Glucose Metabolism in Thoroughbred Weanlings: Regulation by Insulin, Growth Hormone and Insulin-Like Growth Factor-I

Kimberly H. Treiber

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Masters of Sciences
In
Animal and Poultry Sciences

David S. Kronfeld
Joseph H. Herbein
Rebecca K. Splan

December 10th, 2003
Blacksburg, Virginia

Keywords: horse, glucose dynamics, insulin sensitivity, growth hormone, insulin-like growth factor-I, minimal model

Glucose Metabolism in Thoroughbred Weanlings:
Regulation by Insulin, Growth Hormone and Insulin-like Growth Factor-I

by
Kibby. H. Treiber
Department of Animal and Poultry Sciences

ABSTRACT

Diets rich in hydrolyzable carbohydrates induce a hyperglycemic/insulinemic response and may increase the incidence of metabolic disorders associated with some types of laminitis, exertional rhabdomyolysis and osteochondrosis in horses. This study applied the minimal model of glucose and insulin dynamics to determine the effect of diet on metabolites and hormones that regulate glucose metabolism in young horses. Twelve Thoroughbred foals were raised on pasture and supplemented twice daily with a feed high in either sugar and starch (SS) or fat and fiber (FF). As weanlings (age 199 ± 19 d, weight 274 ± 18 kg), the subjects underwent a modified frequent sampling intravenous glucose tolerance test during which they remained in stalls and had access to grass hay and water ad libitum. Samples were colleted at -60, -45, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60 , 70 , 80, 90, 100, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min with a glucose bolus of 300 mg/kg BW at 0 min and an insulin bolus of 1.5 mU/kg BW at 20 min. Plasma was analyzed for glucose, insulin, growth hormone (GH) and insulin-like growth factor-I (IGF-I) concentrations. Insulin sensitivity, glucose effectiveness, acute insulin response to glucose and disposition index were derived using Minmod Millennium and WinSAAM software. Diet groups were compared using the non-parametric Kruskal-Wallis test or the sign test. Time interactions were compared using a mixed model with repeated effects. Rank-ordered linear regression was used for correlations. Basal glucose did not differ between groups ($P = 0.75$). There was nearly a trend towards higher basal ($P = 0.11$), and median insulin was higher in the sugar and starch foals at all 36 sample points ($P = 0.030$). The basal glucose:basal insulin ratio for the sugar and starch supplemented foals was

lower than for fat and fiber foals ($P = 0.025$). Insulin sensitivity (SI) was lower in foals fed sugar and starch than foals fed fat and fiber ($P = 0.007$). Acute insulin response to glucose was directly correlated to weight ($r = 0.78$; $P = 0.003$) and inversely correlated with SI ($r = -0.55$; $P = 0.067$). The glucose:insulin ratio was directly correlated to SI ($r = 0.92$; $P < 0.001$). Growth hormone concentrations were increased from basal from 19 to 180 min after the glucose dose ($P < 0.05$). Basal IGF-I was higher ($P = 0.006$) in the SS group compared to the FF group. Concentrations of total IGF-I increased with time ($P = 0.002$) in the SS group. The change in IGF-I concentration from baseline to the end of the study was positively correlated ($r = 0.72$; $P = 0.008$) to the area under the insulin curve from 0 to 80 min. Basal IGF-I was inversely correlated to SI ($r = 0.71$; $P = 0.015$). These results show that the metabolic response to a diet high in hydrolyzable carbohydrates differs from the response to a fat and fiber meal resembling forage. Weanlings adapted to meals high in glucose equivalents have higher insulin and IGF-I secretion as compared to foals adapted to a fat and fiber feed, possibly contributing to lower insulin sensitivity observed in these foals. Such deviations may contribute to metabolic dysfunction and osteochondrosis in horses fed grain diets.

Key Words: horse, glucose dynamics, insulin sensitivity, growth hormone, insulin-like growth factor-I, minimal model

Acknowledgments

Of course I couldn't have done it all on my own. And even if I could have, it wouldn't have been nearly as fun.

Both my parents have such a passion for the natural world that I'm sure it has passed into my genes, through my life and brought me here. Dad, you've made me feel so proud of myself and showed me that happiness is quintessential. Mom, thanks for teaching me to work... and love it. This is all your fault. I'd also like to thank you both for never buying me a pony. Nevermind the bitterness towards all the little-girl horse-stories that had happy-endings. Here's my happy ending. I've had to work for it and I'm thankful for every step of the journey.

My twin sister has always been my source for perspective, inside and out. This project was my first path away from her, but we run back together like water. I attest to her my competitive edge, my love for the page, my verbosity and my (rare) moments of rebelliousness.

On that note, I should thank my best friend, Allison Smith, the third Angel and my inspiration for *getting the job done*. And my other best friend, Ian Vanbuskirk, for always having faith in me, for letting me relax and be myself, and for giving me a little faith of my own. Thanks to all my dogs, who have always been the best therapy. And thanks to the Exceptional Equestrian Barn, Dottie Billings-Reed, Jim Reed and Crosby for introducing me to horses and nurturing my love for them until my wings were fledged.

Most especially of all, I would like to thank my brother, Colin, who will always be my hero. He knew how to get what he wanted, how to make someone's day, and what the true meaning of a hero is. He taught me that Unless we try, we cannot achieve.

I would like to thank all the students who helped me out more times than I probably know and maintained my sanity, for the most part. Tania Cubitt, for being my roommate, my opposite, and not killing me for being either. Dr. Carey Williams who held my hand through this project and proved that, yes, it all *can* get done. Dara, Ana and Marcelini - for doing the dirty work, picking up all the pieces I left behind, but most especially for being my sunshine through the monsoon. And finally, Dr. Tanja Hess - for her incredible and diverse knowledge, her stamina (she's not an endurance champion for nothing), and her mentor-ship.

I would like to thank the staff here at the MARE Center for their incredible teamwork in spite of all odds, complaints, breakdowns, natural disasters and practical anarchy. Thanks to Dr. Mike Akkers and Pat Boyle for letting me invade their lab and helping me invaluably with the GH and IGF-I assays. You may see me again. Extra special thanks to Louisa Gay for the 10,000 tasks and 10,000 tubes she has helped me with. Thanks to Dr. Rhonda Hoffman for advising me on the Minimal Model and pioneering its use in the horse. Thanks to Dr. Ray C. Boston for his consultation on modeling and statistics. Special thanks to Dr. W. B. Staniar for his knowledge, enthusiasm, direction and lots of time. And finally, thanks to Dr. D. S. Kronfeld for taking a chance on me, for keeping me busy and for looking at the world from the other side of the looking glass. The project is only the beginning of what I've learned.

Table of Contents

List of Tables

List of Figures

List of Appendix Tables

List of Appendix Figures

Appendix B

Introduction

The horse has evolved with a digestive track and metabolic pathways to persist on a high fiber diet consisting of grasses and legumes. This diet is consumed in small portions continuously throughout the day and digests slowly, primarily through fermentation in the hind-gut. Domestication of the horse has led to increased demand for equine performance. Such performance - improved lactation, growth or work - requires an additional source of energy. Thus the domesticated horse has come to be supplemented with feeds having concentrated energy, usually in the form of sugars and starch.

Over 90% of modern equine operations supplement their horses and almost 60% of these supplements are based on concentrates of grain and molasses, which are high in glucose equivalents (USDA, 1998). The hydrolyzable carbohydrates in meals are rapidly digested and absorbed, inducing a sudden increase of glucose in the consumer's bloodstream. This increase ignites a chain reaction of fluxes in regulatory and counter-regulatory hormones associated with glucose-energy allocation, disrupting the natural system and possibly contributing to metabolic disorders like colic, laminitis, exertional rhabdomyolosis and osteochondrosis.

Our laboratory has been developing an alternative supplement, substituting fat in place of carbohydrates as a source of concentrated energy and incorporating fiber to complement the horse's natural diet (Kronfeld, 1996: Hoffman and Kronfeld, 1999). Together, fat and fiber constitute a feed digested and metabolized similar to forage, avoiding metabolic upset while supplying increased energy to the performance horse.

The somatotropic axis responds to energy availability, allocating fuel to and from storage and triggering growth when extra energy is present. The rapid absorption of energy from a meal rich in hydrolyzable carbohydrates is followed by an increase in insulin and then by a rapid decrease in plasma glucose (Jenkins and Jenkins, 1985; Rodiek et al., 1991). This drop in energy may provide a false signal that fuel is low, stimulating growth hormone secretion to promote the

use of alternative stored energy sources like fat (Roth et al., 1963; Sharp et al., 1987). Repeated stimulation of growth hormone secretion following each high-carbohydrate meal could lead to metabolic and growth abnormalities such as insulin resistance (syndrome X) and osteochondrosis (Clarke et al., 1990; Kronfeld et al., 1990; Ralston, 1996).

As in horses, diet may be an important factor in the development of metabolic dysfunction in humans. Low-fat diets which replace calories from fat with calories from hydrolyzable carbohydrates may effect metabolic function in the human and contribute to the present epidemic of insulin resistance and type II diabetes in the human population.

A forage-like diet containing fat and fiber does not trigger abrupt metabolic and hormonal changes (Williams et al., 2001). This cultivates the smooth, consistent regulation of energy and growth for which the horse has evolved. The present study compares the responses of glucose, insulin, growth hormone and IGF-I to a glucose challenge in Thoroughbred weanlings adapted to a diet high in glucose equivalents or one comprised of fat and fiber.

Literature Review

Horse Supplementation

Increased performance in horses requires increased energy. Brood mares, growing foals and working horses not only need to maintain their condition, but devote energy to increasing biomass or physical labor. As a grazing animal, the horse has evolved to constantly take in energy in the form of pasture. The bulk of fiber in pasture limits both energy availability and intake capacity. Therefore horses which require energy above maintenance may require supplementation by a more concentrated, readily available energy source. In addition to concentrated energy, supplements are formulated to provide essential minerals, vitamins, and additional protein to increase nitrogen balance.

Grains offer the most commonly used concentrated energy sources. These are primarily composed of starches, which are rapidly digested and provide ready energy. Grain supplements can be mixed with molasses to improve palatability and texture. These sugars are also rapidly digested.

The horse's digestive tract has evolved to handle fibrous pasture and includes a relatively sparse small intestine for the digestion and absorption of starches and sugars. In contrast, the ample hind-gut provides a substantial environment for the microbial fermentation of plant fibers. When excess hydrolyzable carbohydrates overload the small intestine, the remainder spills into the cecum and large intestine where it is rapidly fermented, increasing the likelihood of colic (Hudson et al., 2001). Increased volatile fatty acid (VFA) production may further alter the balance of the microbial environment, impairing bacterial populations and prompting the release of endotoxins - a chain reaction which can lead to colic or laminitis (Clarke et al., 1990).

Fat and Fiber

To avoid gastric upset, the energy source of the supplement can be furnished by fat rather than sugar and starch. Fat provides more than twice the energy per gram of carbohydrate and has added benefits such as improving coat quality and decreasing excitability (Holland et al., 1996).

Fat stimulates hormones to slow down digestion, encouraging slow, smooth digestion, decreasing insulin stimulation and avoiding abrupt changes in available energy (Welch et al., 1987). Fat adaptation may also reduce the risk of exertional rhabdomyolysis by minimizing excitability and anxiety and by controlling signs of equine polysaccharide storage myopathy which are associated with dysfunctional carbohydrate metabolism (Macleay et al., 1999; Valentine, 2001).

High-fat diets in equines have been shown to improve exercise performance. The use of fat as an energy source can spare muscle glycogen, avoiding depletion which would promote gluconeogenesis and muscle breakdown and providing an alternative source of energy during exercise (Kronfeld et al., 1994; Taylor et al., 1995; Jose-Cunilleras, 2002). During low-intensity treadmill exercise, Arabians have been shown to use less glucose when adapted to a high fat diet, suggesting an improvement in endurance performance (Pagan et al., 2002). Fat supplemented Arabians also had less acidosis during repeated sprints (Graham-Thiers et al., 2001) and apparently more efficient regulation of glycolysis (Taylor et al., 1995; Kronfeld et al., 2000). In another sprint study, fat adapted Thoroughbreds were shown to have lower exercising heart rates, less glucose use, and decreased insulin concentrations during exercise compared to controls (Duren et al., 1999). All these factors indicate more energy efficient metabolic regulation during exercise.

For bulk, fiber can be mixed with the source of fat. Fiber provides additional gastric slowing and stimulates proliferation of a healthy microbial population in the hind-gut (Jenkins and Jenkins, 1985; Kronfeld and Hoffman, 1999). Additional benefits of fiber may include lower serum lipids and decreased risk of insulin resistance and obesity (Hoenig et al., 2001; Pereira et al., 2002; Davy et al., 2003), but these results are confounded due to the complexity of diets and remain controversial.

The fat and fiber supplement is designed to resemble and complement pasture, providing concentrated energy and essential vitamins and minerals without upsetting the natural digestion and metabolism of the horse. Thoroughbred mares fed a fat and fiber meal showed a lower glycemic/insulinemic response compared to the response following a typical grain and molasses meal (Williams et al., 2001). In the same study, the postprandial response to the fat and fiber supplement most closely resembled a response to forage, although the energy density of the fat

and fiber meal was similar to that of common concentrates. Fat and fiber may therefore be more appropriate to the digestive and metabolic systems of the horse, avoiding complications associated with the introduction of foreign diets and abnormal nutritional signals.

Regulation: The Glucose/Insulin System

As a grazer, the horse introduces a slow, steady absorption of glucose from the intestine into the bloodstream for which little insulin is necessary to regulate glucose uptake into the cells and maintain normoglycemia (Stull and Rodiek, 1987). Horses supplemented with meals rich in hydrolyzable carbohydrates, however, the rapidly absorbed glucose load from the intestine triggers extreme responses from regulatory hormones. Increased plasma glucose concentration stimulates the β-cells of the pancreas to secrete more insulin into the bloodstream (Holley and Evans, 1979).

Plasma insulin eventually enters the interstitial fluid where it binds to insulin receptors at the cell membrane and stimulates GLUT-4 insulin-dependant glucose transporters to aggregate at the plasma membrane as shown in human (Bessessen, 2001; Farese, 2001). Insulin may also increase the glucose-shuttling activity of GLUT-4 transporters as has been shown in rat, mouse and human muscle cells (Furtado, 2003). As a consequence, glucose is more efficiently transported from the plasma compartment into the cells, reestablishing normoglycemia.

Insulin responses to an abrupt glucose load may even result in an overshoot of glucose uptake, causing transient hypoglycemia which can effect short-term performance (Stull and Rodiek, 1988). For this reason, rapidly digested carbohydrates are not recommended to athletes just prior to performing (Rodiek et al., 1991; Duren et al, 1999).

Counter-regulation: Growth Hormone and Insulin-like Growth Factor-I

Counter-regulatory hormones oppose the anabolic actions of insulin and promote fuel utilization for growth, maintenance and survival. Counter-regulatory hormones interact with insulin via feedback loops in order to maintain normal glucose metabolism and homeostasis (Figure 1).

Growth hormone is released from the anterior pituitary in a pulsatile fashion and

demonstrates circadian patterns in humans (Friend et al., 1996) and horses (Thompson et al., 1992; Staniar, 2002). Pulsatility is controlled by rhythmic patterns of somatostatin, which withholds GH release, and growth hormone releasing hormone (Tannenbaum, 1990).

Growth hormone affects metabolism directly by promoting the mobilization of fatty acids, antagonizing insulin and sparing glucose for essential glucose-dependent organs like the brain and liver (Kronfeld, 1965; Foster et al., 1988; Breier, 1999). Growth hormone also stimulates the secretion of insulin-like growth factor-I (IGF-I) as shown in humans (Blum et al., 1992; Lee et al., 1997), rat hepatocytes (Johnson et al., 1989) and Thoroughbred horses (Champion et al, 2000; De Kock et al., 2001).

According to the somatomedin hypothesis, IGF-I mediates the growth-promoting effects of GH (Daughaday, 2000). This mediation is demonstrated by transgenic mice which were able to maintain normal growth when engineered to express IGF-I in the absence of GH (Behringer et al., 1990).

Insulin-like growth factor-I is an endocrine or paracrine promoter of cell maturation and proliferation. Injections of IGF-I into equine tendon lesions have been shown to increase mitogenesis and matrix formation (Dahlgren et al., 2002). Fetal and neonatal equine chondrocytes also displayed increased mitogenesis when cultured in the presence of IGF-I (Henson et al., 1997). Although primarily associated with growth, IGF-I also has metabolic effects. It can mimic the effects of insulin by increasing glucose uptake, decreasing hepatic glucose production and decreasing lipid mobilization (Boulware et al., 1992; Froesch et al., 1996).

Growth hormone and IGF-I fluctuate relative to the plane of nutrition. In the fasted state, GH increases to mobilize stored energy as shown in monkeys (Labo-abeal et al., 2002), horses (Christensen et al., 1997) and steers (Breier et al., 1986). The plasma concentration of IGF-I, however, decreases when energy intake is restricted (Smith et al., 1995). The paradoxical relationship between GH and IGF-I during the fasting state may be due to a decreased ability for GH to stimulate IGF-I release (Merimee et al., 1982; Breier et al., 1986;). Such insensitivity to GH would promote the direct metabolic effects of GH and the conservation of energy for maintenance rather than growth.

Feedback

The metabolic system is equipped with a complex feedback system to optimize fuel use for maintenance and production (Figure 1). These mechanisms respond to nutritional 'signals' such as plasma glucose concentrations and respond with the appropriate hormones to allocate energy efficiently (Stoka, 1999; Lado-Abeal et al., 2002). Inappropriate signals could cause the system to respond inappropriately, causing a cascade of regulatory and counter-regulatory changes and leads to metabolic dysfunction.

The exaggerated glucose and insulin responses following a rapidly digested and absorbed carbohydrate meal may constitute an inappropriate signal to counter-regulatory hormones. The sudden decline in plasma glucose concentration may be misidentified as a shortage in energy supply. Growth hormone may be stimulated to spare remaining glucose and prevent hypoglycemia (Roth et al., 1963). During a glucose clamp in type II diabetics, GH was shown to increase just after a decrease in plasma glucose from hyperglycemia to normal concentrations (Sharp et al., 1987). During oral glucose tolerance tests, GH is apparently suppressed with a consequent rebound in GH after approximately 4 hours (Valcavi et al., 1990; Attanasio et al., 1999;). This 'rebound' could also be a GH peak stimulated by the decrease in plasma glucose during the post-absorbtive phase. Thus repeated meals high in glucose equivalents could stimulate repeated GH peaks with consequences to metabolism and growth.

Development of Insulin Resistance

Insulin resistance is a condition in which normal concentrations of insulin provide inadequate stimulation of glucose uptake to maintain normoglycemia. In insulin resistant subjects, insulin secretion by the pancreas is increased in order to compensate for the hormone's inefficiency, resulting in higher concentrations of insulin in the plasma (Kahn, 1978). If the insulin producing β-cells fail to maintain adequate insulin production, blood glucose concentrations increase and the subject is identified as type II diabetic (The expert committee on the diagnosis and classification of diabetes mellitus, 1997).

Insulin resistance may be adaptative during periods of undernourishment. Insulin

resistance would sustain the use of fat as an energy source, sparing muscle glycogen and protein (Jeffcott and Field, 1985). When nutrients are restricted during fetal development, a 'thrifty phenotype may be expressed where the fetus becomes insulin resistant in order to spare glucose energy for the development of essential organs (Hales and Barker, 1992; Flanagan et al., 2000; Ozanne and Hales, 2002). In these individuals, type II diabetes and metabolic syndrome are likely to result when exposed to high-glycemic foods and good nutrition (Cohen et al., 1988).

Certain animals adapted to harsh climates may be more prone to insulin resistance. Ponies are at higher risk for insulin resistance than larger breeds, perhaps because they developed in harsher climates with unreliable food availability (Jeffcott and Field, 1985). Decreased glucose tolerance in camels may also indicate insulin resistance, a condition which might allow camels to better endure nutritional deprivation (Elmahdi et al., 1997).

Increased GH secretion in response to the rapid decrease in glucose at each high-carbohydrate meal may contribute to insulin resistance in grain-fed horses. Growth hormone has been shown to increase insulin resistance in dogs (Bishop et al., 1966), cats and frogs (Houssay and Anderson, 1949) and humans (MacGorman et al., 1981; Bratusch-Marrain, 1982). Humans have demonstrated insulin resistance relative to post-prandial GH secretion and most significantly following a carbohydrate meal (Rosalyn et al., 1969). The mechanism of GH-mediated insulin resistance does not appear to be decreased insulin binding. Adipocytes cells cultured with GH and insulin accumulated less lipids than cells cultured with insulin only despite unaffected insulin-binding to the receptor (Foster et al., 1988). Rather, GH-mediated insulin resistance may result from inhibition of tyrosine kinase which is necessary to phosphorylate the insulin receptor and translate the insulin signal to the intracellular space. Rats treated twice daily with GH developed increased insulin concurrent to a decrease in autophosphorylation of the insulin receptor despite up-regulation of the receptor (Smith et al., 1997).

Metabolic Syndrome (Syndrome X)

Insulin resistance in humans is associated with a myriad of disorders collectively termed metabolic syndrome or syndrome X (Davy et al., 2003; Shen et al., 2003). Hypertension may result from insulin-mediated increases in renal reabsorption or increased stimulation of the

sympathetic nervous system (Reaven, 1988). Dyslipidemia due to insulin resistance in humans contributes to coronary artery disease (Reaven, 1988) and atherothrombotic stroke (Shinozaki et al., 1996). Insulin resistance may even contribute to recurrent pregnancy loss by altering the fetal environment (Craig et al., 2002).

