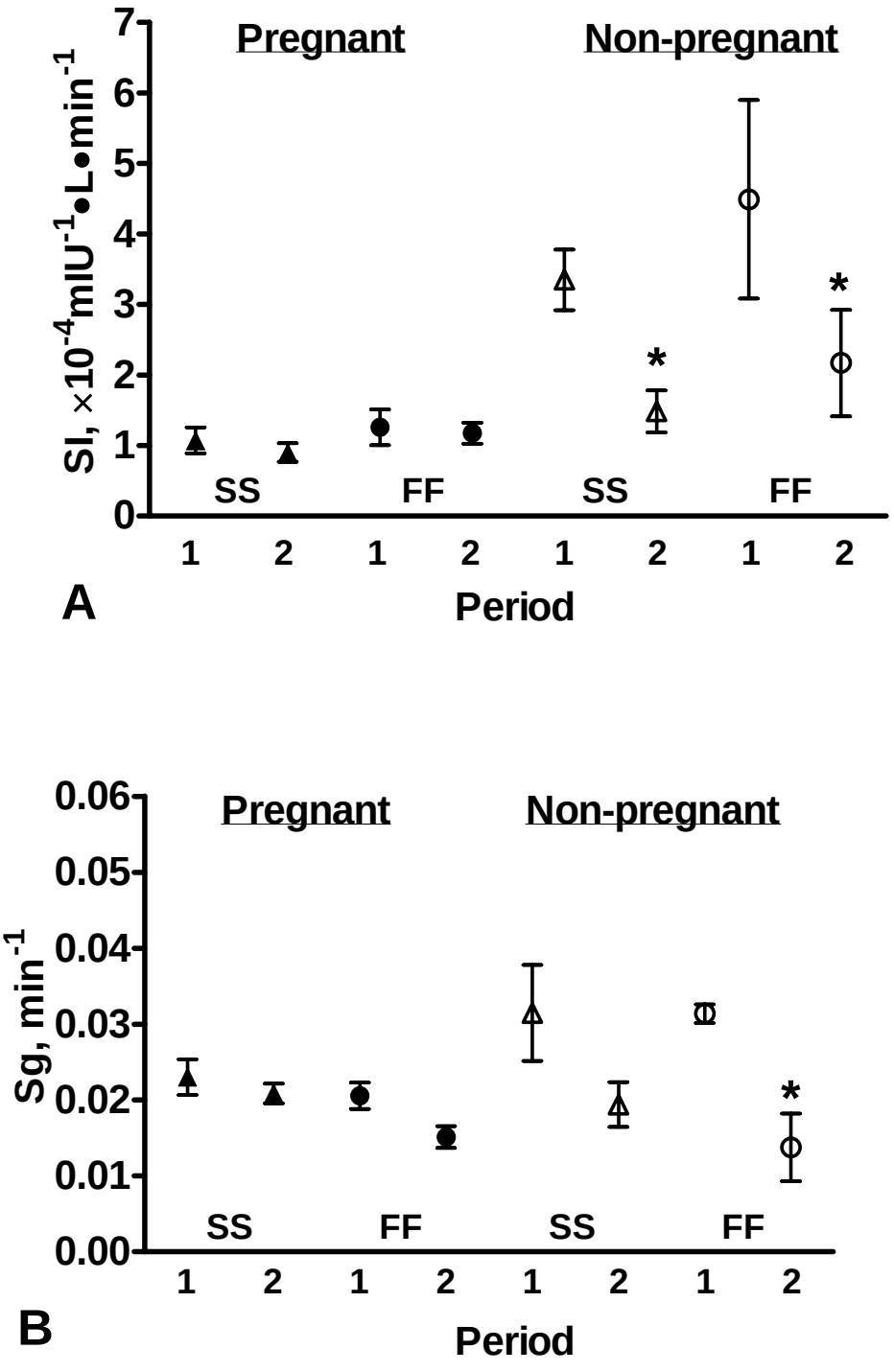

Figure 2. Insulin sensitivity (SI) (A) and glucose effectiveness (Sg) (B) from 28 (Period 1) to 47 (Period 2) wks of gestation in pregnant mares and non-pregnant mares at Period 1 and 2. *, $P < 0.05$ across periods