Although type II diabetes is rare in horses, insulin resistance may be associated with other serious health concerns. Horses experience complications related to syndrome X which are deleterious to health and performance and might be avoided with appropriate dietary management.

Elevated insulin in insulin resistant subjects stimulates fat storage and suppresses lipolysis, increasing adiposity and potentiating health and performance concerns associated with obesity. In insulin resistant, obese subjects, the tissue responds poorly to insulin, causing excess triglycerides to be easily mobilized if nutrition is restricted, leading to hyperlipidemia as commonly observed in overweight ponies (Jeffcott and Field, 1985; Jeffcott et al., 1986).

Hoof separation comparable to laminitis has been induced in hoof-explants restricted from glucose or cultured with inhibitors of glycolysis (Pass et al. 1998). This suggests that restricted glucose metabolism as occurs in an insulin resistant state can contribute to laminitis. In another study, ponies exhibiting chronic laminitis were found to be insulin resistant, a possible contributing factor to their disease (Jeffcott and Field, 1985).

Osteochondrotic lesions may be more likely to develop in foal which are insulin resistant (Ralston, 1996). Osteochondrosis is also associated with foals maintained on high-energy diets (Glade et al., 1984; Kronfeld et al., 1990; Savage et al., 1993). The commonality between insulin resistance and high-energy intake may be an increase in IGF-I (Thissen et al., 1994). Not only does this increased IGF-I promote bone growth as shown in neontal equine cartilage (Henson et al., 1997) and equine tendons (Dahlgren et al., 2002), but it may potentiate insulin-mediated growth promotion. When rats limbs were infused with insulin they showed increased cartilage growth compared to control limbs, but infusion of IGF-I antiserum nullified the growth response (Alarid et al, 1992).

Thus the influence of insulin resistance on abnormal bone development may be twofold, through an increase in both IGF-I and insulin. Rapid growth, IGF-I and insulin may all prevent

suitable maturation of the proliferating chondrocytes, causing weaker bones which may be more likely to develop lesions when stressed (Kronfeld et al., 1990; Henson et al., 1997).

Diet and Insulin Resistance

Numerous etiologies to insulin resistance make causality difficult to separate from a host of interacting possibilities. Insulin resistance is documented in multiple species, including the horse. Animals provide the opportunity to study the causes and effects of abnormal glucose metabolism in a controlled environment and have shown the relationship between diet and insulin resistance more conclusively than in human subjects (Storlien et al., 2000). Human studies have also concentrated on at-risk populations, with less application to the healthy individual and early stages in the development of insulin resistance.

Fiber and Insulin Resistance

Fiber has been considered a possible deterrent to insulin resistance. Fiber may slow passage and absorption of digesta and regulate hormone responses. Fiber also displaces starch which may contribute to insulin resistance by increasing insulin demand and glucose and insulin fluctuations in the plasma.

Elderly women in the highest quintile of fiber consumption were shown to be 22% less likely to develop diabetes than women in the lowest quintile (Meyer et al., 2000). These results are confounded, however, by the fact that women consuming high-fiber diets are less likely to drink or smoke, more likely to exercise and probably better educated than women eating less fiber. Another study showed significantly increased insulin sensitivity in overweight, hyperinsulinemic subjects after 6 wks adaptation to a whole-grain diet (Pereira et al., 2002). In the study, the whole grain diet provided 3% less energy and 11% less starch than the comparable diet, confounding its reported benefit to already overweight, insulin resistant individuals.

In a similar study, 30 normal weight dogs were adapted for eight weeks to diets in which corn-starch was replaced with varying types of fiber (Hoenig et al., 2001). No significant difference was found in postprandial glucose or insulin response between high fiber and control diets, perhaps because all the subjects were healthy and able to use various energy sources

efficiently. This suggests that fiber does not alter glucose absorption and plays at most a minor role in the glucose/insulin system of healthy individuals. Another study in healthy dogs again showed no relationship between the fiber content of different meals and their glycemic/insulinemic response (Nguyen, et al., 1994).

Carbohydrates and Insulin Resistance

Decreasing dietary carbohydrates may be quintessential to avoiding insulin resistance and its consequences. Diets rich in hydrolyzable carbohydrates contribute to increased insulin secretion, a primary factor in the development of tissue resistance to insulin (Kopp, 2003). Constant hyperinsulinemia resulting from a high-carbohydrate diet also contributes to pancreas β-cell dysfunction associated with type II diabetes possibly through amyloid deposits on the islet cells. (Porte, 1991). Meals high in glucose equivalents may also increase obesity which is associated with insulin resistance (Samaha et al., 2003). Wistar rats adapted to high sucrose diets showed an increase in both fat mass and plasma insulin compared to rats fed a standard diet (Wetzler et al., 2003).

Studies on carbohydrate diets, like studies on fiber diets, face numerous complications and sources of confounding. Changes in obesity tend to override other factors contributing to insulin sensitivity. When adapted to twice-daily meals high in hydrolyzable carbohydrates, mature Thoroughbred geldings showed a tendency to be more insulin resistant compared to meals rich in fat and fiber (Hoffman et al., 2003). When the horses were stratified by obesity, horses with normal body conditions were shown to be more sensitive to insulin and to dietary affects on that sensitivity, with the sugar-rich diet being significantly associated with insulin resistance independent of obesity.

Studies, particularly in humans, are further confounded by complex diets. In one human study, subjects adapted 4 months to various diets high in carbohydrates showed no significant difference in insulin resistance compared to subjects adapted to diets high in monounsaturated fats (Wolever and Mehling, 2002). These results were obscured by inconsistent diets, pre-diet variability of insulin resistance between groups, and weight-changes within groups over the course of the study. Another study compared subjects adapted to diets with similar macronutrient

compositions but containing sources of carbohydrates which differed in expected glucose equivalents (Jenkins et al., 1987). Subjects adapted to the diet containing carbohydrate sources with low glucose equivalents – and consequently reduced postprandial hyperglycemia and hyperinsulinemia – were shown to have higher insulin sensitivity. This diet, however, had 7% less calories, 11% less sugar and starch, and 24% higher fiber all of which could be implicated in improved insulin sensitivity. The adaptation period of 2 wks may also have been insufficient for diet effects to manifest. Such complications reflect many of the difficulties in human diet studies.

The use of mathematical models to describe the glucose and insulin systems could help to decrease confounding by providing standards for the characterization of metabolic dysfunction with application to a number of scenarios. The euglycemic/insulinemic clamp has been a common tool to determine glucose volume of distribution and glucose clearance during various hormonal challenges (Pagliassotti et al., 2000; Pereira et al., 2002), but is impractical for use in large study populations. The minimal model offers a simple approach and a detailed description of the glucose and insulin system and can be applied under numerous conditions to assess the causes and effects of metabolic dysfunction.

Minimal Model

The Minimal Model is a two-compartment representation of the dynamic glucose/insulin system (Bergman et al., 1979) (Figure 2). This model was selected over the euglycemic/insulinemic clamp because of its flexibility in terms of sampling and the ease and practicality of the minimal model's application in a variety of scenarios. The Minimal Model also provides a more detailed description of glucose uptake in terms of non-insulin mediated and insulin mediated components.

The first compartment of the minimal model is comprised of the intermixing plasma and interstitial glucose (G). The second compartment constitutes 'remote insulin' or 'insulin action' which is active insulin in the interstitium at the cell membrane (X). Insulin is secreted into the circulation by the β-cells of the pancreas and enters the remote compartment from the bloodstream. The fraction of secreted insulin entering the remote compartment per unit time defines model parameter 3. The fraction of remote insulin cleared per unit time from the remote

compartment defines model parameter 2. The ratio of these parameters - fraction of secreted insulin arriving:fraction cleared - is entitled insulin sensitivity. Essentially this is the proportion of secreted insulin which reaches the remote compartment X and affects glucose uptake by the cells.

Glucose is assumed by the minimal model to be cleared from the plasma/interstitial compartment through two distinct pathways. The first path is non-insulin mediated - or glucose-mediated - glucose disposal. Glucose from the plasma and interstitium enters cells via insulin-independent GLUT-1 transporters located primarily in the membrane of brain, kidney, endothelial and erythrocyte cells (Bergman, 1989). Uptake by the GLUT-1 transporters is dependent on the glucose concentration gradient. The second method of plasma glucose clearance is via insulin mediation, where the fraction of secreted insulin which reaches the remote compartment acts at the cell membrane to stimulate GLUT-4 translocation and activation (Furtado, 2003).

Application of the minimal model involves a manipulation of the dynamics of the glucose/insulin system consisting of the administration of a rapid glucose dose (approximately 300 mg/kg BW) following an overnight fast (Bergman et al., 1997). Clearance of this glucose from the blood plasma is observed for 20 min via frequent sampling. An insulin bolus – large enough to affect glucose clearance but small enough to avoid hypoglycemia – is then administered rapidly into the bloodstream. Frequent venous blood sampling is continued for several hours to observe glucose clearance.

This frequently sampled intravenous glucose test (FSIGT) allows for several determinations. 1) The change in the rate of glucose clearance in response to a known exogenous insulin dose illustrates the capacity of insulin to stimulate the tissue to uptake glucose - i.e. the insulin sensitivity of the tissue. 2) The difference between the rates of insulin-stimulated glucose clearance and total glucose clearance reveals the glucose-mediated component of glucose clearance, which is termed glucose effectiveness. 3) The appropriateness of the endogenous insulin response to glucose can be determined by comparing the insulin sensitivity of the tissue to the amount of insulin secreted. Tissue which has a lower insulin sensitivity requires more insulin to properly clear glucose.

The clearance of glucose according to the minimal model's compartmental representation of glucose/insulin dynamics and the FSIGT procedure is described mathematically by the following equations (Bergman et al., 1981):

$$G'(t) = -G(t)\cdot[Sg + X(t)] + Sg\cdot Gb$$

where G'(t) is the rate ($mg/dl\cdot min^{-1}$) of glucose clearance from the glucose compartment - i.e. the rate of uptake by the cells. The plasma glucose concentration (mg/dL) at time = t is G(t). Glucose effectiveness (Sg) describes glucose-mediated plasma clearance (min^{-1}); Gb is the basal glucose concentration (mg/dL) maintained by hepatic production. Insulin action, X(t), represents the insulin mediated component (min^{-1}) of the plasma glucose clearance rate. This component is further described by:

$$X'(t) = p_3\cdot[I(t)-Ib] - p_2\cdot X(t)$$

where X'(t) is the rate of change in the insulin action, p_3 describes delivery of plasma insulin to the remote interstitial compartment, and p_2 describes the disposal of insulin from the remote compartment.

Insulin sensitivity (SI) is the ratio of these parameters, or the efficiency of insulin to accelerate cellular glucose uptake:

$$SI = p_3/p_2$$

Responsiveness of insulin-secreting β-cells to increased plasma glucose concentration is measured by the acute response of insulin to glucose (AIRg) which is the increase in plasma insulin above basal concentration integrated from 0 to 10 min after the glucose dose (Bergman, 1997). The product of AIRg and SI defines the disposition index (DI) or the appropriateness of the β-cell response relative to the degree of insulin resistance in the tissue.

Application of the Minimal Model

The minimal model was developed to aid the study of insulin resistance and type-II diabetes in humans by quantifying and separating insulin sensitivity (SI) from glucose effectiveness (Sg) (Bergman et al., 1979). Studies of diabetic subjects have shown insulin sensitivity to be approximately 75% lower between normal and diabetic humans (Welch et al., 1990; Ward et al., 1991) and normal and diabetic cats (Feldhan et al., 1999) with a concomitant decrease in Sg for diabetics of both species.

The model is now applied to a number of species and conditions to evaluate the contribution of factors such as race, age and obesity in the etiology of metabolic dysfunction (Bergman, 1989) (Table 1). Measurements of insulin sensitivity determined by the minimal model have been shown to be comparable to results from the euglycemic-clamp in humans (Beard et al., 1986) and dogs (Finegood et al., 1984). The minimal model may also be effective at analyzing glucose and insulin results from oral or meal glucose tolerance test in addition to the FSIGT (Caumo et al., 2000).

Minimal model analysis may provide a new tool for understanding the relationship between insulin resistance and complications in reproduction and development. The model has been used to quantify acute insulin resistance associated with polycystic ovary syndrome in women (Legro et al., 1998). It has been applied to sheep to show inter-breed differences in insulin sensitivity during gestation (Williams et al., 2002). Decreased birth size and weight in men has been shown by the minimal model to correspond to lower SI in adulthood (Flanagan et al., 2000). A modified 90 min sampling schedule was effectively modeled to show lower SI in post-pubertal humans compared to children (Cutfield et al., 1990), possibly indicating an association between insulin resistance and hormones related to reproduction and maturation.

The minimal model reaffirms the strong association between obesity and the development of insulin resistance. Adolescents have been shown to have higher obesity proportional to a decrease in SI (Cutfield et al., 1990). Similarly, obese Thoroughbred geldings had lower SI according to the minimal model than non-obese geldings (Hoffman, 2003). Obese geldings, however, appeared to have higher Sg compared to non-obese horses, a possible compensation for the degree of insulin resistance. Insulin resistant men exhibited similar compensation with higher

Sg compared to men with normal insulin sensitivity (Flanagan et al., 2000). In another study, weight and age matched subjects showed increases in SI and Sg when trained for endurance cycling, indicating the positive effect of exercise on glucose regulation independent of weight benefits (Manetta at al., 2003).

The model failed to show a difference in insulin sensitivity or glucose effectiveness in humans adapted for two weeks to high-fat or high-carbohydrate diets (Wolever and Mehling, 2002). This short period of adaption was probably inadequate for metabolic changes to manifest. The model did show a tendency towards lower insulin sensitivity in geldings after 8 weeks of adaptation to a sugar and starch supplement as compared to a fat and fiber supplement (Hoffman et al., 2003). In calves, the minimal model was used to compare metabolic effects of meal frequency. Six week old heifer calves were raised with milk replacer fed once daily or divided into two meals (Stanley et al., 2002). No difference in insulin sensitivity was observed between feed groups. This may be due to the composition of the milk replacer - high protein and fat - which would be expected to digest slowly and perturb the glucose/insulin system minimally. Accordingly, only a small glucose response was observed following meals of either diet.

The minimal model has also been used to compare differences in glucose and insulin dynamics associated with hormones of the somatotropic axis. The minimal model has supported the positive relationship between IGF-I and SI and a possible insulin-like role for IGF-I in glucose homeostasis (Nyomba et al., 1997). Inversely, increased IGF-I during puberty has been associated with decreased SI (Cook et al., 1993). This paradoxical correlation may be attributable to an increased peripheral effect of GH which would be expected to stimulate IGF-I and also inhibit insulin action.

The minimal model could provide an invaluable instrument in evaluating the effects of hormone treatment on glucose homeostasis, possibly contributing to the discovery of ways to treat insulin resistance and prevent metabolic syndrome. Recombinant IGF-I (rhIGF-I) has been suggested as a possible therapy for severely insulin resistant patients. A subject with IGF-I gene deletion and suffering from insulin resistance was shown by the minimal model to improve SI to within normal range (Woods et al., 2000). In type II diabetics, 6 weeks of rhIGF-I administration improved SI more than 3-fold (Moses et al., 1996). However, significant side effects observed in

this and other studies indicate the need for further research before rhIGF-I therapy is recommended for insulin resistance.

The present study is the first application of the minimal model to examine the relationship between diet, insulin resistance and growth in foals.

Literature Cited

Alarid, E. T., N. L. Schlechter, S. M. Russell and C. S. Nicoll. 1992. Evidence suggesting that insulin-like growth factor-I is necessary for the trophic effects of insulin on cartilage growth in vivo. Endocrinology 130:2305-2309.

Attanasio, R., G. Oppizzi, S. Lodrini, D. Dallabonzana, M. Barausse, P. Orlandi, N. DaRe and R. Cozzi. 1999. Neurosurgery restores late GH rise after glucose-induced suppression in cured acromegalics. European J. Endocrinol. 140:23-28.

Beard, J. C, R. N. Bergman, W. K. Ward and D. Porte, JR. 1986. The insulin sensitivity index in nondiabetic man: Correlation between clamp-derived and IVGTT-derived values. Diabetes 35:362-369.

Behringer, R. R., T. M. Lewin, C. J. Quaife, R. D. Palmiter, R. L. Brinster and A. J. D'ercole. 1990. Expression of insulin-like growth factor-I stimulates normal somatic growth in growth hormone-deficient transgenic mice. Endocrinology 127:1033-1040.

Bergman, R. N., Y. Z. Ider, C. R. Bowden, and C. Cobelli. 1979. Quantitative estimation of insulin sensitivity. Amer. J. Physiol. 236:E667-E677.

Bergman, R. N., L. S. Phillips, and C. Cobelli. 1981. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and β-cell glucose sensitivity from the response of intravenous glucose. J. Clin. Invest. 68:1456-1467.

Bergman, R. N. 1989. Towards physiological understanding of glucose tolerance: Minimal-model approach. Diabetes 38:1512-1527.

Bergman, R. N. 1997. The minimal model: yesterday, today, and tomorrow. The Minimal Model Approach and Determinants of Glucose Tolerance. R. N. Bergman and J. C. Lovejoy, eds. Louisiana State Univ. press, Baton Rouge.

Bessesen, D. H. 2001. The role of carbohydrates in insulin resistance. J. Nutr. 131:1278S-2786S.

Bishop, J. S. R, Steele, N. Altszuler, I. Rathgeb, C. Bjerknes and R. C. Bodo. 1967. Diminished responsiveness to insulin in the growth hormone-treated normal dog. Am. J. Physiol. 212:272-278.

Blum, W. F., K. Albertsson-Wikland, S. Rosburg and M. B. Ranke. 1992. Serum levels of insulin-like growth factor-I (IGF-I) and IGF binding protein 3 reflect spontaneous growth hormone secretion. J. Clin. Endocrinol. Metab. 76:1610-1616.

Boulware, S. D., W. V. Tamborlane, L. S. Matthews and R. S. Sherwin. 1992. Diverse effects of insulin-like growth factor I on glucose, lipid, and amino acid metabolism. Am. J. Physiol. 25:E130-E133.

Bratusch-Marrain, P. R., D. Smith and R. A. DeFronzo. 1981. The effect of growth hormone on glucose metabolism and insulin secretion in man. J. Clin. Endocrinol. Metab. 55:973-982.

Breier, B. H. 1999. Regulation of protein and energy metabolism by the somatotropic axis. Dom. Anim. Endocrinol. 17:209-218.

Champion, Z. J., E. A. James, M.H. Vicker, B. H. Breier and P. J. Casey. 2000. The effects of bovine recombinant growth hormone administration on insulin-like growth factor-I and the haemopoietic system in Thoroughbred geldings. The Veterinary Journal 160:147-152.

Clarke, L. L., M. C. Roberts and T. A. Argenzio. 1990. Feeding and digestive problems in horses: Physiological responses to a concentrated meal. Clin. Nutr. 6:433-450.

Cohen, M. P., E. Stern, Y. Rusecki and A. Zeidler. 1988. High prevalence of diabetes in young adult Ethiopian immigrants to Israel. Diabetes 37:824-828.

Cook, J. S., R. P. Hoffman, M. A. Stene and J. R. Hansen. 1993. Effects of maturational stage on insulin sensitivity during puberty. J. Clin. Endrocrinol. Metab. 77:725-730.

Craig, L. B., R. W. Ke, W. H. Kutteh. 2002. Increased prevalence of insulin resistance in women with a history of recurrent pregnancy loss. Fertility and Sterility 78: 487-490.

Cutfield, W. S., R. N. Bergman, R. K. Menon and M. A. Sperling. 1990. The modified minimal model: Application to measurement of insulin sensitivity in children. J. Clin. Endocrinol. Metab. 70:1644-1650.

Dahlgren, L. A., M. C. H. Van der Meulen, J. E. A. Bertram, G. S. Starrak and A. J. Nixon. 2002. Insulin-like growth factor improves cellular and molecular aspects of healing in a collegenase-induced model of flexor tendinitis. J. Ortho. Res. 20:910-919.

Daughaday, W. H. 2000. Growth hormone axis overview - somatomedin hypothesis. Pediatr. Nephrol. 14:537-540.

Davy, B. M. and C. L. Melby. 2003. The effect of fiber-rich carbohydrates on features of Syndrome X. J. Am. Diet Assoc. 103:86-96.

De Kock, S. S., J. P. Rodgers, B. C. Swanepoel and A. J. Guthrie. 2001. Administration of bovine, porcine and equine growth hormone to the horse effect on insulin-like growth factor-I and selected IGF binding proteins. J. Endocrinol. 171:163-171.

Duren, S. E., J. D. Pagan, P A. Harris and K. G. Crandell. 1999. Time of feeding and fat supplementation affect plasma concentrations of insulin and metabolites during exercise. Equine Vet. J. Suppl. 30:479-484.

Elmahdi, B., H. Sallmann, H. Fuhrmann, W. Von Engelhardt and M. Kaske. 1997. Comparative aspects of glucose tolerance in camels, sheep, and ponies. Comp. Biochem. Physiol. 118A:147-151.

Farese, R. V. 2001. Insulin-sensitive phospholipid signaling systems and glucose transport. Update II. Exp. Biol. Med. 226:283-295.

Feldhahn, J. R., J. S. Rand, and G. Martin. 1999. Insulin sensitivity in normal and diabetic cats. J. Feline Med. Surg. 1:107-115.

Finegood, D. T., G. Pacini, and R. N. Bergman. 1984. The insulin sensitivity index: correlation in dogs between values determined from the intravenous glucose tolerance test and the euglycemic glucose clamp. Diabetes 33:362-368.

Flanagan, D. E., V. M. Moore, I. F. Godsland, R. A. Cockington, J. S. Robinson and D. I. W. Phillips. 2000. Fetal growth and the physiological control of glucose tolerance in adults: a minimal model analysis. Am. J. Physiol. Endocrinol. Metab. 278:E700-E706.

Foster, C. M, P. M. Hale, H. Jing and J. Schwartz. 1988. Effects of human growth hormone on insulin-like stimulated glucose metabolism in 3T3-F442A adipocytes. Endocrinology 123:1082-1088.

Friend, K., A. Iranmanesh, and J. D. Veldhuis. 1996. The orderliness of the growth hormone (GH) release process and the mean mass of GH secreted per burst are highly conserved in individual men on successive days. J. Clin. Endocrinol. Metab. 81:3746-3753.

Froesch, E. R., M. A. Hussain, C. Schmid and J. Zapf. 1996. Insulin-like growth factor I: Physiology, metabolic effects and clinical uses. Diabetes 12:195-215.

Furtado, L. M., V. Poon and A. Klip. 2003. GLUT4 activation: thoughts on possible mechanisms. Acta Physiol. Scand. 178:287-296.

Glade, M. J. And T. H. Belling. 1984. A dietary etiology for osteochondrotic cartilage. Equine Vet. Sci. 6:151-155.

Graham-Thiers, P. M., D. S. Kronfeld, K. A. Kline and D. J. Sklan. 2001. Dietary protein restriction and fat supplementation diminish the acidogenic effect of exercise during repeated sprints in horses. J. Nutr. 131:1959-1964.

Hales, C. N. And D. J. P. Barker. 1992. Type 2 (non-insulin-dependent) diabetes mellitus: the

thrifty phenotype hypothesis. Diabetologia 35:595-601.

Henson, F. M. D., C. Davenport, L. Butler, I. Moran, W. D. Shingleton, L. B. Jeffcott and P. N. Schofield. 1997. Effects of insulin and insulin-like growth factors I and II on the growth of equine fetal and neonatal chondrocytes. Equine Vet. J. 29:441-447.

Hoenig, M., D. Laflamme, D. A. Klaser, M J. Singer, D. C. Ferguson. 2001. Glucose tolerance and lipid profiles in dogs fed different fiber diets. Veterinary Therapeutics 2:160-169.

Hoffman, R. M., R. C. Boston, D. Stefanovski, D. S. Kronfeld, and P. A. Harris. 2003. Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. J. Anim. Sci. 81:2333-2342.

Hoffman, R. M. and D. S. Kronfeld. 1999. Nutrient Requirement for grazing horses: Development of an optimal pasture supplement. Pages 19-37 in Recent Advances in Animal Nutrition. Garnsworthy, P. C. And J. Wiseman, eds. Nottingham University Press, Nottingham, UK.

Holland, J. L., D. S. Kronfeld and T. N. Meacham. 1996. Behavior of horses is affected by soy lecithin and corn oil in the diet. J. Anim. Sci. 74:1252-1255.

Holley, D. C., J. W. Evans. 1979. Secretion of insulin by the nonruminant herbivore (pony) pancreas perfused in vitro. J. Anim. Sci. 49:1021-1029.

Houssay, B. A. and E. Anderson. 1949. Diabetologic action of purified anterior pituitary hormones. Endocrinology 45:627-629.

Hudson, J. M., N. D. Cohen, P. G. Gibbs, J. A. Thompson. 2001. Feeding practice associated with colic in horses. J. Am. Vet. Med. Assoc. 219:1419-1425.

Jeffcott, L. B. And J. R. Field. 1985. Current concepts of hyperlipaemia in horses and ponies. Vet Rec. 116: 461-466.

Jeffcott, L. B., J. R. Field, J. G. McLean, and K.O'Dea. 1986. Glucose tolerance and insulin sensitivity in ponies and Standardbred horses. Equine Vet. J.18:97-101.

Jenkins, D. J. A. and A. L. Jenkins. 1985. Dietary fiber and the glycemic response. Proc. Soc. Exp. Biol. Med. 180:422-431.

Johnson, T. R., B. K. Blossey, C. W. Denko and J. Ilan. 1989. Expression of insulin-like growth factor-I in cultured rat hepatocytes: effects of insulin and growth hormone. Molecular Endocrinology 3:580-587.

Jose-Cunilleras, E., K. W. Hinchcliff, R. A. Sams, S. T. Devor and J. K. Linderman. 2002.

Glycemic index of a meal fed before exercise alters substrate use and glucose flux in exercising horses. J. Appl. Physiol. 92:117-128.

Kopp, W. 2003. High-insulinogenic nutrition - An etiologic factor for obesity and the metabolic syndrome? Metabolism 52:840-844.

Kronfeld, D. S., S. E. Custalow, P. L. Ferrante, L. E. Taylor, D. Moll, T. N. Meacham and W. Tiegs. 2000. Determination of the lactate breakpoint during incremental exercise in horses adapted to dietary corn oil. Am. J. Vet. Res. 61:144-151.

Kronfeld, D. S., T. N. Meacham and S. Donohue. 1990. Dietary aspects of developmental orthopedic disease in young horses. Equine Practice 6:451-465.

Kronfeld, D. S., W. L. Cooper, K. M. Crandell, L. A. Gay, R. M. Hoffman, J. L. Holland, J. A. Wilson, D. Sklan and P. A. Harris. 1996. Supplementation of pasture for growth. Pferdeheilkunde 12:317-319.

Kronfeld, D. S., P. L. Ferrante and D. Grandjean. 1994. Optimal nutrition for athletic performance, with emphasis on fat adaptation in dogs and horses. J. Nutr. 124:2745S-2753S.

Kronfeld, D. S. 1965. Growth hormone-induced ketosis in the cow. J. Dairy Sci. 48:342-346.

Lado-Abeal, J., J. D. Veldhuis and R. L. Norman. 2002. Glucose relays information regarding nutritional status to the neural circuits that control the somatotropic, corticotropic, and gonadotropic axes in adult male Rhesus Macaques. Endocrinology 143:403-410.

Lee, P. D. K., S. K. Durham, V. Martinez, O. Vasconez, D. R. Powell and J. Guevara-Aguirre. 1997. J. Clin. Endorcrinol. Metab. 82:2266-2274.

Legro, R. S., D. Finegood and A. Dunaif. 1998. A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 83:2694-2698.

Longo, K. M., Y. Sun and A. C. Gore. 1998. Insulin-like growth factor-I effects on gonadotropin-releasing hormone biosynthesis in GT1-7 cells. Endocrinology 139:1125-1132.

MacGorman, L. R., R. A. Rizza and J. E. Gerich. 1980. Physiological concentrations of growth hormone exert insulin-like and insulin antagonistic efffects on both hepatic and extrahepatic tissues in man. J. Clin. Endocrinol. Metab. 53:556-559.

MacLeay, J. M., S. J. Valberg, F. De La Corte and J. Pagan. 1999. Recurrent exertional rhabdomyolysis in Thoroughbred racehorses: effects of diet and exercise intensity. AAEP Proceedings 45:325-326.

Merimee, T. J., J. Zapf and E. R. Froesch. 1982. Insulin-like growth factors in the fed and fasted states. J. Clin. Endocrinol. Metab. 55:999-1002.

Meyer, K. A., L. H. Kushi, D. R. Jacobs Jr., J. Slavin, T. A. Sellers and A. R. Folsom. 2000. Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. Am. J. Clin. Nutr. 71:921-930.

Moses, A. C., S. C. J. Young, L. A. Morrow, M. O'Brien and D. R. Clemmons. 1996. Recombinant human insulin-like growth factor I increases insulin sensitivity and improves glycemic control in type II diabetes. Diabetes 45:91-100.

Nguyen, P., H. Dumon, P. Buttin, L. Martin and A. S. Gouro. 1994. Composition of meal influences changes in postprandial incremental glucose and insulin in healthy dogs. J. Nutr. 124:2707S-2711S.

Nyoma, B. L. G., L. Berard and L. J. Murphy. 1997. Free insulin-like growth factor I (IGF-I) in healthy subjects: Relationship with IGF-binding proteins and insulin sensitivity. J. Clin. Endocrinol. Metab. 82:2177-2181.

Ozanne, S. E. And C. N. Hales. 2002. Early programming of glucose-insulin metabolism. Trends in Endocrinol. Metab. 13:368-373.

Pagan, J. D., R. J. Geor, P. A. Harris, K. Hoekstra, S. Gardner, C. Hudson and A. Prince. 2002. Effects of fat adaptation on glucose kinetics and substrate oxidation during low-intensity exercise. Equine Vet. J. Suppl. 34:33-38.

Pagliassotti, M. J., E. C. Gayles, D. A. Podolin, Y. Wei and C. L. Morin. 2000. Developmental stage modifies diet-induced peripheral insulin resistance in rats. Am. J. Physiol. 278:R66-R73.

Pass, M. A., S. Pollitt, and C. C. Pollit. 1998. Decreased glucose metabolism causes separation of hoof lamellae *in vitro*: a trigger for laminitis? Equine Vet. J. 26:133-138.

Pereira, M. A., D. R. Jacobs Jr., J. J. Pins, S. K. Raatz, M. D. Gross, J. L. Slavin and E. R. Seaquist. 2002. Effect of whole grains on insulin senstivity in overweight hyperinsulinemic adults. Am J. Clin. Nutr. 75:848-855.

Ralston, S. L. 1996. Hyperglycemia/hyperinsulinemia after feeding a meal of grain to young horses with osteochondritis dissecans (OCD) lesion. Pferdeheilkunde 12:320-322.

Reaven, G. M. 1988. Role of insulin resistance in human disease. Diabetes 37: 1595-1607.

Report of The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. 1997. Diabetes Care 20:1183-1197.

Rodiek, A., S. Bonvicin, C. Stull and M. Arana. 1991. Glycemic and endocrine responses to corn or alfalfa fed prior to exercise. Equine Exercise Physiol. 3:323-330.

Roth, J., S. M. Glick, R. S. Yalow and S. A. Berson. 1963. Secretion of human growth hormone: physiologic and experimental modification. Metabolism. 12:577-579.

Samaha, F. F., N. Iqbal, P. Seshadri, K. L. Chicano, D. A. Daily, J. McGrory, T. Williams, M. Williams, E. Gracely and L. Stern. 2003. A low-carbohydrate diet as compared with a low-fat diet in severe obesity. N. Engl. J. Med. 348:2074. (Abstr.)

Savage, C. J., R. N. McCarthy and L. B. Jeffcott. 1993. Effects of dietary energy and protein on induction of dyschondroplasia in foals. Equine Vet. J. Suppl. 16:74-79.

Sharp, P. S., V. Mohan, F. Maneschi, F. Vitelli, H. R. Cloke, J. M. Burrin and E. M. Kohner. 1987. Changes in plasma growth hormone in diabetic and nondiabetic subjects during the glucose clamp. Metabolism 36:71-75.

Shen, B, J. F. Todaro, R. Niaura, J. M. McCaffery, J. Zhang, A. Spiro III and K. D. Ward. 2003. Are metabolic risk factors one unified syndrome? Modeling the structure of the metabolic syndrome X. Am. J. Epidemiol. 157:701-711.

Shinozaki, K., H. Naritomi, T. Shimizu, M. Suzuki, M. Ikebuchi, T. Sawada and Y. Harano. 1996. Role of insulin resistance associated with compensatory hyperinsulinemia in ischemic stroke. Stroke 27: 37-43.

Smith, T. R., J. S. Elmendorf, T. S. David and J. Turinsky. 1997. Growth hormone-induced insulin resistance role of the insulin receptor, IRS-1, GLUT-1, and GLUT-4. Am. J. Physiol. 272:E1071-E1079.

Smith, W. J., L. E. Underwood and D. R. Clemmons. 1995. Effects of caloric or protein restriction on insulin-like growth factor-I (IGF-I) and IGF-binding proteins in children and adults. J. Clin. Endocrinol. Metab. 80:443-449.

Stanley, C. C., C. C. Williams, B. F. Jenny, J. M. Fernandez, H. G. Bateman, II, W. A. Nipper, J. C. Lovejoy, D. T. Gantt, and G. E. Goodler. 2002. Effectrs of feeding milk replacer once versus twice daily on glucose metabolism in Holstein and Jersey calves. J. Dairy Sci. 85:2335-2343.

Stoka, A. M. 1999. Phylogeny and evolution of chemical communication: an endocrine approach. J. Mol. Endocrinol. 22:207-225.

Storlien, L. H., J. A. Higgins, T. C. Thomas, M. A. Brown, H. Q. Wang, X. F. Huang and P. L. Else. 2000. Diet composition and insulin action in animal models. Brit. J. Nutr. 83:S85-S90.

Stull, C. and Rodiek, A. V. 1987. Responses of blood glucose, insulin and cortisol concentrations to common equine diets. J. Nutr. 118:206-213.

Tannenbaum, G. S. 1990. Interrelationship of somatostatin and growth hormone-releasing hormone in the genesis of the rhythmic secretion of growth hormone. Acta Paediatr. Scand. (Suppl.) 367:76-80.

Taylor, L. E., P. L. Ferrante, D. S. Kronfeld and T. N. Meacham. 1995. Acid-base variables during incremental exercise in sprint-trained horses fed a high-fat diet. J. Anim. Sci. 73:2009-2018.

Thissen, J., J. Ketelslegers and L. E. Underwood. 1994. Nutritional regulation of the insulin-like growth factors. Endocrine Rev. 15:80-101.

Thompson, D. L., M. S. Rahmanian, C. L. DePew, D. W. Burleigh, C. J. DeSouza and D. R. Colborn. 1992. Growth hormone in mares and stallions: pulsatile secretion, response to growth hormone-releasing hormone and effects of exercise, sexual stimulation and pharmacological agents. J. Anim. Sci. 70:1201-1207.

USDA. 1998. NAHMS Equine '98 Part II: Baseline reference of 1998 equine health and management. Available: www.aphis.usda.gov/vs/ceah/cahm/Equine/eq98des2.htm. Accessed Apr. 22, 2003.

Valcavi, R., M. Zini, C. Dieuguez, I. Portioli and M. F. Scanlon. 1990. Effect of oral glucose on the late growth hormone rise and growth hormone responses to GHRH in normal subjects. Clin. Endocrinology 32:539-543.

Valentine, B. A., R. J. van Saun, K. N. Thompson, and H. F. Hintz. 2001. Role of dietary carbohydrate and fat in horses with equine polysaccharide storage myopathy. J. Am. Vet. Med. Assoc. 219:1537-1544.

Ward, G. M., K. M. Weber, I. M. Walters, P. M. Aitken, B. Lee, J. D. Best, R. C. Boston and F. P. Alford. 1991. A modified minimal model analysis of insulin sensitivity and glucose-mediated glucose disposal in insulin-dependant diabetes. Metabolism 40:4-9.

Welch, I. McL., C. Bruce, S. E. Hill and N. W. Read. 1987. Duodenal and ileal lipid suppresses postprandial blood glucose and insulin responses in man: possible implications for the dietary management of diabetes mellitus. Clin. Sci. 72:209-216.

Welch, S., S. S. P. Gebhart, R. N. Bergman and L. S. Phillips. 1990. Minimal model analysis of intravenous glucose tolerance test-derived insulin sensitivity in diabetic subjects. J. Clin. Endocrinol. Metab. 71:1508-1518.

Wetzler, S., C. Jean, D. Tome and C. Larue-Achagiotis. 2003. A carbohydrate diet rich in sucrose

increased insulin and WAT in macronutrient self-selecting rats. Physiology and behavior 79:695-700.

Williams, C. C., K. J. Calmes, J. M. Fernandez, C. C. Stanley, J. C. Lovejoy, H. G. Bateman, L. R. Gentry, D. T. Gantt, and G. D. Harding. 2002. Glucose metabolism and insulin sensitivity in Gulf Coast and Suffolk ewes during late gestation and early lactation. J. Anim. Sci. 80(Suppl. 1):351. (Abstract)

Williams, C. A., D. S. Kronfeld, W. B. Staniar, and P. A. Harris. 2001. Plasma glucose and insulin responses of Thoroughbred mares fed a meal high in starch and sugar or fat and fiber. J. Anim. Sci. 79:2196-2201.

Wolever, T. M. S. and C. Mehling. 2002. High-carbohydrate-low-glycaemic index dietary advice improves glucose disposition in subjects with impaired glucose tolerance. Brit. J. Nutr. 87:477-487.

Woods, K. A., C. Camacho-Hübner, R. N. Bergman, D. Barter, A. J. L. Clark and M. O. Savage. 2000. Effects of insulin-like growth factor I (IGF-I) therapy on body composition and insulin resistance in IGF-I gene deletion. J. Clin. Endocrinol. Metab. 85:1407-1411.

Yalow, R. S., S. J. Goldsmith and S. A. Berson. 1969. Influence of physiologic fluctuations in plasma growth hormone on glucose tolerance. Diabetes 18:402-408.

Table 1. Sg and SI values determined by the minimal model for humans and other species.

	$Sg \times 10^2$ (min^{-1})	$SI \times 10^4$ (min^{-1} per mU/L)	Reference
Humans			
Adapted high GI (n=11)	1.7 ± 0.2	4.02 ± 0.64	Wolever and Mehling, 2002
Adapted low GI (n=13)	1.7 ± 0.2	3.21 ± 0.68	
Adapted low-carb, high MUFA (n=11)	1.7 ± 0.1	3.99 ± 0.56	
Non-obese IDDM (n=8)	1.6 ± 0.5	2.5 ± 0.6	Ward et al., 1991
Non-obese (n=17)	2.6 ± 0.2	8.3 ± 1.5	
Men (age 19-35) (n=10)	1.7 ± 0.8	8.8 ± 3.0	Beard et al., 1986
Cats			
Normal (n=10)	3.0 ± 0.3	3.22 ± 0.37	Feldhahn et al., 1999
Diabetic (n=5)	1.4 ± 0.3	0.58 ± 0.14	
Dogs			
Normal (n=12)	4.3 ± 0.5	4.3 ± 0.7	Finegood et al., 1984
Ruminants			
6-week Holstein calves (n=18)	2.4 ± 0.003	10.5 ± 1.5	Stanley et al., 2002
6-week Jersey calves (n=15)	2.3 ± 0.003	18.1 ± 3.7	
Sheep in early lactation (n=38)	1.26 ± 1.24	5.27 ± 0.93	Williams et al., 2002
Horses (Thoroughbreds)			
Non-obese geldings (n = 4)	1.43 ± 0.16	1.94 ± 0.19	Hoffman et al., 2003
Obese geldings (n = 3)	3.02 ± 0.22	0.37 ± 0.27	
Weanlings supplemented with a forage-like meal rich in fat and fiber (n=6)	0.756 ± 0.32	3.57 ± 0.46	Treiber, 2003
Weanlings supplemented with a meal high in hydrolyzable carbohydrates (n=6)	0.758 ± 0.56	2.30 ± 0.15	

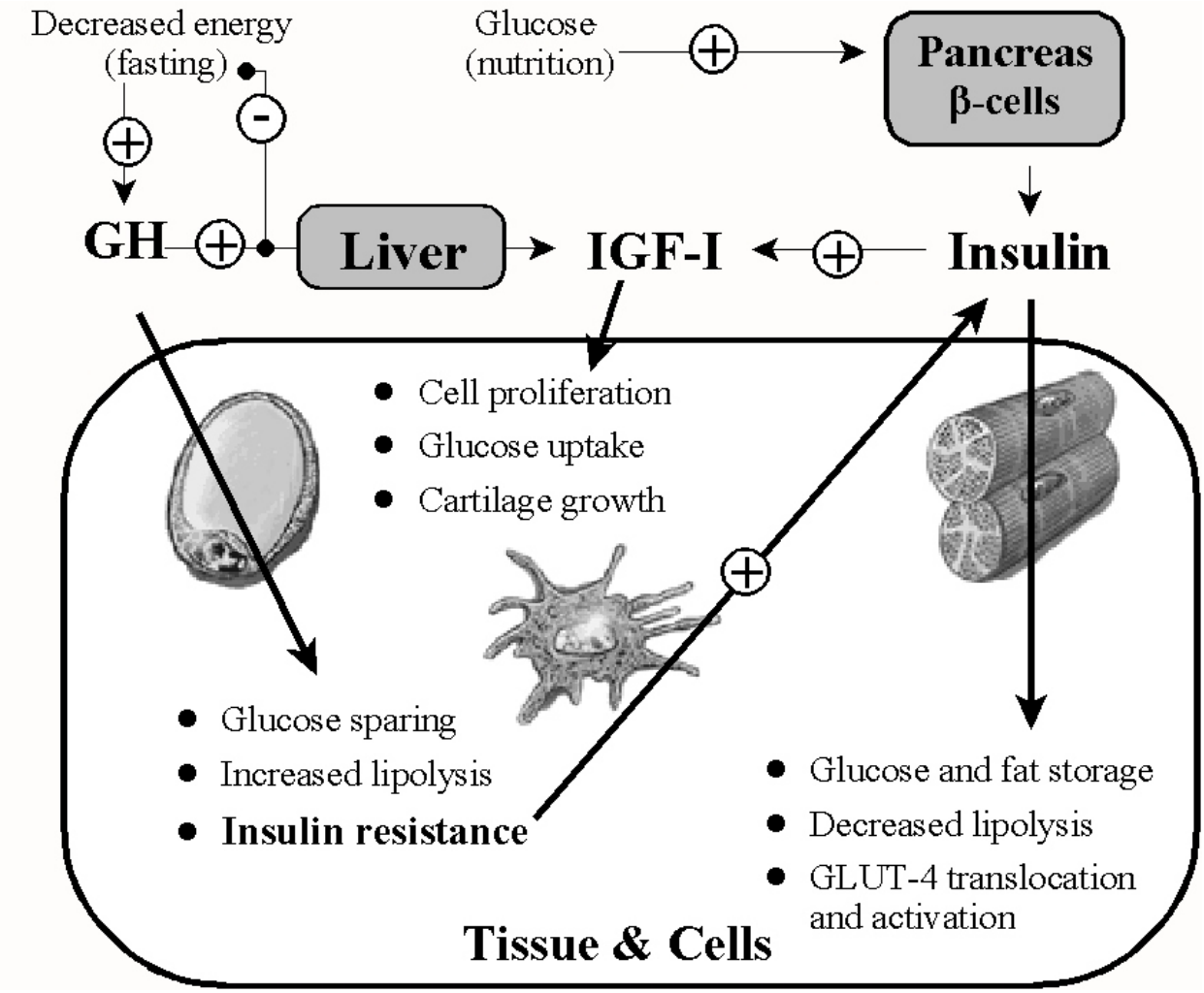

Figure 1. Diagram of metabolic regulation and counter-regulation by insulin, growth hormone and insulin-like growth factor-I. Stimulatory effects are indicated by ⊕; inhibition is indicated by ⊖.