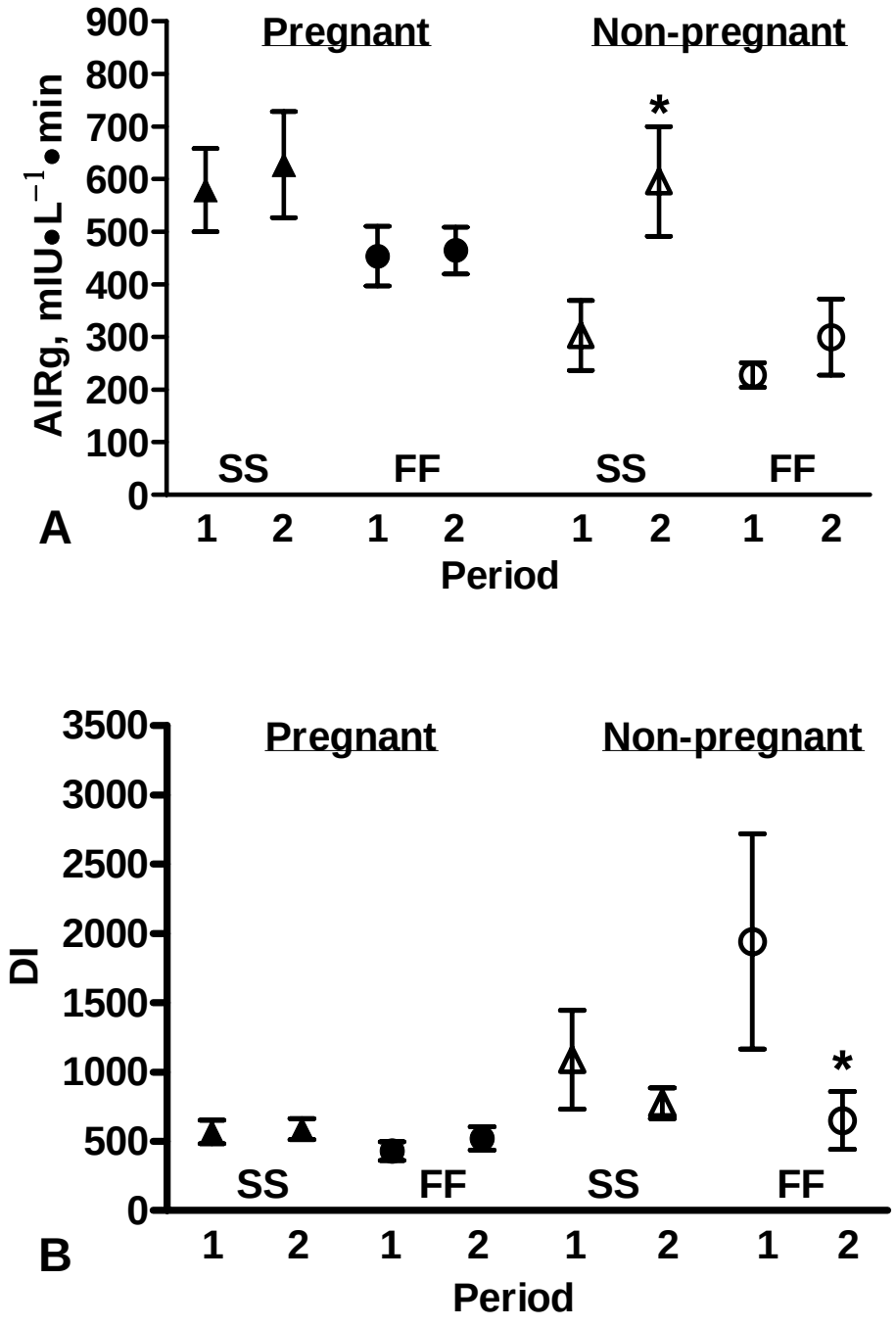

Figure 3. Changes in acute insulin response to glucose (AIRg) (A) and disposition index (DI) (B) in pregnant and non-pregnant mares organized by feed treatment: sugar and starch (SS) and fat and fiber (FF). *, $P < 0.05$ across periods