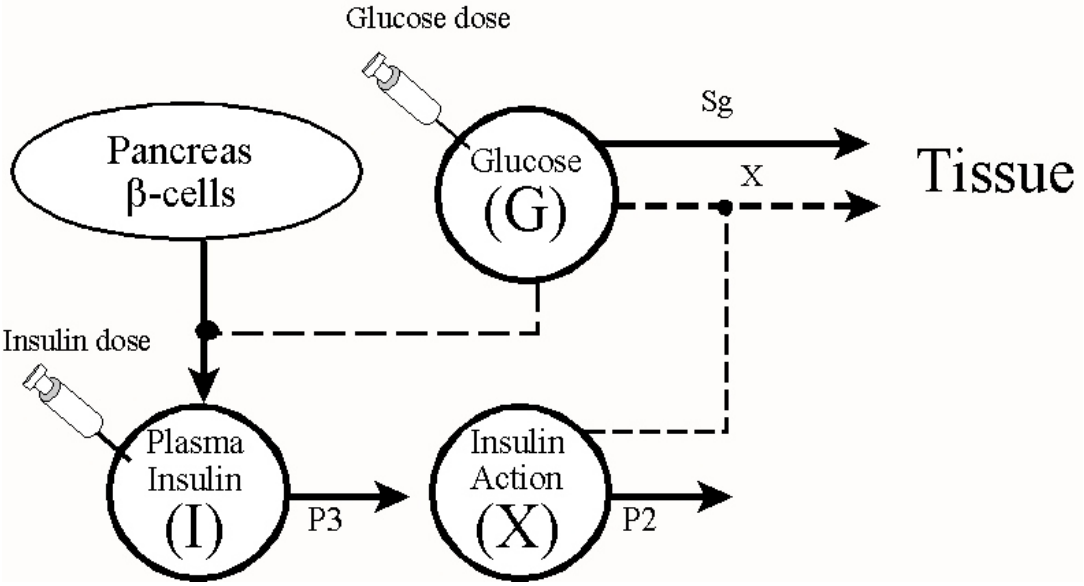

Figure 2. Compartmental interpretation of glucose and insulin dynamics as represented by the minimal model (adapted from Bergman, 1979) in which G represents glucose concentration of the plasma, I is the insulin concentration of the plasma, and X is remote insulin concentration. Glucose-mediated glucose clearance is Sg and insulin-mediated glucose clearance is X. The fractional rate of insulin clearance from the remote compartment is defined as p_2. The contribution of plasma insulin to the remote compartment is defined as p_3. The glucose dose at t = 0 enters the plasma glucose compartment. The insulin dose at t = 20 min enters the plasma insulin compartment. Representative samples are taken for these compartments.

Objective

1. To compare the effects of adaptation to meals rich in sugar and starch or fat and fiber on glucose metabolism and its regulation in Thoroughbred weanlings, focusing on:
    A. Glucose dynamics
    B. Insulin sensitivity
    C. Growth hormone
    D. Insulin-like growth factor-I

2. To apply the Minimal Model of glucose and insulin dynamics to the growing horse.

3. To extend the implications of the minimal model to include hormones of the somatotropic axis

Chapter 1

Insulin Sensitivity in Horses is Decreased by a Sugar and Starch Feed Compared to a Fat and Fiber Feed

ABSTRACT: Diets rich in hydrolyzable carbohydrates induce a hyperglycemic/insulinemic response and may increase metabolic disorders such as type II diabetes and heart disease in humans and some types of laminitis, exertional rhabdomyolysis and osteochondrosis in horses. This study applied the minimal model to determine the effect of diet on glucose and insulin dynamics in young horses. Twelve Thoroughbred foals were raised on pasture and supplemented twice daily with a feed high in either sugar and starch or fat and fiber. As weanlings (age 199 ± 19 d, weight 274 ± 18 kg), the subjects underwent a modified frequently sampled intravenous glucose tolerance test during which they remained in stalls and had access to grass hay and water ad libitum. Samples were colleted at -60, -45, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60 , 70 , 80, 90, 100, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min with a glucose bolus of 300 mg/kg BW at 0 min and an insulin bolus of 1.5 mU/kg BW at 20 min. Plasma was analyzed for glucose and insulin. Insulin sensitivity (SI), glucose effectiveness, acute insulin response to glucose (AIRg) and disposition index were derived using Minmod Millennium and WinSAAM software. Diets were compared using the non-parametric Kruskal-Wallis test or the sign test. Basal glucose did not differ between groups ($P = 0.75$). Insulin levels were higher in the sugar and starch foals at all 36 sample points ($P = 0.030$). The glucose:insulin ratio for the sugar and starch supplemented foals was lower than for fat and fiber foals ($P = 0.025$). Insulin sensitivity was lower in foals fed sugar and starch than foals fed fat and fiber ($P = 0.007$). Acute insulin response to glucose was directly correlated to weight ($r = 0.80$; $P = 0.002$) and inversely correlated with SI ($r = -0.54$; $P = 0.068$). The glucose:insulin ratio was directly correlated to SI ($r = 0.63$; $P = 0.036$). These results show that foals adapted to a supplement high in sugar and starch had lower insulin sensitivity compared to foals adapted to a fat and fiber feed. Foals adapted to sugar and starch appeared to compensate for a lower sensitivity to insulin by increasing insulin secretion. Feeding meals high in glucose equivalents may increase the risk of

developing insulin resistance and associated disorders in horses.

Keywords: horse, glucose dynamics, insulin sensitivity, low-glycemic index, minimal model

Introduction

Insulin resistance in equids is a risk factor for obesity and hyperlipidemia (Jeffcott and Field, 1985: Jeffcott et al., 1986) and possibly certain forms of laminitis (Pass et al. 1998), exertional rhabdomyolysis (Valentine et al., 2001) and osteochrondrosis (Ralston, 1996). In humans, insulin resistance is associated with diets high in hydrolyzable carbohydrates and may contribute to disorders such as non-insulin-dependent diabetes, hypertension and coronary artery disease, atherothrombotic stroke, and recurrent pregnancy loss (Reaven, 1988; Shinozaki et al., 1996; Craig et al., 2002). The minimal model representation of glucose and insulin dynamics has been applied to humans in order to clarify the etiology of these disorders in terms of glucose and insulin metabolism (Bergman et al., 1981; Beard et al., 1986). It may be equally beneficial in quantifying the regulation of glucose metabolism in the horse (Hoffman et al., 2003).

The minimal model differentiates insulin- and glucose-mediated components of plasma glucose uptake in order to describe the efficiency of the insulin response to an intravenous glucose dose. It is used here to compare adaptation of foals to twice daily meals rich in hydrolyzable carbohydrates (sugar and starch) (SS) or meals resembling forage and containing fat and fiber (FF). Previous studies have demonstrated that glycemic/insulinemic responses are large following meals high in glucose equivalents compared to the response following meals composed of fat and fiber or hay (Stull and Rodiek, 1988; Williams et al., 2001). This deviation of post-prandial hormones impacts the glucose regulatory system, possibly contributing to a decrease in tissue sensitivity to insulin. In this study, foals adapted to a SS supplement were expected to have a lower sensitivity to insulin with a compensatory increase in insulin secretion following a glucose challenge.

Materials and Methods

The study was conducted at the Middleburg Agricultural Research and Extension (MARE) Center and approved by the Institution's Animal Care and Use Committee.

Twelve Thoroughbred foals were raised on 60 acres of mixed bluegrass/clover pasture. They were supplemented with twice daily meals, six receiving the SS feed and six receiving the FF feed. Diet groups were fed collectively but in individual pans, with each pan containing an single portion. Prior to weaning, foals shared meals with their dams. After weaning, the subjects had access to 3.2 kg/d feed per individual pan, a portion intended to approximate a 1:1 supplement:pasture ratio of DE.

The supplements were formulated to be isocaloric and isonitrogenous with vitamin and mineral contents designed to complement the pasture and fulfill present recommendations (NRC, 1989; Hoffman et al., 2001). Pasture and supplements were sampled monthly and submitted to a commercial laboratory for proximate and mineral analysis (Table 1). A pilot study indicated that the glycemic index of the SS supplement was higher ($P = 0.001$) than FF, with glucose AUC at 143.9 ± 4.1 and 11.4 ± 5.4 $g \cdot min \cdot L^{-1}$ for SS and FF, respectively.

The experiment consisted of a modified frequently sampled intravenous glucose tolerance test (FSIGT) administered to each horse (Caumo et al., 2000; Hoffman et al., 2003).
Three horses were randomly assigned to each of four days for sample collection. On the day prior to each test, the three subjects were weighed on an electronic scale (TYREL Platform, Model TC-10S, Allweights Hamilton Scale Corp., Richmond, VA). Catheters were inserted into the jugular vein and the horses were stalled overnight. The catheters were flushed with heparin in isotonic saline (10 U heparin /mL saline) that evening and again in the morning. Basal samples were collected 60 and 45 min prior to the glucose dose. To simulate a non-fasting grazing state the stalled horses had access to grass hay and water ad libitum throughout the experiment.

The modified FSIGTs were initiated at 0900 with a bolus of 300 mg/kg BW of glucose through the catheter (Dextrose Solution 50%, Phoenix Pharmaceutical, Inc. St. Joseph, MO). Twenty minutes after the glucose bolus an insulin bolus of 1.5 mU/kg BW was rapidly administered (within 30 sec) through the catheter (Humulin R, Eli Lilly and Company, Indianapolis, IN).

Thirty-six venous samples were collected from each horse over the 6 h FSIGT. Basal samples were taken 60 and 45 min before the glucose dose. Blood samples were drawn at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60 , 70 , 80, 90, 100, 120, 150,

180, 210, 240, 270, 300, 330 and 360 min after the glucose bolus. The blood samples were immediately transferred to heparinized sample tubes (Vacutainer, Fisher Health Care, Chicago, IL) and placed in ice water until centrifuged. Plasma was removed within 30 min of collection and frozen at -4° C until analysis.

Plasma glucose samples were analyzed by colorimetric assay (Beckman Instruments, Glucose Procedure #16-UV, Sigma Diagnostics, St. Louis, MO). Insulin was determined using a RIA (Coat-A-Count Insulin, Diagnostic Products, Los Angeles, CA) previously validated for equine insulin (Freestone et al., 1991). The intraassay CV of duplicate samples was <1% for glucose and 5% for insulin.

Minimal Model Analysis

The glucose and insulin curves were interpreted according to the minimal model of glucose dynamics (Figure 1) as described by the following equations (Bergman et al., 1981):

$$G'(t) = -G(t)\cdot[Sg + X(t)] + Sg\cdot Gb$$

where G'(t) is the rate ($mg/dl\cdot min^{-1}$) of plasma glucose uptake by the tissue. Glucose effectiveness (Sg) describes one component of this plasma clearance (min^{-1}) which is the capacity of the cells to take up glucose without insulin mediation. The plasma glucose concentration (mg/dL) at time = t is G(t); Gb is the basal glucose concentration (mg/dL) maintained by hepatic production. Insulin action, X(t), represents the insulin mediated component (min^{-1}) of the plasma glucose clearance rate via the acceleration of glucose uptake in response to an increment change in the insulin concentration. This component is further described by:

$$X'(t) = p_3\cdot[I(t)-Ib] - p_2\cdot X(t)$$

where X'(t) is the rate of change of the insulin action, p_3 describes delivery of insulin to the interstitium, and p_2 describes the disposal of insulin from the interstitium.

Insulin sensitivity (SI) is the ratio of these parameters:

$$SI = p_3/p_2$$

and represents the efficiency of insulin to accelerate glucose uptake by the cells.

Responsiveness of β-cells to the glucose load is described by the acute response of insulin to glucose (AIRg) which is the increase in plasma insulin above basal concentration integrated from 0 to 10 min after the glucose dose (Bergman, 1997). The product of AIRg and SI determines the disposition index (DI) or the appropriateness of the β-cell response relative to the degree of insulin resistance in the tissue.

For this study, SI, Sg, DI, and AIRg were calculated using MinMod Millenium (Ver. 5.10, BeBos Assoc., 2001) and WinSAAM (Ver. 3.0.1, Greif and Boston, 1997) software.

Statistics

Feed groups were compared by the non-parametric sign test (n=36) and the Kruskal-Wallis analysis of variance (STATA 8, 2003). Because tests were non-parametric, the data were reported as means and ranges rather than means and standard errors. Associations were determined by robust linear regression.

Table 1. Partial proximate analysis of supplements, pasture and hay[2]. Data are summarized as means ± SE.

Nutrient	SS(n=3)	FF(n=3)	Pasture (n=6)	Hay[1] (n=2)
DM%	89.6 ± 2.1	91.3 ± 0.7	30.6 ± 2.1	76.8 ± 12.2
DE, MCAL/kg	3.28 ± 0.11	2.82 ± 0.40	2.40 ± 0.07	2.20 ± 0.03
CP, %	14.8 ± 0.6	14.4 ± 1.4	17.7 ± 0.9	16.6 ± 0.1
NDF, %	21.3 ± 1.0[a]	44.0 ± 1.0[b]	57.6 ±1.9	56.5 ± 2.3
Starch, %	40.4 ±2.1[a]	3.9 ± 1.2[b]	1.2 ± 0.2	2.8 ± 0.4
Sugar, %	8.5 ± 0.4	8.4 ± 0.4	8.2 ± 1.4	6.2 ± 0.7
Fat, %	3.0 ± 0.1[c]	9.7 ± 0.9[d]	3.6 ± 0.1	2.0 ± 0.3

[1] Foals had access to hay ad libitum while in the stalls on the day of sampling

[a,b] Supplement means differ ($P < 0.001$)

[c,d] Supplement means differ ($P < 0.05$)

[2] Dairy One DHIA Forage Testing Laboratory, Ithaca, NY

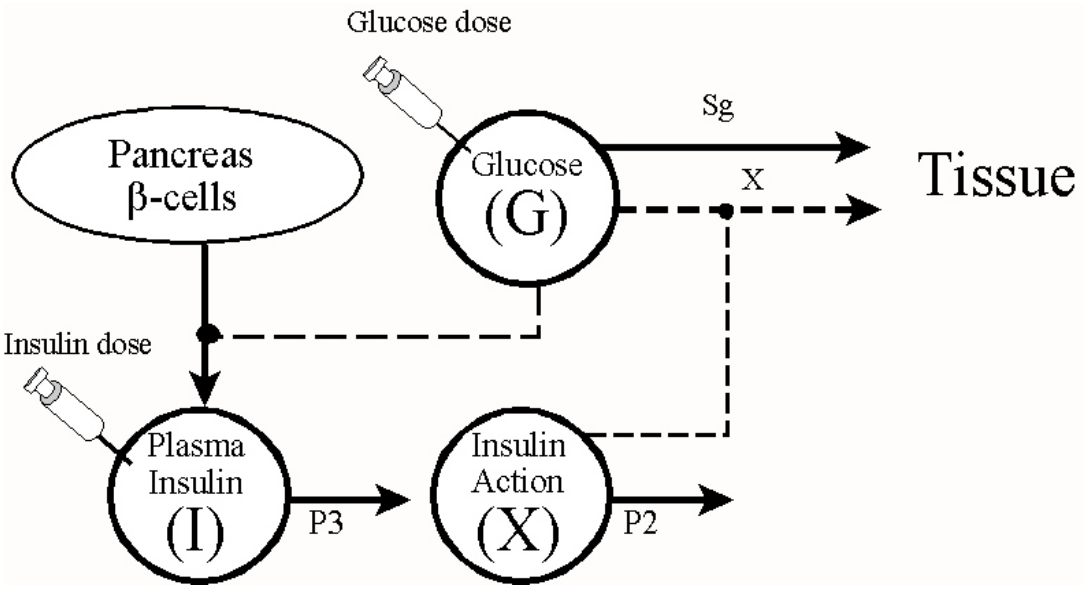

Figure 1. Compartmental interpretation of glucose and insulin dynamics as represented by the minimal model (adapted from Bergman, 1979) in which G represents glucose concentration of the plasma, I is the insulin concentration of the plasma, and X is remote insulin concentration. Glucose-mediated glucose clearance is Sg and insulin-mediated glucose clearance is X. The fractional rate of insulin clearance from the remote compartment is defined as p_2. The contribution of plasma insulin to the remote compartment is defined as p_3. The glucose dose at t = 0 enters the plasma glucose compartment. The insulin dose at t = 20 min enters the plasma insulin compartment. Samples represent these compartments.

Results

On the day of the test the median age was 196 d with a range from 177 to 235 d. The horses had been weaned a median of 30 d prior with a range from 25 to 36 d. No differences were found between diet groups in median body weight (273 kg; range 242 to 318; $P = 0.75$), age (196 d; range 177 to 235; $P = 0.81$), or number of days since weaning (30 d; 25 to 36; $P = 0.94$). All weanlings were considered to be in good health and had similar body condition scores of 5 or 6 (Henneke et al., 1983).

Basal glucose did not differ ($P = 0.75$) between feeds (Figure 2). Median insulin values were higher ($P = 0.030$) at all 36 sample points (Figure 3) and median basal insulin was 22% higher ($P = 0.11$) in the SS group compared to the FF group. Compared to the FF group, the basal glucose:insulin ratio was 20% lower ($P = 0.025$) and SI was 23% lower ($P = 0.007$) for SS foals than for FF foals. Feeds were not found to differ with respect to Sg, AIRg or DI ($P = 1.00$, $P = 0.15$, $P = 0.26$, respectively) (Table 2).

Acute insulin response to glucose tended towards an inverse correlation ($r = 0.54$; $P = 0.068$) to SI (Figure 4). The basal glucose:basal insulin ratio was directly correlated ($r = 0.63$; $P = 0.036$) to SI.

Table 2. Diet effects on basal glucose, basal insulin, glucose:insulin ratio, glucose effectiveness (Sg), insulin sensitivity (SI), acute insulin response to glucose (AIRg) and disposition index (DI) of Thoroughbred foals supplemented with meals rich in either hydrolyzable carbohydrates (SS) or fat and fiber (FF).

Minimal Model Variable	**SS median (range)**	**FF median (range)**	***P***
Basal glucose (mg/dL)	122.9 (114.1 to156.7)	121.8 (112.5 to147.3)	0.75
Basal insulin (mU/L)	5.03 (4.52 to 10.36)	4.12 (1.78 to 5.57)	0.11
Glucose:insulin ratio	24.0 (14.9 to 27.5)	30.0 (24.5 to 64.9)	0.025
$Sg \times 10^3$ (min^{-1})	7.43 (6.03 to 10.1)	7.53 (6.15 to 8.52)	1.00
$SI \times 10^4$ (min^{-1} per mU/L)	2.33 (1.76 to 2.78)	3.03 (2.76 to 5.94)	0.007
AIRg (mU/L·10 min)	144 (111 to 241)	113 (60 to 173)	0.15
$DI \times 10^4$	311.5 (216 to 612)	381.5 (317 to 477)	0.26

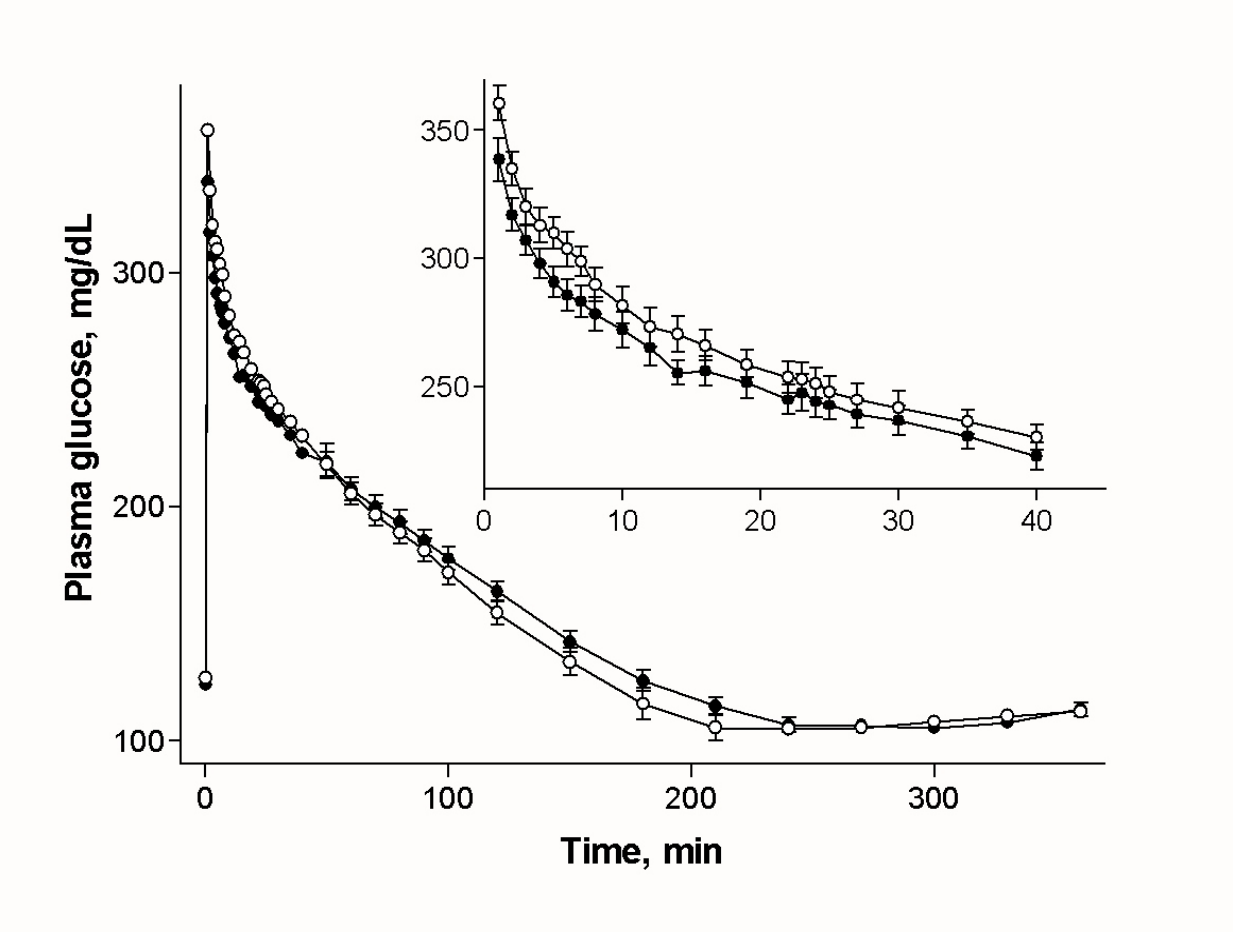

Figure 2. Plasma glucose concentrations in Thoroughbred weanlings during an FSIGT with 300mg/kg glucose administered intravenously at 0 min and 1.5 mU/kg insulin administered intravenously at 20 min. Horses were adapted to a diets high in glucose equivalents (SS, ○) or fat and fiber (FF, ●). Data represented by mean and SE.

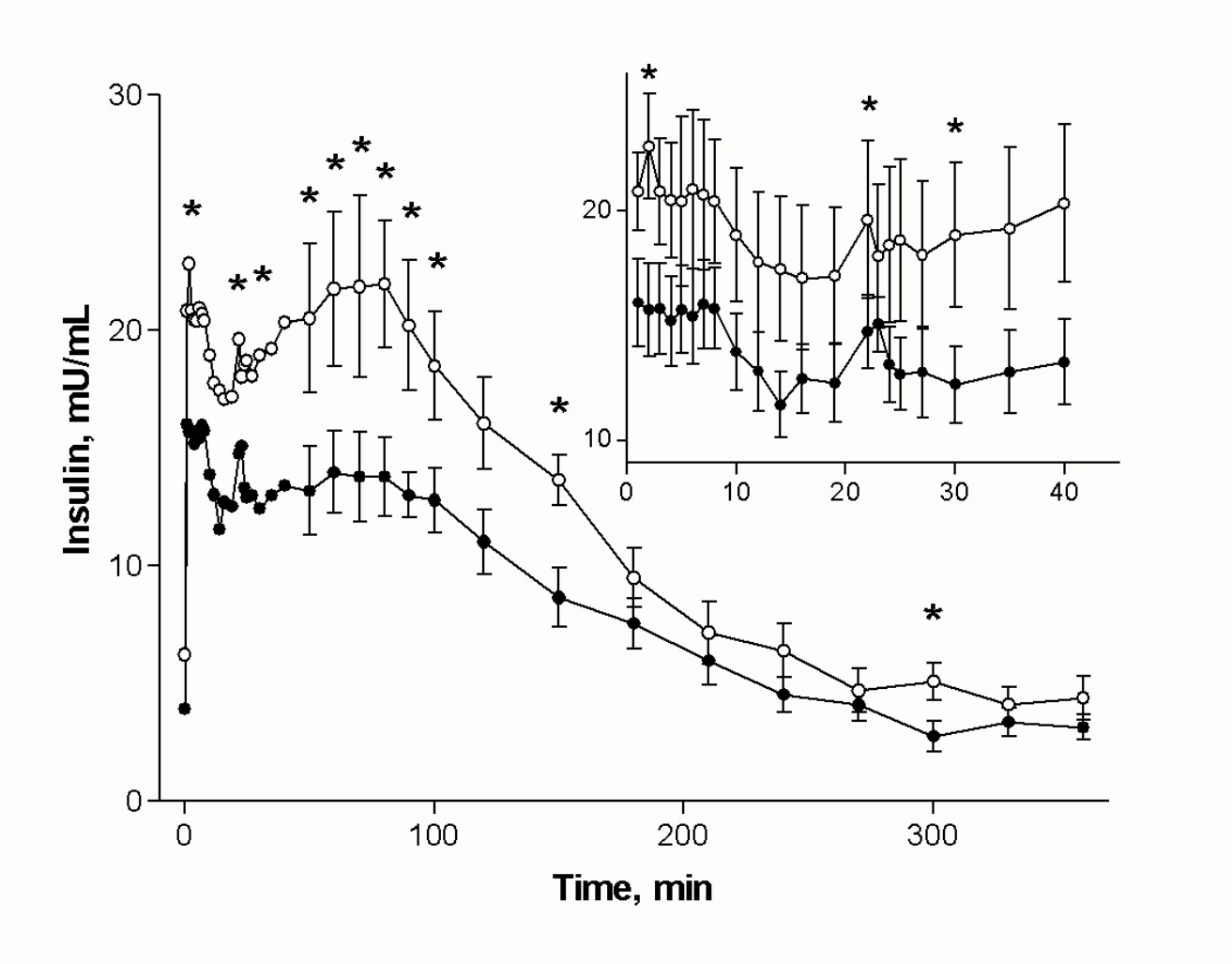

Figure 3. Plasma insulin concentrations in Thoroughbred weanlings during an FSIGT with 300mg/kg glucose administered intravenously at 0 min and 1.5 mU/kg insulin administered intravenously at 20 min. Horses were adapted to a diets high in glucose equivalents (SS, ○) or fat and fiber (FF, ●). Data represented by mean and SE. A * indicates significant differences where $P < 0.055$. Insulin concentrations were higher in SS weanlings for all 36 time points ($P < 0.030$).

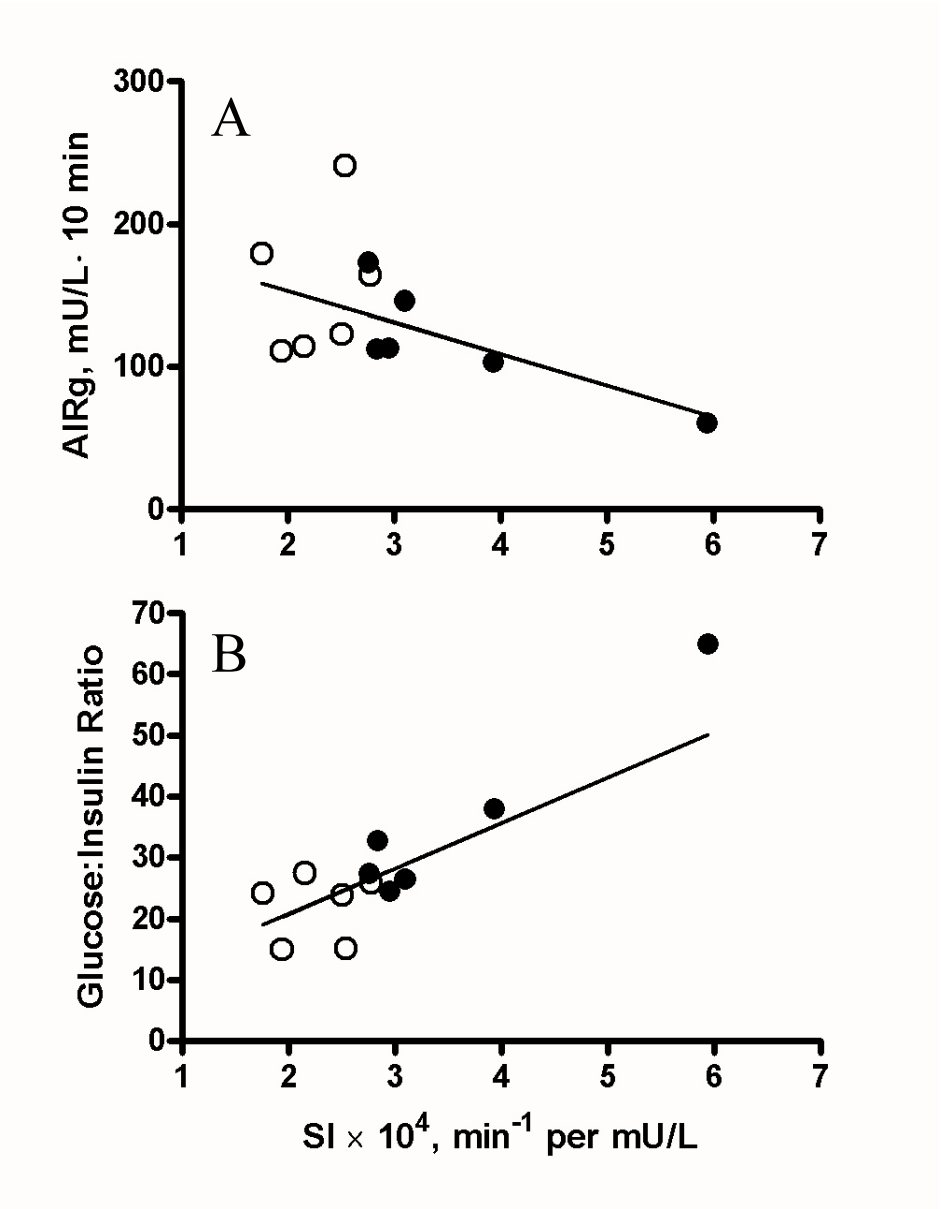

Figure 4. Correlations between SI and AIRg (A; r = 0.54), and SI and glucose:insulin ratio (B; r = 0.63) of Thoroughbred weanlings. Horses were adapted to a diets high in glucose equivalents (SS, ○) or fat and fiber (FF, ●).

Discussion

The results show lower insulin sensitivity in Thoroughbred weanlings adapted to a feed high in glucose equivalents as compared to weanlings adapted to a feed rich in fat and fiber. Application of the minimal model indicates adaptation to these differing diets affects the insulin-mediated component of glucose clearance (SI) but not the glucose-mediated component (Sg).

Large glycemic and insulinemic responses such as that following the SS meal may increase stimulation of counter-regulatory hormone which antagonize insulin-action and could alter insulin-sensitivity of tissue as has been shown in humans (Yalow et al., 1969; Jenkins, 1987). Mature Thoroughbred geldings having normal body condition scores also demonstrated a trend towards decreased SI when adapted to the SS diet (Hoffman et al., 2003). Similarly, insulin sensitivity decreased in insulin resistant humans over 4 months of adaptation to diets with high glycemic indices while SI increased in subjects adapted to diets with low glycemic indices (Wolever and Mehling, 2002). The complexity and diversity of meals within each diet group in this and other human studies, however, increases confounding and reduces the capacity to clearly distinguish diet effects. Animal models like the present experiment provide a useful alternative in controlling diet for application to both animal and human welfare (Storlien, 2000).

Glucose effectiveness did not differ between diet groups. This suggests that the glucose-mediated component of glucose uptake is not regulated by adaptation to a feed with high glucose equivalents. In this experiment, compensation with increased insulin secretion sufficiently regulated glucose uptake, therefore glucose-mediated components probably remained unstressed by either ration and required no adjustment. Holstein or Jersey calves showed no difference in Sg when fed milk-replacer as or twice daily meals (Stanley, 2002). Humans adapted to diets with different glycemic indices also showed no significant change in Sg from baseline (Wolever and Mehling, 2002). Nevertheless, non-insulin-dependent GLUT-1 glucose transporters may represent a component of Sg and are capable of compensatory changes when nutrition is inadequate (Sadiq et al., 1999; Flanagan, 2000). In cases of extreme insulin resistance, Sg may also be required to compensate in order to sufficiently clear glucose from the plasma (Hoffman et

al., 2003). Such changes in Sg are not indicated by the scope of the present experiment. While consistent for all foals, Sg was substantially smaller in this study than values reported for older horses and other species (Feldhahn et al., 1999; Stanley et al., 2002; Hoffman et al., 2003).

Thoroughbred weanlings adapted to the SS meal had a significantly lower glucose:insulin ratio than foals adapted to the FF meal. These findings further indicate lower insulin sensitivity in the SS group and correlate closely to SI as derived from the minimal model, demonstrating the capacity of static measurements such as basal glucose:insulin ratio to estimate properties of the dynamic system. The correlation between basal glucose:basal insulin ratio and SI was also observed in women with polycystic ovary syndrome (Legro et al. 1998). In horses with pituitary adenoma, resultant insulin resistance was demonstrated by a low glucose:insulin ratio (Garcia and Beech, 1986). Accordingly, basal concentrations of glucose and insulin may be useful in providing a preliminary investigation of the metabolic system.

Lower SI in SS foals tended to be correlated with an increased insulin response, which probably represents a compensation for the decreased response of the tissue to insulin. Such compensation could be a precursor to elevated circulating insulin levels which may play a role in the development of metabolic disorders. Mature Thoroughbred geldings showed a similar increase in AIRg when SI was decreased (Hoffman et al., 2003) as did calves (Stanley et al., 2002) and humans (Welch et al., 1990).

Because of the inverse relationship of AIRg and SI, their product, DI, was consistent across all 12 weanlings. Uniform DIs suggest that each weanling's insulin response was appropriate to the insulin sensitivity of its tissue. This was expected as all the horses effectively cleared the glucose dose and were considered to be healthy. One human study recorded an increase in DI in human subjects fed a low-glycemic index diet while DI in subjects fed a high-glycemic index diet did not change (Wolever and Mehling, 2002). Final DI values in humans for the two diet groups, however, did not differ.

The results of this study are consistent with other studies regarding the effects of diets with high glucose equivalents on glucose and insulin dynamics. Foals adapted to SS feed showed lower insulin sensitivity but no difference in non-insulin mediated glucose clearance. In humans, diets with high glycemic indices are also implicated in decreased insulin sensitivity (Wolever and

Mehling, 2002). Such diets may therefore play a role in the disorders associated with insulin resistance and the predominance of obesity in humans, just as feeds rich in hydrolyzable carbohydrates may be detrimental to horses. As in humans, proper diet management may reduce the horse's risk of developing disorders associated with insulin resistance.

Implications

Growing horses fed a diet high in sugar and starch have an increased risk of developing insulin resistance, a metabolic disorder associated with obesity, laminitis, exertional rhabdomyolysis and osteochondrosis. The majority of equine operations in the US feed supplements based on grains and molasses. Developing feeds that replace sugars and starches with fat and fiber may help to reduce the occurrence of metabolic disorders in equines.

Literature Cited

Beard, J. C., R. N. Bergman, W. K. Ward, and D. Porte, Jr. 1986. The insulin sensitivity index in nondiabetic man: correlation between clamp-derived and IVGTT-derived values. Diabetes 35:362-369.

Bergman, R. N., Y. Z. Ider, C. R. Bowden, and C. Cobelli. 1979. Quantitative estimation of insulin sensitivity. Amer. J. Physiol. 236:E667-E677.

Bergman, R. N., L. S. Phillips, and C. Cobelli. 1981. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and β-cell glucose sensitivity from the response of intravenous glucose. J. Clin. Invest. 68:1456-1467.

Bergman, R. N. 1997. The minimal model: yesterday, today, and tomorrow. Pages 3-50 in The Minimal Model Approach and Determinants of Glucose Tolerance. R. N. Bergman and J. C. Lovejoy, eds. Louisiana State Univ. press, Baton Rouge.

Caumo, A., R. N. Bergman, and C. Cobelli. 2000. Insulin sensitivity from meal tolerance tests in normal subjects: a minimal model index. J. Clin. Endocrin. Metab. 85:4396-4402.

Craig, L. B., R. W. Ke, W. H. Kutteh. 2002. Increased prevalence of insulin resistance in women with a history of recurrent pregnancy loss. Fertility and Sterility 78:487-490.

Feldhahn, J. R., J. S. Rand, and G. Martin. 1999. Insulin sensitivity index: correlation in dogs between values determined from the intravenous glucose tolerance test and the euglycemic glucose clamp. Diabetes 33:362-368.

Finegood, D. T., G. Pacini, and R. N. Bergman. 1984. The insulin sensitivity index: correlation in dogs between values determined from the intravenous glucose tolerance test and the euglycemic glucose clamp. Diabetes 33:362-368.

Flanagan, D. E. 2000. Fetal growth and the physiological control of glucose tolerance in adults: a minimal model analysis. Am. J. Phys. 278:E700-E706.

Freestone, J. F., K. J. Wolfsheimer, S. G. Kamerling, G. Church, J. Hamra, and C. Bagwell. 1991. Exercise induced hormonal and metabolic changes in Thoroughbred horses: effects of conditioning and acepromazine. Equine Vet. J. 23:219-223.

Garcia, M. C. and J. Beech. 1986. Equine intravenous glucose tolerance test: glucose and insulin responses of healthy horses fed grain or hay and of horses with pituitary adenoma. Am. J. Vet. Res. 47:570-572.

Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet. J. 15:371-372.

Hoffman, R. M., J. A. Wilson, D. S. Kronfeld, W. L. Cooper, L. A. Lawrence, D. Sklan and P. A. Harris. 2001. Hydrolyzable carbohydrates in pasture, hay, and horse feeds: Direct assay and seasonal variation. J. Anim. Sci. 79:500-506.

Hoffman, R. M., R. C. Boston, D. Stefanovski, D. S. Kronfeld, and P. A. Harris. 2003. Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. J. Anim. Sci. 81:2333-2342.

Jeffcott, L. B. And J. R. Field. 1985. Current concepts of hyperlipaemia in horses and ponies. Vet Rec. 116: 461-466.

Jeffcott, L. B., J. R. Field, J. G. McLean, and K.O'Dea. 1986. Glucose tolerance and insulin sensitivity in ponies and Standardbred horses. Equine Vet. J.18:97-101.

Jenkins, D. J. A. 1987. Metabolic effect of a low-glycemic-index diet. Am. J. Clin. Nutr. 46:968-975.

Legro, R. S., D. Finegood, and A. Dunaif. 1998. A fasting glycose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. J. Clin. Endocrinol. & Metab. 83:2694-2698.

NRC. 1989. Pages 39-48 in Nutrient Requirements of Horses. 5th rev. ed. Natl. Acad. Press, Washington, DC.

Pass, M. A., S. Pollitt, and C. C. Pollit. 1998. Decreased glucose metabolism causes separation of hoof lamellae *in vitro*: a trigger for laminitis? Equine Vet. J. 26:133-138.

Ralston, S. L. 1996. Hyperglycemia/hyperinsulinemia after feeding a meal of grain to young horses with osteochondritis dissecans (OCD) lesion. Pferdeheilkunde 12:320-322.

Reaven, G. M. 1988. Role of insulin resistance in human disease. Diabetes 37:1595-1607.

Sadiq, H. F., U. G. Das, T. F. Tracey, and S. U. Devaskar. 1999. Intra-uterine growth restriction differentially regulates perinatal brain and skeletal muscle glucose transporters. Brain Res. 823:96-103.

Shinozaki, K., H. Naritomi, T. Shimizu, M. Suzuki, M Ikebuchi, T. Sawada and Y. Harano. 1996. Role of insulin resistance associated with compensatory hyperinsulinemia in ischemic stroke. Stroke. 27:37-43.

Stanley, C. C., C. C. Williams, B. F. Jenny, J. M. Fernandez, H. G. Bateman, II, W. A. Nipper, J. C. Lovejoy, D. T. Gantt, and G. E. Goodler. 2002. Effectrs of feeding milk replacer once versus twice daily on glucose metabolism in Holstein and Jersey calves. J. Dairy Sci. 85:2335-2343.

Statacorp. 2003. Stata Statistical Software: Release 8.0. College Station, TX: Stata Corporation.

Storlien, L. H., J. A. Higgins, T. C. Thomas, M. A. Brown, H. Q. Wang, X. F. Huang, and P. L. Else. 2000. Diet composition and insulin action in animal models. Br. J. Nutr. 83:S85-S90.