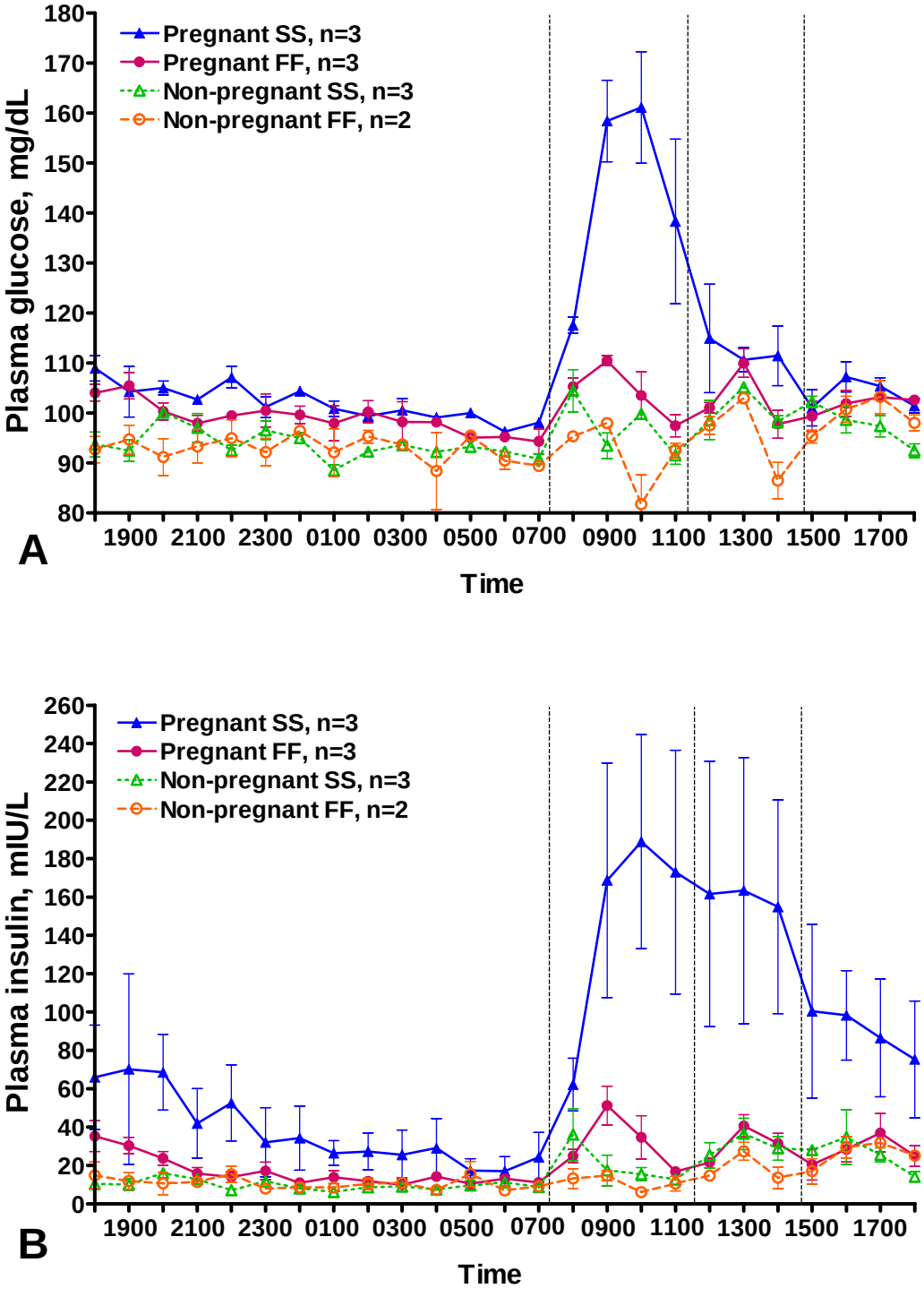

Figure 4. Circadian plasma glucose (A) and insulin (B) patterns in response to feeding (vertical dashed lines) in grazing pregnant and non-pregnant mares supplemented with a high sugar and starch (SS) or fat and fiber (FF) feed after 11 wks adaptation to feeding.

CHAPTER III

Glucose and insulin dynamics in neonatal foals following maternal dietary treatment

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Abstract

Fetal programming refers to changes in development that occur in response to environment during in utero development, presumably changes that are appropriate adaptations for the environment in which that animal will have to survive following parturition. Metabolic changes occurring during fetal and neonatal growth that are not appropriate to the environment in which animals later live may predispose to development of insulin resistance, which is associated with obesity and laminitis in mature horses. This study characterized insulin and glucose dynamics in neonatal foals by the minimal model approach and examined the influence of maternal diet on these measures of neonatal insulin and glucose dynamics. Twenty late gestation mares maintained on pasture were supplemented two-thirds of energy requirements with feed high in either sugar and starch (**SS**) or fat and fiber (**FF**). Foals were born spontaneously and an intravenous glucose tolerance test applied at 5 ± 1 d of age. Insulin sensitivity (**SI**), glucose effectiveness (**Sg**), acute insulin response to glucose (**AIRg**) and disposition index (**DI**) were determined by the minimal model of insulin and glucose dynamics. Foals exhibited high basal glucose, SI and Sg relative to mature horses, indicating a large capacity for glucose uptake with or without insulin. Basal glucose concentrations were higher and basal insulin concentrations tended to be higher in SS than FF foals ($P = 0.016$ and $P = 0.071$, respectively). Insulin sensitivity (mean $\times 10^{-4}$ $mIU^{-1}\cdot L\cdot min^{-1}$) ranged widely (4.5 – 72) and was substantially higher than values observed in mature horses

(median 1.8; 95% reference interval 0.2 – 5.9). Variation in SI was not attributable to maternal dietary treatments, sex, or birthweight. The neonate clearly demonstrates higher insulin dependent and independent glucose clearance than mature horses, indicating the importance of glucose as an energy substrate in young, growing and suckling foals, but factors affecting variation in the parameters measured remain unclear.

***Keywords**: fetal programming, glucose and insulin dynamics, maternal nutrition, minimal model, neonatal foal*

Introduction

Fetal programming describes changes in development occurring in utero that are presumably adaptive to the environment in which the offspring will enter following parturition. Work in other species suggests that maternal nutrition during pregnancy may impact fetal development, specifically development of systems controlling glucose and insulin dynamics (Armitage et al., 2004; Nathanielsz, 2006). Thus, mismanagement of maternal nutrition during gestation, resulting in maladaptive changes in fetal development, could predispose the offspring to metabolic disorders (e.g. insulin resistance) later in life. Undernutrition during gestation in sheep resulted in glucose intolerance and depleted insulin response to glucose in 250 d old offspring (Ford et al., 2007). In horses, insulin resistance is associated with increased risk for development of obesity and laminitis (Hoffman et al., 2003a; Treiber et al., 2006b; Vick et al., 2007). Predisposition to such disorders in horses could be related to influences of maternal nutrition on fetal development. Furthermore, little data describes glucose and insulin dynamics in healthy neonatal foals, the basis of understanding healthy changes in metabolism associated with development in horses. Thus, the objectives of this study were to characterize glucose and insulin dynamics by the minimal model approach in healthy neonatal foals and to examine the effect of dietary energy composition fed during the last third of gestation on glucose and insulin dynamics in neonatal foals. Evaluation of insulin sensitivity and other parameters of insulin and glucose dynamics by the minimal model approach has not been conducted in neonatal foals. We hypothesized that foals born from dams fed high starch feed relative to foals born from dams fed a high fat

and fiber feed in late gestation would exhibit lowered insulin sensitivity and altered glucose dynamics, responses observed in mature horses fed high glycemic diets (Hoffman et al., 2003a; Pratt et al., 2006; Williams et al., 2001).

Materials and Methods

Animals and Management

Pregnant Thoroughbred mares (n=20) of similar age and body condition were maintained on pastures at the Virginia Tech Middleburg Agricultural Research and Extension Center (Table 1). Pastures were a mix of grass and legume forage species and mixed grass and legume hay was provided in winter months as needed based on pasture conditions. Beginning at 28 ± 3 wks gestation, mares were provided two-thirds of DE requirements from feed high in either sugar and starch (**SS**: n=10) or fat and fiber (**FF**: n=11) (Table 2) (NRC, 1989). The remainder of mares' energy requirement was assumed to be provided by forage. Feeds were isocaloric, isonitrogenous and formulated to provide adequate vitamins and minerals (NRC, 1989). Daily rations were divided into three equal portions offered at approximately 0700, 1130, and 1430 into individual pans arranged in a large circle in each pasture, with groups separated into different pastures according to feed and pregnancy status. Mares were rotated monthly among pastures with similar botanical composition to ensure equivalent pasture conditions for all groups. Pastures, hay, and experimental feeds were sampled throughout the study period and analyzed for nutrient content (Dairy One Forage Laboratory, Ithaca, NY).