Stull, C. L., A. V. Rodiek. 1988. Responses of blood glucose, insulin, and cortisol concentrations to common equine diets. J. Nutr. 118: 206-213.

USDA:APHIS:VS. 1998. Equine '98 Part II: Baseline reference of 1998 equine health and management. NAHMS. Pg. 33-42.

Valentine, B. A., R. J. van Saun, K. N. Thompson, and H. F. Hintz. 2001. Role of dietary carbohydrate and fat in horses with equine polysaccharide storage myopathy. J. Am. Vet. Med. Assoc. 219:1537-1544.

Welch, S., S. S. P. Gebhart, R. N. Bergman and L. S. Phillips. 1990. Minimal model analysis of intravenous glucose tolerance test-derived insulin sensitivity in diabetic subjects. J. Clin. Endocrinol. & Metab. 71:1508-1518.

Williams, C., D. S. Kronfeld, W. B. Staniar, and P. A. Harris. 2001. Plasma glucose and insulin responses of Thoroughbred mares fed a meal high in starch and sugar or fat and fiber. J. Anim. Sci. 79:2196-2201.

Wolever, T. M. S., and C. Mehling. 2002. High-carbohydrate-low-glycemic indec dietary advice improves glucose disposition index in subjects with impaired glucose tolerance. Br. J. Nutr. 87:477-487.

Yalow, R. S., S. J. Goldsmith and S. A. Berson. 1969. Influence of physiologic fluctuations in plasma growth hormone on glucose tolerance. Diabetes 18:402-408.

Chapter 2

Growth Hormone and Insulin-like Growth Factor-I Concentrations in Growing Thoroughbreds Adapted to Supplements Rich in Sugar and Starch or Fat and Fiber

ABSTRACT: Diets rich in hydrolyzable carbohydrates induce a hyperglycemic/insulinemic response and may increase metabolic disorders associated with some types of laminitis, hyperlipidemia, exertional rhabdomyolysis and osteochondrosis in horses. This study revisited samples from a frequently sampled glucose tolerance test to determine the effect of diet on the counter-regulation of hyperglycemia by growth hormone (GH) and insulin-like growth factor-I (IGF-I). Twelve Thoroughbred foals were raised on pasture and supplemented twice daily with a feed high in either sugar and starch (SS) or fat and fiber (FF). As weanlings (age 199 ± 19 d, weight 274 ± 18 kg), the subjects underwent a modified frequent sampling intravenous glucose tolerance test during which they remained in stalls and had access to grass hay and water ad libitum. Samples were collected at -60, -45, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60 , 70 , 80, 90, 100, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min with a glucose bolus of 300 mg/kg BW at 0 min and an insulin bolus of 1.5 mU/kg BW at 20 min. Plasma was analyzed for glucose, insulin, growth hormone and insulin-like growth factor-I (IGF-I) concentrations. Diet groups were compared using the non-parametric Kruskal-Wallis test. Time interactions for IGF-I were compared using a mixed model with repeated effects. Rank-ordered linear regression was used for correlations. Growth hormone concentrations were increased from 19 to 180 min after the glucose dose ($P < 0.05$). The area under the GH curve from 0 to 360 minutes tended to be inversely correlated to SI (r = 0.55; P = 0.08). Basal IGF-I was higher ($P = 0.006$) in the SS group compared to the FF group. Concentrations of IGF-I increased with time ($P = 0.002$) in the SS group. The change in IGF-I concentration from baseline to the end of the study was positively correlated (r = 0.68; $P = 0.02$) to the area under the insulin curve from 0 to 80 min. Basal IGF-I was inversely correlated to SI (r = 0.52; $P = 0.10$). These results suggest that weanlings adapted to meals high in glucose equivalents have higher

IGF-I secretion as compared to weanlings adapted to a fat and fiber supplement. This deviation may be associated with insulin resistance, metabolic dysfunction and osteochondrosis in horses fed grain diets.

Keywords: horse, diet, insulin sensitivity, growth hormone, insulin, insulin-like growth factor-I

Introduction

Hyperglycemic/hyperinsulinemic responses are exaggerated following grain meals rich in hydrolyzable carbohydrates like sugars and starch (SS) as compared to meals of hay or supplements rich in fat and fiber (FF) (Stull and Rodiek, 1988; Williams et al., 2001). Deviation from the nutritional signals and metabolic response elicited by a forage diet suggests that the grain meal could impact counter-regulatory hormone responses, further contributing to metabolic dysfunction including conditions of hyperlipidemia (Jeffcott and Field, 1985: Jeffcott et al., 1986) and certain forms of laminitis (Pass et al. 1998), exertional rhabdomyolysis (Valentine et al., 2001) and osteochrondrosis (Ralston, 1996).

This study compares the response of GH and IGF-I to glucose and insulin challenges in Thoroughbred weanlings raised on pasture and supplemented with twice daily meals rich in sugar and starch (SS) or fat and fiber (FF). It was conducted simultaneously with an application of the minimal model of glucose and insulin dynamics (Treiber, 2003). Repeated hyperglycemic/insulinemic meal responses were expected to hyper-stimulate counter-regulatory hormones, therefore foals adapted to the SS supplement were expected to have an exaggerated GH response and higher IGF-I concentrations.

Materials and Methods

Twelve Thoroughbred foals were raised on pasture and adapted to twice daily meals of sugar and starch (n=6) or fat and fiber (n=6) as described in the previous chapter. As weanlings, each horse underwent a modified frequently sampled intravenous glucose tolerance test (FSIGT) for comparison of glucose and insulin dynamics by the minimal model (see Chapter 1).

Growth hormone and IGF-I were analyzed for samples collected during the FSIGT by double-antibody RIAs as previously described (Staniar, 2002). Growth hormone was analyzed at sampling points 0, 19, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300 and 360 which represent the time in minutes since the glucose bolus (300 mg/kg at 0 min). Insulin-like growth factor-I was

analyzed for sampling points 0, 60, 120, 180, 240, 300, and 360. Insulin-like growth factor-I was extracted with acid-ethanol to remove binding proteins. Interrassay CV was 6% for GH and 13% for IGF-I.

Basal GH was estimated for each horse as the mean of values within the lowest quartile of all sampling points from 0 to 360 min. This estimation takes into account a higher percentage of the data points than previous studies, which had more frequent sampling (Veldhuis et al., 1995; Friend et al., 1996). Outliers were determined by the Z-test. One horse was determined to be an outlier based on its basal GH levels ($P = 0.02$) and was removed from analysis. One horse in the FF group was determined to be an outlier for IGF-I ($P = 0.007$) and dropped from analysis of individual sampling points.

Statistics

Feed groups were compared by the non-parametric Kruskal-Wallis analysis of variance (STATA 8, 2003). The area under the insulin curve was determined by trapezoid approximation. Time interactions for IGF-I were compared using a mixed model with repeated effects. Robust linear regression was used for correlations.

Results

The diet groups were similar in body weight with a median of 273 kg and a range of 242 to 318 kg ($P = 0.75$). On the day of testing the median weanling age was 196 d with a range from 177 to 235 d. The horses had been weaned a median of 30 d prior with a range from 25 to 36 days. All weanlings were considered to be in good health and had body condition scores of 5 or 6.

Characteristics of the GH curves between diet groups are compared in Table 1. Growth hormone concentrations did not differ ($P > 0.05$) by diet for any sampling point within the 180 min immediately following the glucose dose. The patten of GH concentration for all 12 weanlings across the 360 min of the study is shown in Figure 1. The area under the GH curve from 0 to 360 min tended to an inverse correlation with SI ($r = 0.55$; $P = 0.08$).

Plasma concentrations of IGF-I are shown in Figure 2. Basal IGF-I is compared between

diet groups in Table 1. Concentrations of IGF-I remained constant ($P = 0.75$) throughout the study in the FF group, but increased 18% ($P = 0.002$) in the SS group following the glucose challenge (Figure 2).

Total area under the insulin curve was 27% greater from 0 to 80 min in SS weanlings compared to FF weanlings (Figure 3). The increase in IGF-I from baseline to the end of the study was positively correlated ($r = 0.68$; $P = 0.02$) to insulin AUC from 0 to 80 min. Basal IGF-I tended to be correlated to SI ($r = 0.52$; $P = 0.10$).

Table 1. Properties of somatotropic hormones following a glucose challenge (300 mg/kg) in Thoroughbred weanlings adapted to the SS or FF diet. Data are summarized by median and range.

	SS Median (range)	**FF Median (range)**	***P*-value**
Basal GH (ng/mL)	0.259 (0.169 to 0.391)	0.413 (0.229 to 0.557)	0.068
Maximum Peak GH (ng/mL)	8.13 (2.41 to 12.95)	6.77 (1.80 to 11.10)	0.27
Time of Maximum Peak (min)	60 (19 to 120)	90 (30 to 180)	0.58
GH AUC (0-180 min) (ng/mL·180 min)	556 (122 to 670)	522 (90 to 871)	0.86
GH AUC (0-360 min) (ng/mL·360 min)	613 (337 to 1284)	722 (522 to 1196)	0.47
Basal IGF-I (ng/mL)	187.8 (167.8 to 195.8)	146.4 (110.7 to 156.8)	0.006
Insulin AUC (0-80 min) (mU/L·80 min)	1400 (1069 to 2813)	1126 (530 to 1470)	0.055
$SI \times 10^{4\dagger}$ (min^{-1} per mU/L)	2.33 (1.76 to 2.78)	3.03 (2.76 to 5.94)	0.007

† Treiber, 2003

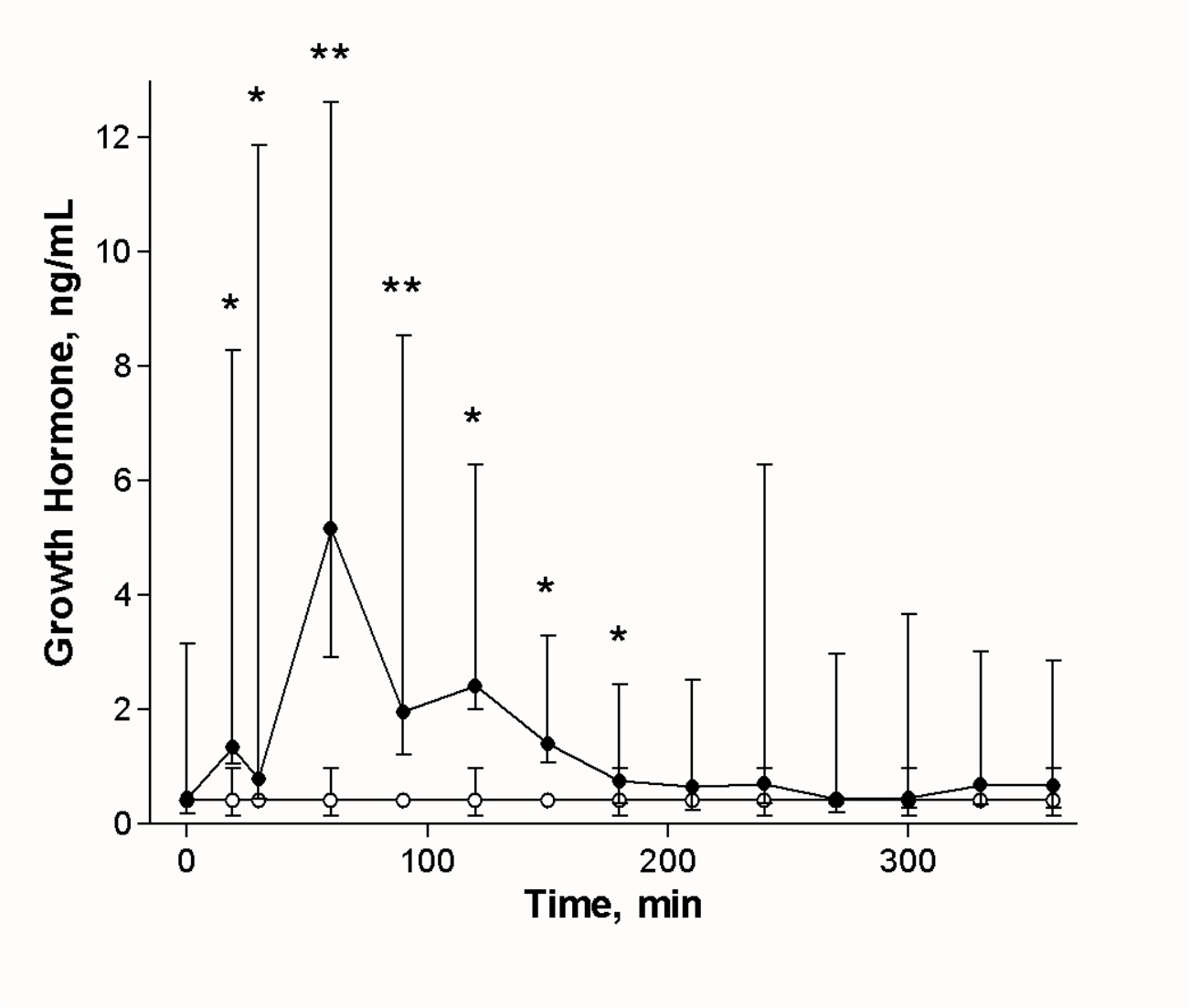

Figure 1. Median plasma growth hormone concentrations (●) of 12 Thoroughbred weanlings from 0 to 360 min following an i.v. glucose dose (300 mg/kg). Error bars represent the interquartile range of GH concentrations at each sampling point. Basal GH is indicated (○) with error bars representing the interquartile range for basal GH levels. Basal GH was calculated as the median of GH concentrations below the 25th percentile for each horse. Growth hormone concentrations at each sampling point is compared to basal concentration with significantly higher values indicated by * when $P < 0.05$ and ** when $P < 0.01$.

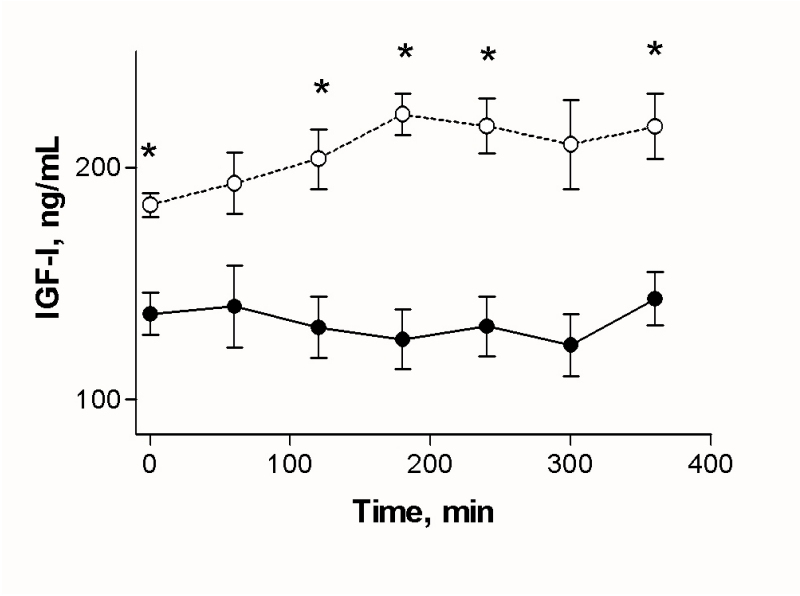

Figure 2. Plasma IGF-I concentrations of Thoroughbred weanlings adapted to a diet high in hydrolyzable carbohydrates or fat and fiber. Data are represented by mean and SE (* indicates a significant difference where $P < 0.05$).

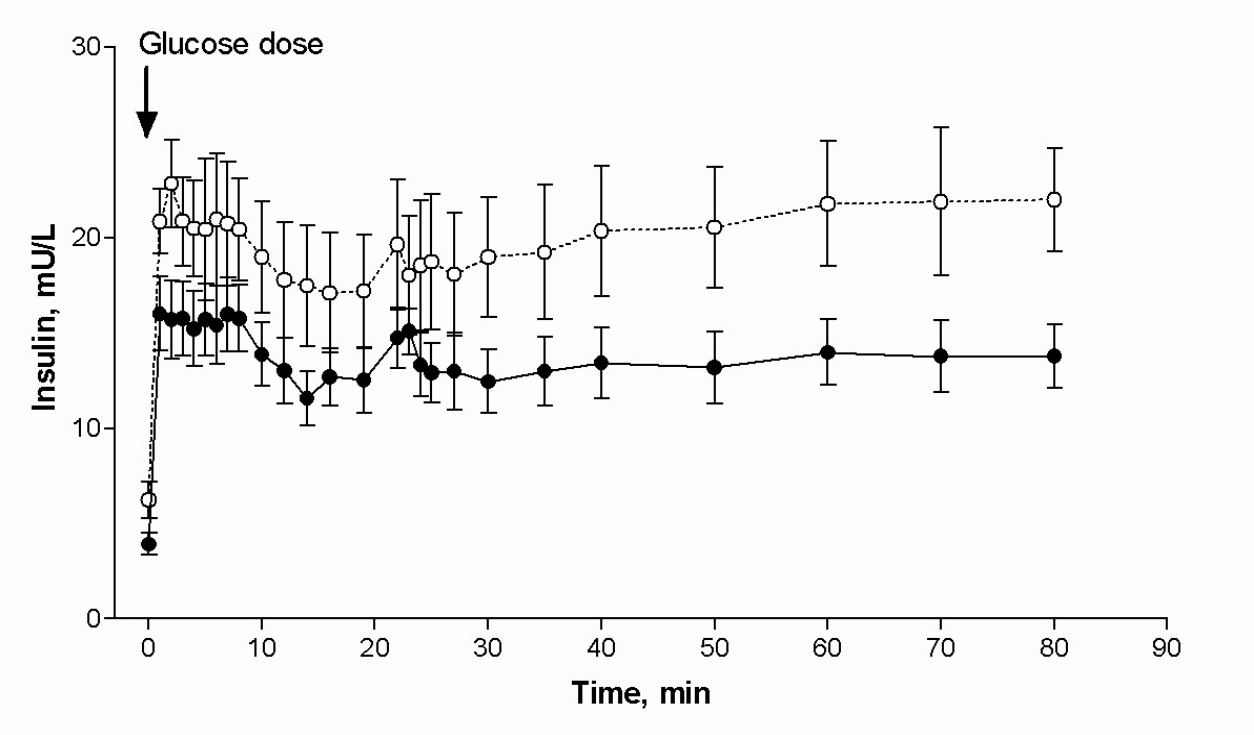

Figure 3. Plasma insulin concentrations from 0 to 80 min after an i.v. glucose bolus (300 mg/kg) for Thoroughbred weanlings adapted to a diet high in hydrolyzable carbohydrates (○) or fat and fiber (●). Data are represented by mean and SE.

Discussion

These results show that Thoroughbred weanlings adapted to a supplement rich in hydrolyzable carbohydrates have higher circulating IGF-I than weanlings adapted to a forage-like diet and that rapid clearance of glucose from the plasma is associated with a peak in GH secretion.

The pulsatile nature of GH release from the anterior pituitary makes the hormone difficult to interpret statistically, particularly when sampling is infrequent. The differences in IGF-I observed in weanlings in this study may best indicate long-term GH patterns as IGF-I is regulated by GH. Growth hormone has been shown to directly stimulate IGF-I release in rat hepatocytes (Johnson et al., 1989). Twenty-four hour GH secretion has been correlated to IGF-I in humans (Blum et al., 1992) and administration of recombinant bovine growth hormone has led to proportional increases of IGF-I in Thoroughbred geldings (Champion et al., 2000). The higher IGF-I concentrations observed in the SS weanlings in this study may therefore indicate increased GH secretion in these weanlings.

Higher basal plasma insulin concentrations could also contribute to the higher plasma IGF-I concentrations observed in SS weanlings. As previously published, basal glucose and glucose clearance did not differ between diet groups, but insulin concentrations after a glucose challenge averaged 41% higher in the SS group compared to the FF group (Treiber, 2003).. Insulin has been shown to stimulate IGF-I mRNA release in cultured rat hepatocytes (Phillips et al., 1991). Insulin may reverse a post-GH-receptor defect, mediating IGF-I indirectly through the GH pathway by increasing the potency of GH to stimulate IGF-I release (Bereket et al., 1994). However, insulin-mediated IGF-I increase has been shown to occur prior to changes in GH, indicating a possible direct effect of insulin on hepatic IGF-I release (Bereket et al., 1994).

Insulin response to the glucose bolus was also greater in the SS group and may have stimulated the transient increase in IGF-I levels observed throughout the FSIGT in this study. Accordingly, the increase in plasma IGF-I during the study was proportional to the insulin AUC within the first 80 min following the glucose dose.

The GH patterns in this study showed an increase in GH following an intravenous glucose

bolus. This GH peak may be a response to the sudden decline in plasma glucose concentration as glucose was cleared into the cells (Roth et al., 1963; Sharp et al., 1987). Horses adapted to a meal high in glucose equivalents, such as the SS meal, experience similar dramatic changes in plasma glucose concentrations (Williams et al., 2001), and may therefore exhibit comparable GH peaks following each meal. Repeated GH secretion following each SS meal could be responsible for both increased IGF-I plasma concentrations and the reduced insulin sensitivity observed in SS adapted horses compared to FF-adapted horses (Treiber, 2003).