Foals were born spontaneously at pasture and then brought into a stall with their dam for the first 24 to 36 h of life to allow closer observations of neonatal health and activity. Birthweight was recorded within 12 h. Blood samples collected between 12 to 24 h post parturition confirmed normal serum IgG concentrations (SNAP Foal, IDEXX Laboratories Inc.). Mares and foals were moved to a grass and legume paddock after 24 to 36 h. Mares were fed the same as during late gestation; no appreciable intake of feed by the neonatal foal was observed.

Frequently Sampled Intravenous Glucose Tolerance Test Procedure

When foals were 5 ± 1 d of age, an insulin modified frequently sampled intravenous glucose tolerance test (FSIGT) was conducted. On the morning of the FSIGT, mares and foals were brought into the barn between 0700 and 0800 and foal BW measured. Foals were lightly restrained without sedation and a jugular catheter (16 ga) placed following aseptic preparation and local analgesia of the overlying skin. Foals were allowed a rest period of at least 30 min with their dam and allowed to suckle normally in order to reduce potential effects of stress. The catheterization procedure was completed within approximately 20 min and did not cause observable stress to the foals. Hay and water, but not a morning feed ration, were provided in the stall. Following the rest period, baseline blood samples were taken -30 min, -15 min and immediately prior to intravenous glucose administration (300 mg/kg BW 50% dextrose, Vedco Inc., St. Joseph, MO), when the timed test began. Blood samples were then taken at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 min following glucose administration. At 20 min post-glucose, an insulin dose (10 mIU/kg BW Humulin R, Lilly, Lake Forest, IL) was administered and blood samples continued at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 100, 120, 150, and 180 min. The 10 IU/kg BW insulin administration, half the dose normally used in adult horses, was chosen to avoid inducing excessive hypoglycemia in the young foals. Blood was immediately transferred to sodium heparin blood collection tubes and placed in ice water. Within 30 min, tubes were centrifuged for 10 min at 3000 g and 4º C. Plasma was removed and stored frozen at -20º C.

Biochemical assays & data analysis

Glucose and insulin concentrations were measured in all baseline and FSIGT blood samples. Plasma glucose concentrations were measured by the glucose oxidase method using a chemical autoanalyzer (Kit # 442640, Beckman Synchron CX5CE, Brea, CA). Plasma insulin was measured using a commercially available radioimmunoassay kit (Coat-A-Count Insulin, Diagnostic Products Corp., Los Angeles, CA). Intra-assay CV was 6.4% for this insulin assay. Parameters of the minimal model of insulin and glucose

dynamics; insulin sensitivity (**SI**), glucose effectiveness (**Sg**), acute insulin response to glucose (**AIRg**), disposition index (**DI**); were determined by MinMod Millenium 5.10 software. Differences were determined by analysis of variance (StataCorp, College Station, TX).

Results

Pregnant mares during feeding were observed to generally remain at one feed pan containing a single ration and complete their meals in approximately 30-45 min, with SS meals usually consumed 10 to 15 min more quickly than FF meals. Mare age, BW and BCS data prior to feed treatment and approximately 2 wks prior to foaling are presented in Table 1. Results of nutrient analysis of feeds, pasture and hay are presented in Table 2. All foals were clinically normal during the study period. Birthweight was not different between SS (56.9 ± 6.7 kg) and FF (57.5 ± 6.5 kg) foals. Average daily gain between birth and the FSIGT was 1.8 ± 0.7 kg BW, except for one FF foal which gained poorly due to insufficient mare milk production. The FSIGTs were accomplished at 5 ± 1 d of age and day of age at FSIGT had no effect on the variables measured ($P > 0.10$).

Basal plasma glucose was higher in foals from mares fed SS during late gestation (**SS foals**) than FF foals ($P = 0.016$) (Figure 1A). A trend for higher basal plasma insulin in SS foals was also observed ($P = 0.071$) (Figure 1B). Both SI and Sg were not significantly affected by feed treatment ($P = 0.178$ and $P = 0.480$, respectively) (Figure 2 A, B). Observations of SI in all foals ranged widely from 4.5 to 72 $\times 10^{-4}$ $mIU^{-1} \bullet L \bullet min^{-1}$ and Sg ranged from 0.003 to 0.071 min^{-1}. Acute insulin response to glucose was not significantly different between feed groups ($P = 0.144$) and ranged from 59 to 210 $mIU \bullet L^{-1} \bullet min$ among all foals (Figure 3A). Disposition index (dimensionless) was higher in SS than FF foals ($P = 0.035$) ranging from 1150 to 6280 and 502 to 3540 in SS and FF foals, respectively (Figure 3B). Variation in SI, Sg, AIRg and DI was not attributable to foal sex, birth weight, day of age measured, average daily gain, or any other variable measured in this study ($P > 0.05$).