Basal growth hormone concentrations in the plasma has not previously been shown to be associated with nutritional status (Breier et al., 1986; Thissen et al., 1994). The tendency for basal growth hormone to differ in weanlings adapted to different diets in this study may be associated with gender differences, although the subjects were prepubescent and colts were castrated at three weeks of age. Gender dimorphism of basal GH secretion has been previously reported in the horse and other species (Thompson et al., 1992; Veldhuis et al., 1995) and are associated with different growth patterns in males and females (Tannenbaum, 1990). At the same time, nutritional signals have been shown to alter the pulsatile pattern of GH. This capacity to alter patterns of somatostatin and growth hormone releasing hormone which govern GH release from the anterior pituitary might extend to alter chronic basal GH levels (Tannenbaum, 1990). Low basal GH levels and frequent GH peaks have been associated with rapid growth (Tannenbaum, 1990) and could contribute to developmental problems like osteochondrosis.

The association between GH secretion over the 360 minutes of the study and the insulin sensitivity index, SI, may be indicative of a GH effect on tissue responsiveness to insulin. The SI is a parameter of the Minimal Model which describes the efficiency of insulin to affect the tissue and accelerate glucose uptake into the cells (Caumo et al., 2000). The SI for the weanlings in this study was determined in a previous study (Treiber, 2003).

Chronically raised GH has been shown to decrease insulin sensitivity and impart diabetogenic conditions in many species. Hyperglycemia, glycosuria and polyuria were attributed to GH injections in partially pancreatized dogs, cats and frogs (Houssay and Anderson, 1949). In normal dogs, daily GH treatment decreased insulin-mediated glucose uptake during insulin infusion (Bishop et al., 1966). Humans showed impaired glucose tolerance in oral tests performed

2-3 hrs after a rise in GH (Yalow et al., 1969). The degree of glucose intolerance was relative to the GH peak amplitude, which was greatest following a meal high in hydrolyzable carbohydrates.

During GH infusions at physiological levels and in cultured adipocyte cells, insulin resistance has been shown to increase, while maintaining normal insulin binding to the insulin receptor (MacGorman et al., 1981; Bratusch-Marrain et al., 1982; Foster et al., 1988). More recently, insulin resistance in rats treated with GH has been shown to result from decreased GLUT-1 in adipocytes and decreased phosphorylation of the insulin receptor by tyrosine kinase (Smith et al., 1997; Yakar et al., 2001). Growth hormone may therefore prevent activation of insulin-mediated glucose uptake by the bound receptor, leading to insulin resistance.

The somatotropic axis is closely tied to metabolic function and energy regulation. Feeds high in glucose equivalents are rapidly digested and absorbed, providing a sudden increase of plasma glucose which rapidly declines as the glucose is cleared into the cells (Stull and Rodiek, 1988; Williams et al., 2001). This fluctuation of nutritional signals lead to a cascade of regulatory and counter-regulatory hormone responses which may be responsible for altered GH and IGF-I levels in many species (Thissen et al., 1994), including grain-fed horses (Staniar, 2002). Insulin-like growth factor-I may be the key to the association between glucose intolerance and OCD (Ralston, 1996). Increased IGF-I could induce excess cartilage proliferation while suppressing chondrocyte maturation into bone, leading to weaknesses which could become lesions when exposed to physical stress (Henson et al., 1997).

This study shows that IGF-I is higher in Thoroughbred weanlings adapted to a diet rich in hydrolyzable carbohydrates. The increase in IGF-I concentrations observed in SS fed weanlings may indicate increased GH secretion. This study also shows that a peak in GH is associated with a rapid fall in plasma glucose. The rapid fall in glucose following a rapidly digested grain meal may therefore regularly stimulate GH secretion. This increased GH secretion could be responsible for lower insulin resistance observed in Thoroughbred weanlings adapted to the SS. Grain meals high in hydrolyzable carbohydrates may therefore contribute to metabolic and growth problems in young horse.

Implications

Growing horses fed a diet high in sugar and starch may have increased growth hormone secretion, increasing the risk of insulin resistance, metabolic dysfunction and osteochondrosis. Developing feeds that replace sugars and starches with fat and fiber may help to reduce the occurrence of endocrine and metabolic disorders in equines.

Literature Cited

Bereket, A., C. H. Lang, S. L. Blethen, M. C. Gelato, J. Fan, R. A. Frost and T. A. Wilson. 1994. Effect of insulin on the insulin-like growth factor system in children with new-onset insulin-dependent diabetes mellitus. J. Clin. Endocinol. & Metab. 80:1312-1317.

Bishop, J. S. R, Steele, N. Altszuler, I. Rathgeb, C. Bjerknes and R. C. Bodo. 1967. Diminished responsiveness to insulin in the growth hormone-treated normal dog. Am. J. Physiol. 212:272-278.

Blum, W. F., K. Albertsson-Wikland, S. Rosburg and M. B. Ranke. 1992. Serum levels of insulin-like growth factor-I (IGF-I) and IGF binding protein 3 reflect spontaneous growth hormone secretion. J. Clin. Endocrinol. Metab. 76:1610-1616.

Bratusch-Marrain, P. R., D. Smith and R. A. DeFronzo. 1981. The effect of growth hormone on glucose metabolism and insulin secretion in man. J. Clin. Endocrinol. Metab. 55:973-982.

Breier, B. H., J. J. Bass, J. H. Butler and P. D. Gluckman. 1986. The somatotrophic axis in young steers: influence of nutritional status on pulsatile release of growth hormone and circulating concentrations of insulin-like growth factor-I. J. Endocr. 111:209-215.

Brismar, K, E. Fernqvist-Forbes, J. Wahren and K. Hall. 1994. Effect of insulin on the hepatic production of insulin-like growth factor-binding protein-1 (IGFBP-1), IGFBP-3, and IGF-I in insulin-dependent diabetes. J. Clin. Endocrinol. & Metab. 79:872-878.

Caumo, A., R. N. Bergman, and C. Cobelli. 2000. Insulin sensitivity from meal tolerance tests in normal subjects: a minimal model index. J. Clin. Endocrin. Metab. 85:4396-4402.

Champion, Z. J., E. A. James, M.H. Vicker, B. H. Breier and P. J. Casey. 2000. The effects of bovine recombinant growth hormone administration on insulin-like growth factor-I and the haemopoietic system in Thoroughbred geldings. The Veterinary Journal 160:147-152.

Dimitriades, G., M. Parry-Billings, S. Bevan, D. Dunger, T. Piva, U. Krause, G. Wegener and E. A. Newsholme. 1992. Effects of insulin-like growth factor I on the rates of glucose transport and utilization in rat skeletal muscle in vitro. Biochem. J. 285:269-274.

Foster, C. M., P. M. Hale, H. Jing and J. Schwartz. 1988. Effects of human growth hormone on insulin-stimulated glucose metabolism in 3T3-F442A adipocytes. Endocrinology 123:1082-1088.

Freestone, J. F., K. J. Wolfsheimer, S. G. Kamerling, G. Church, J. Hamra, and C. Bagwell. 1991. Exercise induced hormonal and metabolic changes in Thoroughbred horses: effects of conditioning and acepromazine. Equine Vet. J. 23:219-223.

Friend, K. A. Iranmanesh and J. D. Veldhuis. 1996. The orderliness of the growth hormone (GH) release process and the mean mass of GH secreted per burst are highly conserved in individual men on successive days. J. Clin. Endocrinol. Metab. 81: 3746-3753.

Henson, F. M. D., C. Davenport, L. Butler, I. Moran, W. D. Shingleton, L. B. Jeffcott and P. N. Schofield. 1997. Effects of insulin and insulin-like growth factors I and II on the growth of equine fetal and neonatal chondrocytes. Equine Vet. J. 29:441-447.

Henneke, D. R., G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet. J. 15:371-372.

Hoffman, R. M., R. C. Boston, D. Stefanovski, D. S. Kronfeld, and P. A. Harris. 2003. Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. J. Anim. Sci. 81:2333-2342.

Houssay, B. A. and E. Anderson. 1949. Diabetologic action of purified anterior pituitary hormones. Endrocrinology 45:627-629.

Jeffcott, L. B. and J. R. Field. 1985. Current concepts of hyperlipaemia in horses and ponies. Vet Rec. 116: 461-466.

Jeffcott, L. B., J. R. Field, J. G. McLean, and K.O'Dea. 1986. Glucose tolerance and insulin sensitivity in ponies and Standardbred horses. Equine Vet. J.18:97-101.

Johnson, T. R., B. K. Blossey, C. W. Denko and J. Ilan. 1989. Expression of insulin-like growth factor-I in cultured rat hepatocytes: effects of insulin and growth hormone. Molecular Endocrinology 3:580-587.

MacGorman, L. R., R. A. Rizza and J. E. Gerich. 1981. Physiological concentrations of growth hormone exert insulin-like and insulin antagonistic efffects on both hepatic and extrahepatic tissues in man. J. Clin. Endocrinol. Metab. 53:556-559.

NRC. 1989. Pages 39-48 in Nutrient Requirements of Horses. 5th rev. ed. Natl. Acad. Press, Washington, DC.

Pass, M. A., S. Pollitt, and C. C. Pollit. 1998. Decreased glucose metabolism causes separation of hoof lamellae *in vitro*: a trigger for laminitis? Equine Vet. J. 26:133-138.

Phillips, L. S., S. Goldstein and C. I. Pao. 1991. Nutrition and Somatomedin: XXVI. Molecular regulation of IGF-I by insulin in cultured rat hepatocytes. Diabetes 40:1525-1531.

Ralston, S. L. 1996. Hyperglycemia/hyperinsulinemia after feeding a meal of grain to young horses with osteochondritis dissecans (OCD) lesion. Pferdeheilkunde 12:320-322.

Roth, J., S. M. Glick, R. S. Yalow and S. A. Berson. 1963. Secretion of human growth hormone: physiologic and experimental modification. Metabolism. 12:577-579.

Sharp, P. S., V. Mohan, F. Maneschi, F. Vitelli, H. R. Cloke, J. M. Burrin and E. M. Kohner. 1987. Changes in plasma growth hormone in diabetic and nondiabetic subjects during the glucose clamp. Metabolism 36:71-75.

Smith, T. R., J. S. Elmendorf, T. S. David and J. Turinsky. 1997. Growth hormone-induced insulin resistance role of the insulin receptor, IRS-1, GLUT-1, and GLUT-4. Am. J. Physiol. 272:E1071-E1079.

Staniar, W. B. 2002. Growth and somatotropic axis in young Thoroughbreds. Ph.D. Diss., Virginia Polytechnic and State Univ., Blacksburg.

Stull, C. L., A. V. Rodiek. 1988. Responses of blood glucose, insulin, and cortisol concentrations to common equine diets. J. Nutr. 118: 206-213.

Tannenbaum, G. S. 1990. Interrelationship of somatostation and growth hormone-releasing hormone in the genesis of the rhythmic secretion of growth hormone. Acta Paediatr. Scand. Suppl. 367: 76-80.

Thissen, J. P., J. M. Ketelslegers and L. E. Underwood. 1994. Nutritional regulation of the insulin-lie growth factors. Endocrine Reviews 15:80-101.

Thompson, D. L., M. S. Rahmanian, C. L. DePew, D. W. Burleigh, C. J. DeSouza and D. R. Colborn. 1992. Growth hormone in mares and stallions: pulsatile secretion, response to growth hormone-releasing hormone, and effects of exercise, sexual stimulation and pharmocological agents. J. Anim. Sci. 70:1201-1207.

Treiber, K. H. 2003. Glucose metabolism in Thoroughbred weanlings: Regulation by insulin, growth hormone and insulin-like growth factor-I. M.S. Thesis, Virginia Polytechnic Institute and State Univ. Blacksburg.

Valentine, B. A., R. J. van Saun, K. N. Thompson, and H. F. Hintz. 2001. Role of dietary carbohydrate and fat in horses with equine polysaccharide storage myopathy. J. Am. Vet. Med. Assoc. 219:1537-1544.

Velduis, J. D., A. Y. Liem, S. South, A. Weltman, J. Weltman, D. A. Clemmons, R. Abbot, T. Mulligan, M. L. Johnson, S. Pincus, M. Straume and A. Iranmesh. 1995. Differential impact of age, sex steroid hormones and obesity on basal versus pulsatile growth hormone secretion in men as assessed in an ultrasensitive chemiluminescence assay. J. Clin. Endocrinol. Metab. 80: 3209-3222.

Williams, C. A., D. S. Kronfeld, W. B. Staniar, and P. A. Harris. 2001. Plasma glucose and

insulin responses of Thoroughbred mares fed a meal high in starch and sugar or fat and fiber. J. Anim. Sci. 79:2196-2201.

Yakar, S., J. Liu, A. M. Fernandez, Y. Wu, A. V. Schally, J. Frystyk, S. D. Chernausek, W. Mejia and D. Le Roith. 2001. Liver-specific IGF-I gene deletion leads to muscle insulin insensitivity. Diabetes 50:1110-1118.

Yalow, R. S., S. J. Goldsmith and S. A. Berson. 1969. Influence of physiologic fluctuations in plasma growth hormone on glucose tolerance. Diabetes 18:402-408.

Table 1. Partial proximate analysis of supplements, pasture and hay[2]. Data are summarized as means ± SE.

Nutrient	SS(n=3)	FF(n=3)	Pasture (n=6)	Hay[1] (n=2)
DM%	89.6 ± 2.1	91.3 ± 0.7	30.6 ± 2.1	76.8 ± 12.2
DE, MCAL/kg	3.28 ± 0.11	2.82 ± 0.40	2.40 ± 0.07	2.20 ± 0.03
CP, %	14.8 ± 0.6	14.4 ± 1.4	17.7 ± 0.9	16.6 ± 0.1
ADF, %	11.5 ± 0.8[a]	29.5 ± 0.6[b]	33.0 ± 1.1	38.0 ± 1.15
NDF, %	21.3 ± 1.0[a]	44.0 ± 1.0[b]	57.6 ±1.9	56.5 ± 2.3
NFC[4], %	54.1 ± 0.7[a]	25.4 ± 2.1[b]	17.6 ± 1.1	21.5 ± 2.4
NSC[3], %	49.0 ± 1.7[a]	12.3 ± 1.6[b]	9.4 ± 1.5	8.9 ± 1.0
Starch, %	40.4 ±2.1[a]	3.9 ± 1.2[b]	1.2 ± 0.2	2.8 ± 0.4
Sugar, %	8.5 ± 0.4	8.4 ± 0.4	8.2 ± 1.4	6.2 ± 0.7
Fat, %	3.0 ± 0.1[c]	9.7 ± 0.9[d]	3.6 ± 0.1	2.0 ± 0.3
Ash, %	7.66 ± 0.66	7.94 ± 0.21	8.9 ± 0.32	8.30 ± 0.27
Ca, %	1.34 ± 0.19	1.54 ± 0.09	0.54 ± 0.01	1.00 ± 0.06
P, %	0.64 ±0.02	0.67 ± 0.16	0.34 ± 0.03	0.30 ± 0.03
Mg, %	0.18 ± 0.02	0.19 ± 0.01	0.35 ± 0.004	0.20 ± 0.02
K, %	1.17 ± 0.06[c]	1.34 ± 0.02[d]	2.37 ± 0.15	2.50 ± 0.13
Na, %	0.224 ± 0.043	0.290 ± 0.082	0.023 ± 0.001	0.012 ± 0.001

[1] Foals had access to hay ad libitum while in the stalls on the day of sampling
[2] Dairy One DHIA Forage Testing Laboratory, Ithaca, NY
[3] Non-structural carbohydrates: NSC = suger + starch
[4] Non-fiber carbohydrates: NFC = 100 - water - ash - CP - fat - NDF.
[a,b] Supplement means differ ($P < 0.001$)
[c,d] Supplement means differ ($P < 0.05$)

Horse	Diet	Sex	Days Post Weaning	Age (d)	BW (kg)	Sire	Day	BC	SI ×10^4 (min^{-1} per mU/L)	Sg ×10^3 (min^{-1})	AIRg ($mU \cdot min \cdot L^{-1}$)	DI ×10^4	Basal Gluc: Basal Ins
18	FF	M	30	203	268	BB	3	5	2.95	8.52	113	334	24.48
19	FF	F	32	235	283	CP	4	5	2.76	7.43	173	477	27.41
26	FF	M	27	206	242	CP	1	5	5.94	7.77	60	359	64.94
93	FF	F	27	177	280	CP	1	5	3.10	6.15	146	451	26.47
114	FF	M	32	198	284	BB	4	5	3.93	7.62	103	404	37.93
119	FF	F	25	179	271	CP	2	6	2.84	7.36	112	317	32.69
12	SS	F	36	233	318	CP	4	5	2.54	7.04	241	612	15.12
97	SS	M	30	193	270	CP	3	6	2.51	6.03	123	308	23.88
110	SS	F	29	183	275	BB	2	5	2.78	7.82	164	457	25.82
128	SS	F	25	192	257	CP	2	6	1.94	10.10	111	216	14.92
129	SS	M	27	203	278	CP	1	5	2.15	6.32	114	245	27.45
134	SS	F	30	190	265	CP	3	5	1.76	8.20	179	315	24.16
Mean	**-**	**-**	**29.17**	**199.3**	**274.3**	**-**	**-**	**5.3**	**2.93**	**7.53**	**136.6**	**374.6**	**28.77**
SEM	-	-	0.93	5.4	5.2	-	-	0.1	0.32	0.33	13.6	32.0	3.76
Median	-	-	29.50	195.5	273.0	-	-	5.0	2.77	7.53	118.5	346.5	26.15
FF Mean	**-**	**-**	**28.83**	**199.7**	**271.3**	**-**	**-**	**5.2**	**3.59**	**7.48**	**117.8**	**390.3**	**35.65**
FF SEM	-	-	1.19	8.7	6.4	-	-	0.2	0.50	0.31	15.8	26.4	6.19
SS Mean	**-**	**-**	**29.50**	**199.0**	**277.2**	**-**	**-**	**5.3**	**2.28**	**7.59**	**155.3**	**358.8**	**21.89**
SS SEM	-	-	1.52	7.3	8.7	-	-	0.2	0.16	0.61	20.6	61.0	2.24
FF Median	-	-	28.50	200.5	275.5	-	-	5.0	3.03	7.53	112.5	381.5	30.05
SS Median	-	-	29.50	192.5	272.5	-	-	5.0	2.33	7.43	143.5	311.5	24.02
P - value FF vs. SS	**-**	**-**	**0.94**	**0.81**	**0.75**	**-**	**1.00**	**0.52**	**0.0065**	**1.00**	**0.15**	**0.26**	**0.025**

Table 2. Population analysis and minimal model determinations for the 12 weanlings used in the study. Data used for Chapters 1 and 2.

SS Diet	Horse # / Glucose (mg/dL)									
Time	12	97	110	128	129	134	**Mean**	STDEV	SEM	Median
-60	156.83	119.19	123.44	113.34	123.32	122.28	-	-	-	-
-45	156.59	119.12	123.41	114.94	124.61	122.50	-	-	-	-
-5	158.53	.	.	.	.	.	-	-	-	-
0*	156.71	119.15	123.42	114.14	123.96	122.39	**126.73**	15.42	6.29	122.91
1	363.08	356.79	329.18	375.67	367.77	372.16	**360.77**	16.85	6.88	365.42
2	357.21	332.41	308.16	336.23	344.07	331.83	**334.98**	16.21	6.62	334.32
3	346.72	318.81	294.15	317.66	321.62	322.46	**320.24**	16.72	6.82	320.22
4	341.78	308.51	289.53	314.74	312.01	311.83	**313.06**	16.76	6.84	311.92
5	333.13	294.96	.	306.78	306.92	306.92	**309.89**	14.02	6.27	306.92
6	330.56	291.74	.	299.70	298.59	298.59	**303.64**	15.36	6.87	298.59
7	321.77	289.92	.	293.68	296.88	296.88	**298.96**	12.99	5.81	293.68
8	321.17	281.74	275.95	282.77	286.89	289.16	**289.61**	16.12	6.58	284.83
10	315.03	275.37	265.80	272.54	280.03	281.24	**281.67**	17.26	7.05	277.70
12	307.35	264.89	250.71	268.53	271.37	276.36	**273.20**	18.85	7.70	269.95
14	301.64	263.85	250.67	263.00	267.44	275.00	**270.27**	17.28	7.05	265.65
16	293.25	258.49	251.18	259.35	264.25	269.98	**266.08**	14.71	6.00	261.80
19	282.36	250.90	246.99	251.04	256.59	264.71	**258.76**	13.09	5.34	253.81
22	283.07	246.98	240.18	244.59	252.22	254.51	**253.59**	15.34	6.26	249.60
23	282.63	242.53	241.29	242.64	252.99	255.32	**252.90**	15.73	6.42	247.81
24	279.64	244.25	237.46	244.64	249.03	252.78	**251.30**	14.81	6.04	246.84
25	275.37	241.11	232.45	241.01	248.97	247.65	**247.76**	14.75	6.02	244.38
27	269.73	240.23	225.93	235.49	246.40	250.61	**244.73**	14.98	6.11	243.31
30	267.97	237.32	220.84	235.89	242.77	245.57	**241.72**	15.45	6.31	240.04
35	257.16	231.44	221.79	230.54	239.75	236.30	**236.16**	11.95	4.88	233.87
40	250.23	229.79	213.59	225.28	231.33	230.46	**230.11**	11.86	4.84	230.12
50	235.86	216.82	201.24	207.99	223.66	223.11	**218.11**	12.33	5.03	219.96
60	220.38	214.40	191.02	195.13	200.88	211.48	**205.55**	11.62	4.74	206.18
70	207.39	207.05	177.37	189.06	198.51	199.39	**196.46**	11.52	4.70	198.95
80	197.62	200.08	169.81	180.25	192.52	193.85	**189.02**	11.64	4.75	193.18
90	182.78	196.13	161.72	173.81	186.24	187.21	**181.31**	12.01	4.90	184.51
100	171.45	190.88	151.35	169.49	173.89	174.09	**171.86**	12.63	5.16	172.67
120	151.30	173.06	135.89	149.56	160.30	158.02	**154.69**	12.42	5.07	154.66
150	123.92	150.10	112.06	133.76	148.50	134.17	**133.75**	14.50	5.92	133.96
180	98.00	134.33	95.26	117.48	132.52	118.21	**115.96**	16.55	6.76	117.84
210	85.35	119.36	93.86	105.83	119.80	110.58	**105.79**	13.88	5.67	108.20
240	101.40	105.79	104.63	104.72	110.37	104.13	**105.17**	2.94	1.20	104.68
270	110.47	101.78	108.90	102.95	108.18	102.35	**105.77**	3.83	1.56	105.56
300	112.83	106.37	109.67	103.32	109.35	107.94	**108.25**	3.23	1.32	108.64
330	119.13	108.84	111.58	104.60	113.20	107.04	**110.73**	5.14	2.10	110.21
360	123.25	111.97	113.04	108.79	112.07	105.91	**112.50**	5.89	2.41	112.02

Table 3. Plasma glucose concentrations by time for individual weanlings adapted to the SS supplement. Times are relative to the administration of a glucose bolus (time = 0). The * indicates that the 0 time point was calculated as the average of the -60 min and -45 min (and -5 min for horse 12) basal samples. Data for Chapter 1.