Discussion

This study is the first to apply the minimal model of glucose and insulin dynamics to neonatal foals. The results indicate that carbohydrate composition of feed provided to mares in late gestation impacts basal glucose and insulin concentrations, but does not appear to influence minimal model estimates of peripheral insulin sensitivity and beta-cell function in neonatal foals. In both SS and FF foals basal glucose concentrations were markedly higher than observed in healthy mature horses. This could reflect the importance of glucose as an energy substrate in neonatal foals consuming lactose rich milk relative to a mature horse consuming a high fiber, forage-based diet. Lower basal glucose concentrations in FF foals may reflect adaptation to alternate energy substrate utilization (e.g. fatty acids), thus decreasing the relative requirement for glucose as an energy substrate. Fatty acid metabolism becomes an important component of feto-placental energy metabolism nearing parturition in horses and in undernourished mares (Fowden et al., 2000a; Stammers et al., 1995). Mares fed FF feed may also utilize this alternative fuel as less glucose is available from FF than SS feed following digestion in the foregut and fatty acid utilization likely provides a higher proportion of the energy requirement in these mares. The trend for lower basal insulin concentrations in FF foals likely reflects the lower basal glucose concentrations observed in these foals. Neither group of foals exhibited basal insulin concentrations distinct from those observed in mature horses indicating the competency of the neonatal pancreas relative to maturity. Other work evaluating basal glucose and insulin concentrations in 5 d pony foals (Holdstock et al., 2004) reported similar glucose (160 mg/dL), but higher insulin (20 mIU/L) concentrations than observed in 5 d Thoroughbred foals in the present study. No nutritional information was provided regarding the pony mares and foals, so differences in basal insulin concentrations could be due to different nutritional regimens during gestation and/or inherent differences between ponies and horses.

Based on previous observations of lowered SI in response to consumption of a high glycemic diet in horses, we hypothesized that SI would be lower in SS foals (Hoffman et al., 2003a; Pratt et al., 2006; Treiber et al., 2005a). The same mechanisms that link high

glycemic diets to diminished SI in mature horses could function similarly in the developing fetus. Differences in mature versus fetal physiology and the response of fetal blood glucose and insulin concentrations to changes in maternal blood glucose and insulin concentrations associated with consuming a high glycemic diet (i.e. placental physiology) could play a role in mediating such effects, however. Data reported here indicate that neonatal SI is not affected by maternal dietary carbohydrate composition. Other factors potentially impairing our ability to detect differences in foal SI due to maternal diet may be those contributing to variability in measurement of SI in these young foals (e.g. suckling during testing). Also, effects of maternal diet during gestation may become apparent with age and be imperceptible in measurements made at this young age. Relative to mature horses, SI was considerably higher in the neonatal foals of this study. A 95% confidence interval for SI developed in mature Thoroughbred and Arabian horses is 0.16 to 5.88, a range above which all our observations in neonates fell except two (4.50, 5.65) (Treiber et al., 2005b). The lowest SI value was observed in the foal that exhibited poor weight gain leading up to the FSIGT. Glucose effectiveness, or insulin-independent glucose clearance, was also not affected by maternal dietary treatment and was substantially higher in neonates compared to mature horses (Treiber et al., 2005b). A 95% confidence interval for Sg in mature horses was 0.0012 to 0.0295, a range below the mean of 0.0369 observed in these neonates. Therefore, insulin mediated and non-insulin mediated glucose clearance is greater in neonatal foals than observed in mature horses.

Mean AIRg was not significantly different between feed groups, and 85% of observations fell within the lowest two quintiles developed for AIRg in mature horses. Low AIRg is appropriate given the high SI exhibited in these foals. However, AIRg, like basal insulin levels, is more similar to that observed in mature animals than any other parameter evaluated here (SI, Sg, DI), indicating function of the endocrine pancreas is more similar to that of mature animals than other mechanisms controlling glucose homeostasis. Indeed, evaluation of equine fetuses in utero showed no change in insulin concentrations and an increase in beta cell response to glucose leading up to parturition, indicating maturation of these systems during the final stages of gestation (Fowden et al., 1980). It is interesting that compared to mature horses, neonatal foals maintain greater

insulin mediated and non-insulin mediated glucose clearance rates and comparable insulin secretion, yet still maintain basal blood glucose concentrations up to twice that of mature horses. Like the β cells of the pancreas, specialized neurons of the brain contain glucose sensors which respond to changes in blood glucose concentrations. Thus, glucose homeostasis is maintained through both peripheral endocrine responses (e.g. insulin secretion) and centrally controlled responses influencing energy homeostasis (e.g. food intake and energy expenditure) (Levin et al., 1999; Penicaud et al., 2002). These mechanisms apparently operate at a different glucose set-point in neonatal versus mature horses to maintain such different blood glucose concentrations. It is also possible that mechanisms controlling rates of hepatic glucose production and storage are different in neonates, contributing to the maintenance of higher blood glucose concentrations despite insulin action.

Disposition index from the minimal model estimates overall insulin mediated glucose clearance ability, defined as SI × AIRg. Overall, 75% of observations in the foals fell above the highest reference quintile established for mature horses. Even though no significant differences were observed in SI or AIRg, the variables used for calculation of DI, SS foals exhibited higher DI than FF foals. This comes from the combination of relatively lower insulin sensitivity and AIRg observed in FF foals. Typically this parameter is used to characterize an insulin resistant state that is either compensated by increased AIRg or uncompensated. Since both groups are very insulin sensitive, interpretation of the difference in DI is difficult.

The application of the FSIGT procedure for evaluation of glucose and insulin dynamics is challenging in neonates because of their frequent suckling. We chose to allow suckling to best characterize minimal model parameters in unstressed animals. As we did not quantify the duration of suckling episodes, quantity of milk consumed, or evaluate milk composition, it is impossible to determine the effect suckling had on the variability in measurement of SI and other minimal model parameters. Nonetheless, suckling is likely one of the factors contributing to the large variation observed in these young foals.

Implications

To further understand the impact of nutrition on postnatal development of the glucoregulatory system, measurement of glucose and insulin dynamics in foals of all ages under controlled nutritional conditions is needed. Also, data helping elucidate the normal maturation of this system from gestation through postnatal development will contribute to better nutritional management of these young foals to aid healthy development. Understanding the long term effects of nutrition during gestation and nutrition during the postnatal period could also help avoid predisposition to insulin resistance and obesity in horses.

Table 1. Mean and standard deviation of mare age, body weight (BW) and body condition score (BCS) evaluated at mid (28 ± 3 wks) and late (47 ± 1 wks) gestation, prior to administration of test diets: high sugar and starch (SS) and high fat and fiber (FF).

		SS			FF		
		Mean	SD	N	Mean	SD	N
Age, yrs		9.6	2.7	11	9.6	2.9	11
BW, kg	Mid	601	35.2	11	595	44.3	11
	Late	662	45.3	11	644	61.3	11
BCS	Mid	6.1	0.6	11	6.3	0.6	11
	Late	6.2	0.4	8	5.6	0.6	9

Table 2. Nutrient analysis of high sugar and starch (SS) and high fat and fiber (FF) feeds, pastures and hay. Mean and standard deviation are represented for crude protein (CP), crude fat, non-structural carbohydrates (NSC), acid detergent fiber (ADF), neutral detergent fiber (NDF), and digestible energy (DE) on a % dry matter basis.

	SS (n=20)		FF (n=20)		Pasture (n=17)		Hay (n=2)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
% CP	14.5	1.5	15.5	0.7	19.4	5.6	16.5	0.07
% Crude fat	3.4	0.6	13.9	2.2	2.8	0.8	2.7	0.1
% NSC	50.4	5.7	13.0	2.9	10.8	3.2	8.3	0.5
% ADF	10.0	1.7	28.2	2.6	31.8	6.2	39.9	2.1
% NDF	17.6	2.7	42.2	3.0	60.1	8.2	54.6	0.4
DE, Mcal/kg	3.0	0.3	3.0	0.2	1.2	0.2	0.98	0.02

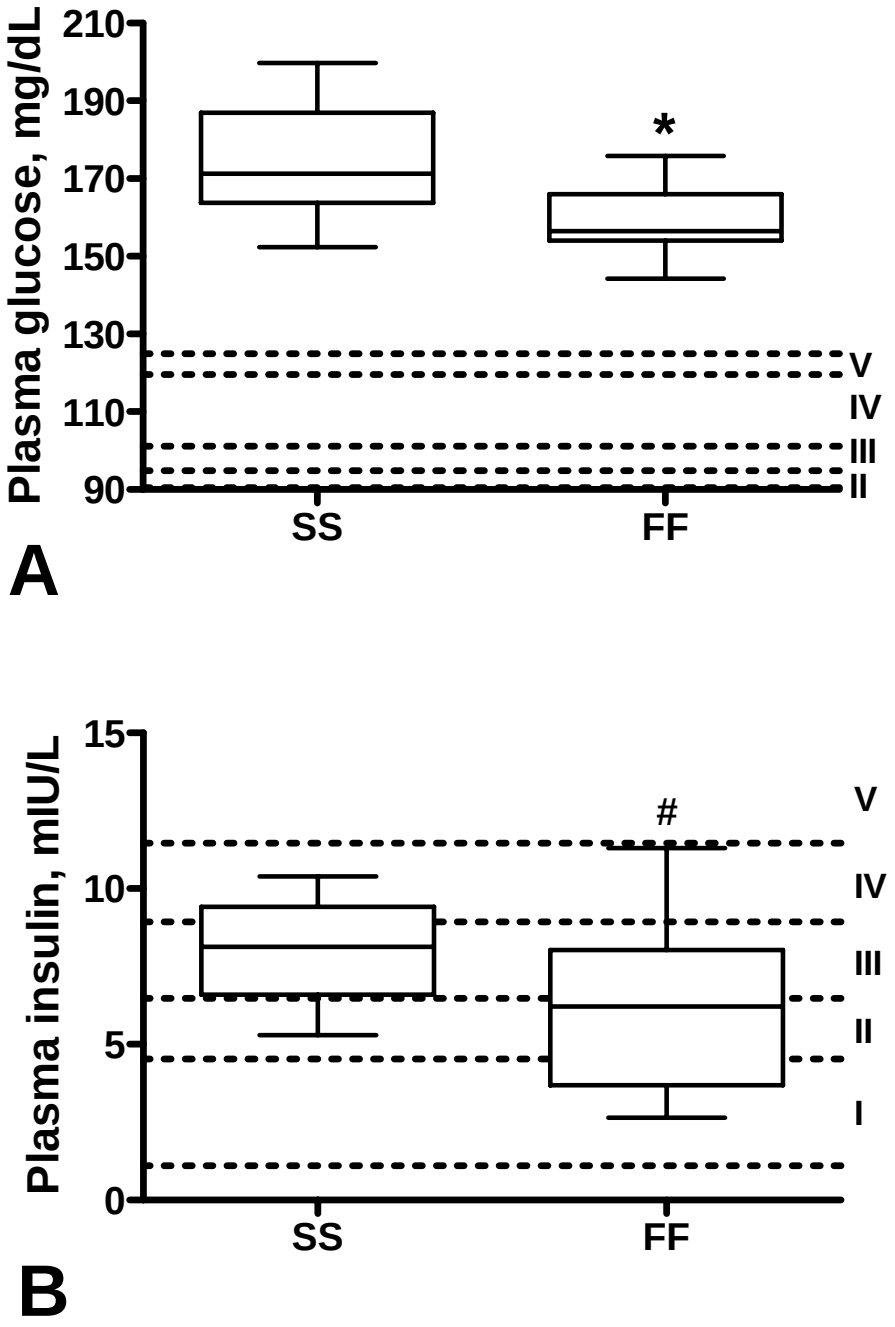

Figure 1. Basal plasma glucose (A) and insulin (B) concentrations in 5 d foals born from mares fed high sugar and starch (SS) and high fat and fiber (FF) feed during late gestation, relative to quintiles (---) developed in mature horses (Treiber et al., 2005b). Box and whisker plots represent median, 25th and 75th percentiles, and highest and lowest values. Significant differences (*, $P < 0.05$) and trends (#, $P < 0.10$) between groups are indicated.

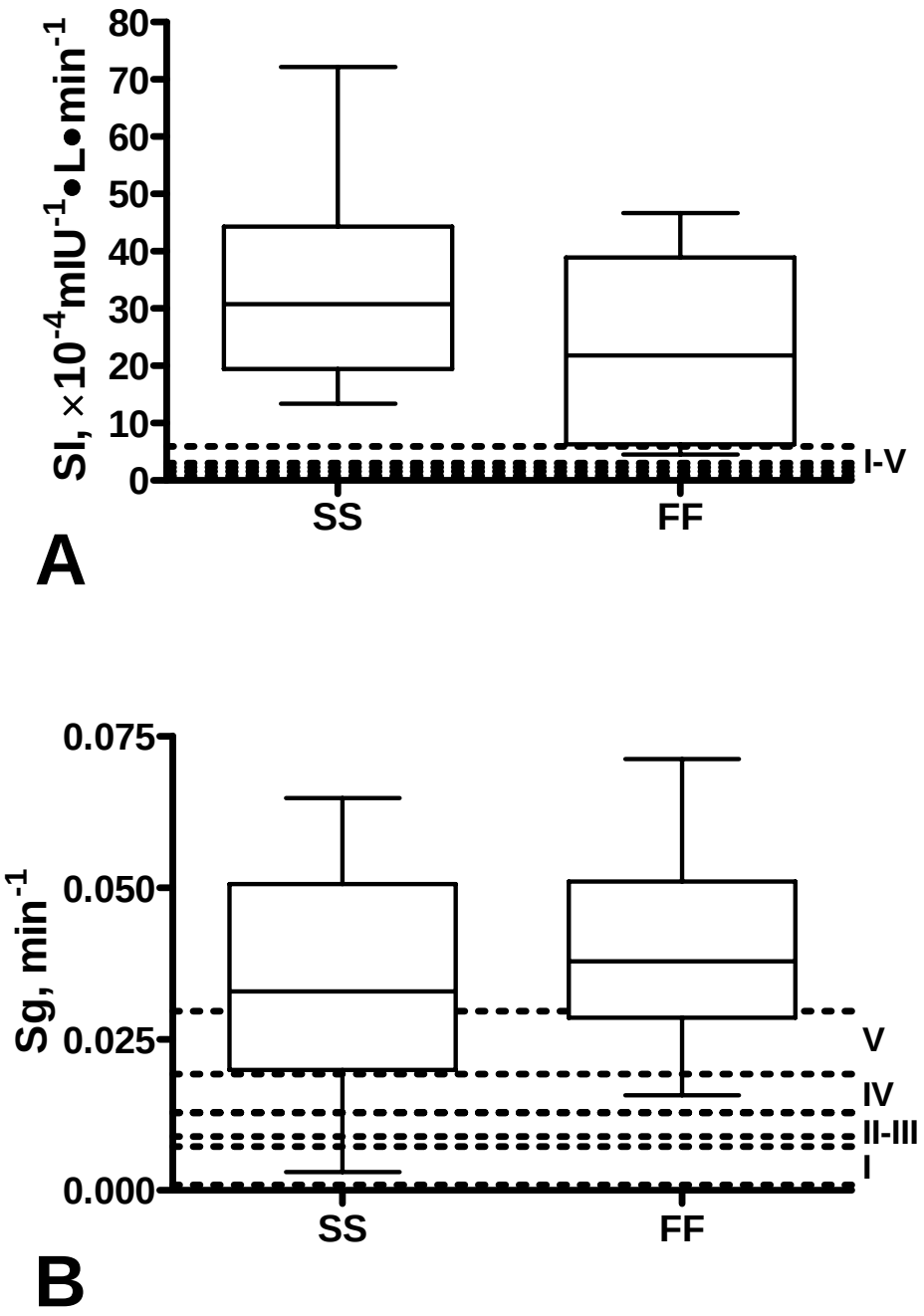

Figure 2. Insulin sensitivity (SI) (A) and glucose effectiveness (Sg) (B) in foals born from mares fed high sugar and starch (SS) and high fat and fiber (FF) feed during late gestation, relative to quintiles (---) developed in mature horses (Treiber et al., 2005b). Box and whisker plots represent median, 25th and 75th percentiles, and highest and lowest values.

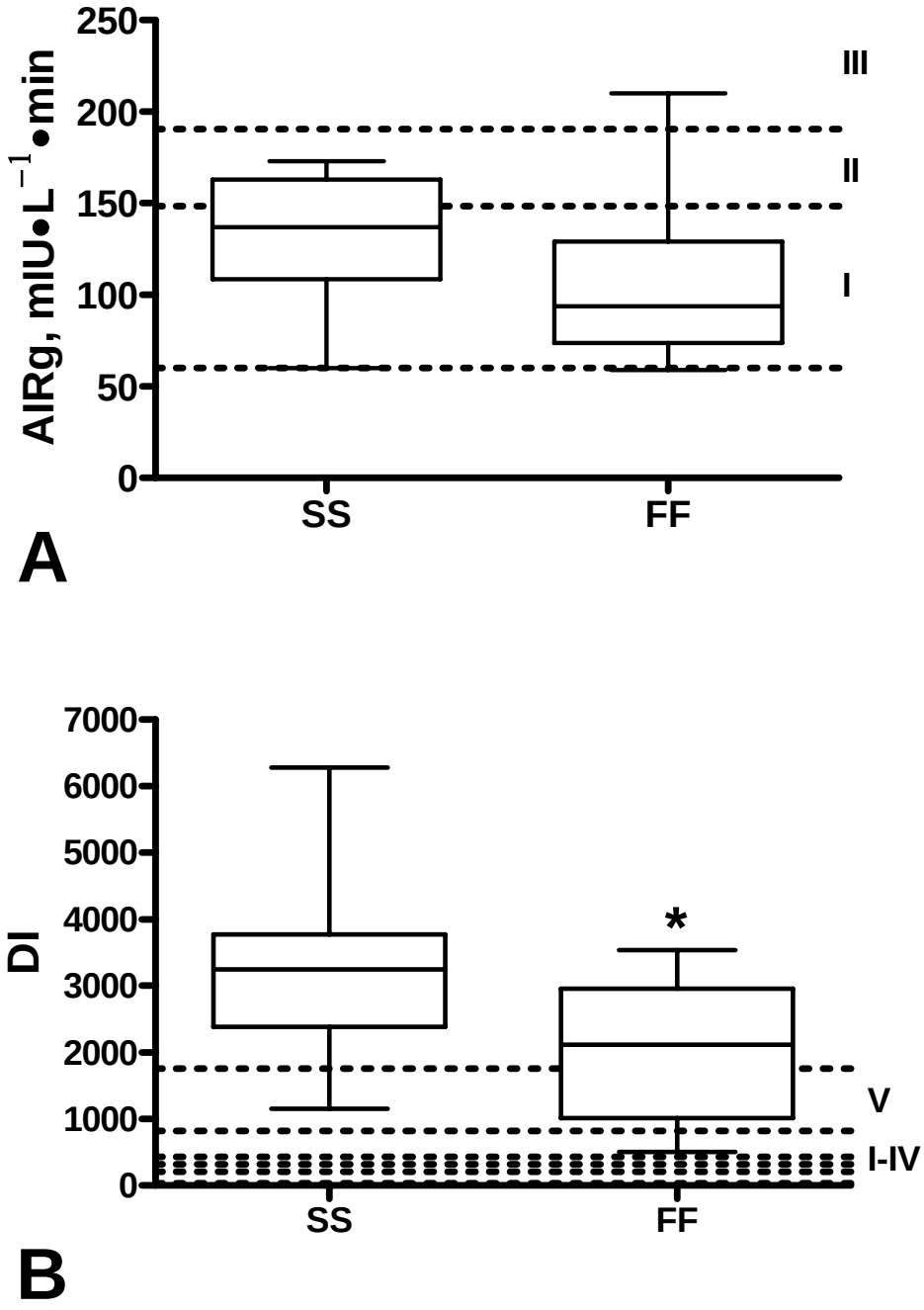

Figure 3. Acute insulin response to glucose (AIRg) (A) and disposition index (DI) (B) in foals born from mares fed high sugar and starch (SS) and high fat and fiber (FF) feedduring late gestation, relative to quintiles (---) developed in mature horses (Treiber et al., 2005b). Box and whisker plots represent median, 25th and 75th percentiles, and highest and lowest values. Significant differences (*, $P < 0.05$) between groups are indicated.

CHAPTER IV

Conclusions

Mares in late gestation and neonatal foals represent two unique metabolic conditions, evidencing the adaptive nature of the glucoregulatory role of insulin and overall energy homeostasis. These studies demonstrated lower SI, Sg and higher AIRg in pregnant mares relative to non-pregnant mares, as well as higher SI, Sg and lower AIRg in neonatal foals relative to a mature equine population. Thus, insulin sensitivity is high in the beginning of life and glucose clearance from circulation is rapid. Presumably, insulin sensitivity and glucose clearance rate decline with age to reach levels normally observed in mature horses. Observations in these studies suggest, then, that as a horse encounters different physiologic (e.g. pregnancy) and nutritional conditions (e.g. high NSC feed), insulin sensitivity and glucose dynamics change in response to these conditions. Decreased SI in pregnancy likely ensures ample glucose availability to the insulin insensitive, but highly glucose reliant placenta which mediates glucose transfer to the rapidly growing fetus. However, the change in SI, Sg and AIRg observed in non-pregnant horses following feed treatment and seasonal changes in pasture quality and quantity suggest that nutrition can impact insulin sensitivity and glucose dynamics to a similar magnitude as pregnancy in horses. Thus, both nutrition and physiologic state should be considered when assessing metabolic parameters in animals. Further consideration to point of gestation, season, body condition and age should be given when evaluating and comparing data regarding insulin sensitivity and glucose dynamics in horses. Research in pregnant mares earlier in gestation, relative to non-pregnant controls, is warranted to better characterize temporal changes in glucose and insulin dynamics associated with gestation. Studies examining the development of glucose and insulin dynamics in aging foals, as well as in response to events such as weaning and castration, are needed to continue to characterize the unique and likely dynamic metabolic status of young horses. This characterization will provide a stronger foundation on which to prescribe nutritional recommendations for young horses that could better ensure their healthy development and longevity.

CHAPTER V

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