FF Diet	Horse # / Glucose (mg/dL)									
Time	18	19	26	93	114	119	**Mean**	STDEV	SEM	Median
-60	125.10	124.65	115.10	145.53	113.65	120.33	-	-	-	-
-45	121.60	125.07	115.77	149.13	111.38	120.33	-	-	-	-
0*	123.35	124.86	115.43	147.33	112.51	120.33	**123.97**	12.36	5.05	121.84
1	331.33	363.60	312.82	353.15	318.64	352.56	**338.68**	20.72	8.46	341.94
2	316.17	325.44	301.03	332.31	296.77	330.46	**317.03**	15.18	6.20	320.80
3	305.96	309.24	294.37	323.00	287.37	321.75	**306.95**	14.31	5.84	307.60
4	299.31	291.37	286.47	318.16	281.49	310.71	**297.92**	14.28	5.83	295.34
5	288.04	283.71	282.79	312.94	273.30	304.06	**290.81**	14.79	6.04	285.88
6	280.52	279.44	278.12	309.27	268.84	298.85	**285.84**	15.08	6.15	279.98
7	278.38	276.68	279.24	304.87	262.99	296.58	**283.12**	15.09	6.16	278.81
8	272.44	272.90	269.53	300.36	259.49	295.52	**278.37**	15.98	6.53	272.67
10	267.75	265.43	265.98	298.58	249.74	285.36	**272.14**	17.19	7.02	266.86
12	261.22	255.57	255.73	290.94	245.76	282.37	**265.26**	17.51	7.15	258.47
14	255.47	257.09	256.36	247.42	240.79	274.78	**255.32**	11.46	4.68	255.91
16**	255.42	246.40	248.68	278.33	239.85	267.48	**256.02**	14.40	5.88	252.05
19**	250.92	244.42	244.67	275.83	234.56	257.93	**251.39**	14.26	5.82	247.80
22**	241.40	237.42	239.29	269.06	230.03	251.57	**244.79**	13.78	5.62	240.34
23**	245.09	236.80	238.83	273.50	228.82	262.70	**247.62**	17.04	6.96	241.96
24**	242.96	234.15	237.44	271.37	228.48	250.35	**244.12**	15.30	6.25	240.20
25**	244.22	232.49	234.48	265.81	228.15	251.18	**242.72**	14.09	5.75	239.35
27	242.88	229.59	232.23	262.92	225.27	243.04	**239.32**	13.61	5.55	237.56
30	235.61	228.71	226.55	258.81	221.99	247.90	**236.59**	14.13	5.77	232.16
35	230.70	220.38	223.64	251.33	219.43	237.58	**230.51**	12.30	5.02	227.17
40	220.80	211.19	216.27	244.61	211.70	232.29	**222.81**	13.19	5.39	218.54
50	216.58	204.99	206.71	236.69	199.85	224.24	**219.26**	18.08	7.38	215.47
60	208.78	195.46	204.69	225.82	195.21	215.38	**207.55**	11.86	4.84	206.74
70	204.70	187.24	193.60	217.67	188.87	207.76	**199.97**	12.02	4.91	199.15
80	196.69	181.69	185.70	211.92	180.40	202.84	**193.21**	12.71	5.19	191.19
90	189.07	177.01	180.54	202.19	170.59	192.73	**185.35**	11.51	4.70	184.80
100	183.37	165.91	172.71	195.52	164.07	185.60	**177.86**	12.34	5.04	178.04
120	169.11	152.03	159.16	177.43	153.24	172.41	**163.89**	10.58	4.32	164.13
150	151.99	128.47	136.79	154.00	133.63	148.97	**142.31**	10.70	4.37	142.88
180	139.25	110.99	119.82	135.38	118.05	130.85	**125.72**	11.07	4.52	125.33
210	124.62	101.37	110.15	124.09	108.31	120.52	**114.84**	9.59	3.91	115.33
240	116.03	97.44	103.18	114.91	102.34	106.25	**106.69**	7.37	3.01	104.71
270	111.06	99.87	105.15	110.41	99.04	105.75	**106.44**	4.54	2.03	105.75
300	105.41	109.39	102.42	112.36	96.21	107.40	**105.53**	5.69	2.32	106.40
330	111.49	112.02	105.93	108.82	97.21	110.57	**107.67**	5.58	2.28	109.69
360	116.16	114.63	113.14	121.11	100.32	115.73	**113.51**	7.00	2.86	115.18

Table 4. Plasma glucose concentrations by time for individual weanlings adapted to the FF supplement. Times are relative to the administration of a glucose bolus (time = 0). The * indicates that the 0 time point was calculated as the average of the -60 min and -45 min (and -5 min for horse 12) basal samples. Data for Chapter 1.

SS Diet	Horse # / Insulin (µU/mL)									
Time	12	97	110	128	129	134	**Mean**	STDEV	SEM	Median
-60	6.00	4.89	4.45	7.14	3.64	5.73	**-**	-	-	-
-45	10.36	5.09	5.11	8.16	5.40	4.40	**-**	-	-	-
-5	14.73	.	.	.	.	.	**-**	-	-	-
0*	10.36	4.99	4.78	7.65	4.52	5.07	**6.23**	2.33	0.95	5.03
1	27.39	22.27	19.01	18.93	15.24	22.15	**20.83**	4.12	1.68	20.58
2	32.44	21.45	22.40	21.53	15.05	24.05	**22.82**	5.62	2.30	21.96
3	30.82	18.20	20.16	18.70	14.07	23.09	**20.84**	5.70	2.33	19.43
4	31.75	19.38	19.26	17.26	13.66	21.45	**20.46**	6.12	2.50	19.32
5	34.65	18.61	.	15.13	14.17	19.48	**20.41**	8.27	3.70	18.61
6	34.45	18.75	.	18.21	14.44	18.82	**20.93**	7.77	3.47	18.75
7	33.18	19.92	.	16.92	14.39	19.16	**20.71**	7.30	3.26	19.16
8	33.21	18.65	19.46	16.22	14.71	20.19	**20.41**	6.60	2.70	19.05
10	32.82	15.34	19.39	15.58	12.56	18.02	**18.95**	7.19	2.94	16.80
12	32.53	16.16	15.12	13.92	11.96	16.84	**17.76**	7.44	3.04	15.64
14	32.93	14.57	15.85	13.20	12.25	15.91	**17.45**	7.72	3.15	15.21
16**	32.39	14.34	16.39	13.46	11.20	14.61	**17.07**	7.69	3.14	14.47
19**	31.34	15.02	16.21	15.07	9.93	14.96	**17.17**	7.31	2.99	15.04
22**	35.04	19.20	20.22	19.39	14.06	19.40	**19.62**	8.36	3.41	19.30
23**	33.25	16.60	16.99	16.39	13.17	15.73	**18.02**	7.63	3.11	16.06
24**	34.79	15.66	18.37	16.33	11.09	14.92	**18.52**	8.32	3.40	15.99
25**	36.10	15.23	18.86	14.43	12.66	16.15	**18.72**	8.68	3.54	15.69
27	33.59	15.98	17.57	14.76	11.38	15.06	**18.06**	7.88	3.22	15.52
30	34.23	16.02	18.64	16.16	12.72	15.95	**18.95**	7.72	3.15	16.09
35	36.20	15.05	20.32	15.13	13.39	15.23	**19.22**	8.64	3.53	15.18
40	35.89	17.22	23.92	14.16	14.73	16.07	**20.33**	8.39	3.43	16.65
50	35.12	17.35	22.39	17.11	13.05	18.02	**20.51**	7.75	3.16	17.68
60	36.84	18.35	23.67	18.78	13.69	19.15	**21.75**	8.04	3.28	18.97
70	40.23	19.91	21.98	18.29	12.93	17.79	**21.86**	9.49	3.87	19.10
80	35.35	19.65	20.10	19.74	17.58	19.33	**21.96**	6.62	2.70	19.70
90	33.80	18.94	17.87	17.91	15.36	17.38	**20.21**	6.76	2.76	17.89
100	29.37	19.58	15.40	17.51	14.35	14.71	**18.49**	5.68	2.32	16.46
120	22.88	20.47	11.56	16.96	11.90	12.52	**16.05**	4.83	1.97	14.74
150	17.14	15.39	11.25	15.23	11.97	10.82	**13.63**	2.62	1.07	13.60
180	12.39	10.58	5.87	13.37	7.11	7.61	**9.49**	3.06	1.25	9.10
210	6.36	8.99	2.15	11.03	9.04	5.35	**7.15**	3.19	1.30	7.68
240	4.51	10.81	2.71	6.89	7.63	5.76	**6.39**	2.79	1.14	6.33
270	2.73	7.27	2.24	6.78	6.22	2.97	**4.70**	2.29	0.93	4.60
300	5.71	8.04	3.94	6.17	3.51	3.12	**5.08**	1.90	0.77	4.82
330	4.97	6.98	3.96	4.56	2.57	1.53	**4.10**	1.91	0.78	4.26
360	7.27	6.18	2.56	5.91	2.39	2.03	**4.39**	2.31	0.94	4.24
AUC 80	**1984**	**999**	**1205**	**724**	**708**	**997**	**1103**	**471**	**192**	**998**

Table 5. Plasma insulin concentrations by time for individual weanlings adapted to the SS supplement. Times are relative to the administration of a glucose bolus (time = 0). The * indicates that time point 0 was derived from the average of the -60 min, -45 min and -5 min samples. The ** indicates sample points at times 16, 19, 22, 23, 24, and 25 min were corrected by averaging the difference between the first and second assay of these samples and adding this to the value of the second assay (corrected value = assay2 value + 2.265). Data for Chapter 1.

FF Diet	Horse # / Insulin (µU/mL)									
Time	18	19	26	93	114	119	**Mean**	STDEV	SEM	Median
-60	5.59	4.48	2.44	5.31	3.22	3.13	**-**	-	-	-
-45	4.49	4.64	1.11	5.82	2.71	4.23	**-**	-	-	-
-5	.	.	.	.	.	.	**-**	-	-	-.
0*	5.04	4.56	1.78	5.57	2.97	3.68	**3.93**	1.41	0.58	4.12
1	15.71	22.16	8.18	19.14	15.74	15.09	**16.00**	4.69	1.91	15.72
2	14.85	23.38	8.04	17.97	14.49	15.43	**15.69**	5.00	2.04	15.14
3	17.03	22.78	7.94	16.94	14.78	14.92	**15.73**	4.80	1.96	15.93
4	16.39	22.25	7.29	16.35	14.69	14.24	**15.20**	4.82	1.97	15.52
5	17.84	21.67	7.87	17.42	15.56	13.76	**15.69**	4.65	1.90	16.49
6	16.99	21.98	6.71	17.33	15.60	13.79	**15.40**	5.05	2.06	16.29
7	16.71	22.11	7.65	18.11	15.92	15.14	**15.94**	4.75	1.94	16.32
8	17.38	20.92	7.88	16.05	16.29	15.93	**15.74**	4.29	1.75	16.17
10	17.04	15.90	6.22	17.06	13.47	13.46	**13.86**	4.08	1.66	14.68
12	16.03	15.07	5.34	17.04	12.50	12.07	**13.01**	4.23	1.73	13.78
14	15.23	14.59	5.49	12.09	11.79	10.16	**11.56**	3.52	1.44	11.94
16**	16.76	15.33	6.89	14.78	12.07	10.26	**12.68**	3.68	1.50	13.43
19**	16.98	16.85	6.74	14.00	11.65	8.86	**12.51**	4.21	1.72	12.82
22**	18.63	17.96	8.56	15.79	15.69	11.79	**14.74**	3.86	1.58	15.74
23**	18.66	17.95	13.51	16.16	12.03	12.09	**15.07**	2.93	1.20	14.83
24**	16.67	16.64	6.60	16.43	12.53	10.95	**13.30**	4.08	1.67	14.48
25**	15.71	17.44	6.65	14.51	12.11	10.97	**12.90**	3.86	1.58	13.31
27	18.05	17.09	5.09	15.17	12.59	9.84	**12.97**	4.89	2.00	13.88
30	14.40	16.03	4.83	15.34	12.05	11.95	**12.43**	4.09	1.67	13.22
35	14.36	17.93	5.33	16.31	11.67	12.34	**12.99**	4.43	1.81	13.35
40	15.66	19.07	5.71	15.44	11.74	12.84	**13.41**	4.55	1.86	14.14
50	17.61	17.08	5.82	16.26	10.85	11.50	**13.19**	4.62	1.89	13.88
60	16.97	19.34	7.90	15.91	10.85	12.88	**13.98**	4.23	1.73	14.40
70	14.76	20.22	7.20	17.12	12.38	10.92	**13.77**	4.63	1.89	13.57
80	15.00	19.48	6.83	14.07	14.12	13.16	**13.78**	4.07	1.66	14.10
90	13.93	15.96	8.95	12.97	13.56	12.64	**13.00**	2.30	0.94	13.27
100	13.68	16.09	6.37	14.32	13.33	12.84	**12.77**	3.33	1.36	13.50
120	13.58	14.66	5.47	9.30	10.39	12.67	**11.01**	3.37	1.38	11.53
150	11.03	11.34	3.71	8.52	6.49	10.81	**8.65**	3.05	1.25	9.66
180	10.13	7.22	3.62	8.84	5.48	9.95	**7.54**	2.61	1.06	8.03
210	10.33	5.56	3.13	3.93	7.17	5.77	**5.98**	2.56	1.05	5.66
240	6.77	4.02	1.99	3.28	4.82	6.30	**4.53**	1.82	0.74	4.42
270	6.43	2.32	2.40	3.73	4.46	5.18	**4.09**	1.61	0.66	4.09
300	3.41	5.39	0.70	1.86	2.05	3.14	**2.76**	1.62	0.66	2.60
330	5.19	4.78	1.31	2.40	2.82	3.66	**3.36**	1.47	0.60	3.24
360	4.80	3.30	1.22	2.15	4.33	2.97	**3.13**	1.33	0.54	3.14
AUC 80	**887**	**1106**	**388**	**822**	**748**	**670**	**770**	**239**	**98**	**785**

Table 6. Plasma insulin concentrations by time for individual weanlings adapted to the SS supplement. Times are relative to the administration of a glucose bolus (time = 0). The * indicates that time point 0 was derived from the average of the -60 min, -45 min and -5 min samples. The ** indicates sample points at times 16, 19, 22, 23, 24, and 25 min were corrected by averaging the difference between the first and second assay of these samples and adding this to the value of the second assay (corrected value = assay2 value + 2.265). Data for Chapter 1.

Horse	AIRg	DI	SI	Sg	GB	IB	P(2)	P(3)	G(0)	GEZI	Beta-Cell function	Insulin Resistance	Rsquared
	[mu.l^-1.min]	[]	[(mu/l)^-1.min^-1]	[min^-1]	[mg/dl]	[mu/l]	[min^-1]	[(mu/l)^-1.min^-2]	[mg/dl]	[min^-1]	[mu/mM]	[mM.mu/l^2]	[%]
12	2.41E+02	6.12E+02	2.54	7.28E-03	1.23E+02	7.27	1.73E-02	4.40E-06	3.23E+02	5.43E-03	4.35E+01	2.21E+00	98.95
18	1.13E+02	3.34E+02	2.95	8.52E-03	1.16E+02	4.80	4.11E-03	1.21E-06	2.81E+02	7.10E-03	3.25E+01	1.38E+00	98.95
19	1.73E+02	4.76E+02	2.76	7.43E-03	1.15E+02	3.30	1.57E-02	4.32E-06	2.69E+02	6.53E-03	2.30E+01	9.34E-01	98.78
26	6.04E+01	3.59E+02	5.94	7.77E-03	1.13E+02	1.22	1.16E-02	6.88E-06	2.71E+02	7.04E-03	8.79E+00	3.42E-01	99.19
93	1.46E+02	4.52E+02	3.10	6.15E-03	1.21E+02	2.15	9.99E-03	3.10E-06	2.96E+02	5.49E-03	1.33E+01	6.44E-01	98.38
97	1.23E+02	3.08E+02	2.51	6.03E-03	1.12E+02	6.18	1.17E-02	2.94E-06	2.74E+02	4.48E-03	4.54E+01	1.71E+00	98.68
110	1.64E+02	4.65E+02	2.83	7.57E-03	1.13E+02	2.56	2.47E-02	6.99E-06	2.72E+02	6.85E-03	1.84E+01	7.15E-01	98.94
114	1.03E+02	4.08E+02	3.97	7.63E-03	1.00E+02	4.33	7.15E-03	2.84E-06	2.61E+02	5.91E-03	4.17E+01	1.07E+00	99.63
119	1.12E+02	3.17E+02	2.83	7.34E-03	1.16E+02	2.97	1.41E-02	4.00E-06	2.90E+02	6.50E-03	2.03E+01	8.50E-01	98.81
128	1.11E+02	2.16E+02	1.94	1.01E-02	1.09E+02	5.91	1.36E-02	2.63E-06	2.88E+02	8.97E-03	4.65E+01	1.59E+00	99.55
129	1.14E+02	2.53E+02	2.22	5.63E-03	1.12E+02	2.39	4.17E-01	9.25E-05	2.89E+02	5.10E-03	1.75E+01	6.60E-01	99.48
134	1.79E+02	3.14E+02	1.76	8.22E-03	1.06E+02	2.03	1.53E-02	2.70E-06	2.95E+02	7.87E-03	1.70E+01	5.30E-01	99.5

Table 7. Results from MinMod Millenium(Ver. 5.10, BeBos Assoc., 2001). Data for Chapter 1.

SS Diet	Horse # / IGF-I (ng/mL)								
Time	12	97	110	128	129	134	**Mean**	SEM	Median
0	194.62	185.95	169.40	189.70	195.80	167.75	**183.87**	5.05	187.82
60	209.94	193.28	235.92	191.51	190.61	137.53	**193.13**	13.18	192.40
120	182.49	249.50	235.93	189.45	194.17	170.43	**203.66**	12.89	191.81
180	205.33	232.18	261.05	206.15	224.31	207.99	**222.83**	8.86	216.15
240	190.57	269.13	223.81	215.79	216.42	190.87	**217.77**	11.75	216.11
300	206.19	293.58	178.71	213.99	212.22	154.99	**209.95**	19.17	209.21
360	223.00	268.56	242.06	196.80	202.24	173.23	**217.65**	13.98	212.62
Horse Mean	**201.74**	**241.74**	**220.98**	**200.48**	**205.11**	**171.83**	-	-	
SEM	5.52	16.47	13.90	4.66	5.20	9.35	-	-	
ΔIGF-I (0-360 min)	28.38	82.61	72.66	7.10	6.45	5.47	33.78	14.36	17.74
FF Diet									
Time	18	19	26	93	114	119	**Mean**	SEM	Median
0	110.65	156.81	150.82	120.52	146.45	248.06	**137.05**	9.05	146.45
60	113.69	180.69	148.12	87.38	170.86	242.96	**140.15**	17.51	148.12
120	107.97	158.87	144.69	91.70	152.34	239.06	**131.11**	13.22	144.69
180	123.56	125.01	105.01	102.67	173.77	238.73	**126.00**	12.79	123.56
240	123.30	144.96	136.21	87.94	165.66	222.79	**131.61**	12.91	136.21
300	96.08	142.94	136.09	87.66	155.38	297.71	**123.63**	13.39	136.09
360	118.37	169.19	113.71	153.20	162.94	217.95	**143.48**	11.51	153.20
Horse Mean	**113.37**	**154.07**	**133.52**	**104.44**	**161.06**	**243.90**	-	-	
SEM	3.95	7.49	7.18	10.06	4.10	10.65	-	-	
ΔIGF-I (0-360 min)	7.72	12.37	-37.11	32.69	16.50	-30.11	0.343	11.31	10.05

Table 8. Plasma insulin-like growth factor-I concentrations by time for individual weanlings adapted to the FF or SS supplement. Times are relative to the administration of a glucose bolus (time = 0). Horse 119 was determined to be an outlier and is not included in the determination of mean time values. Data for Chapter 2.

FF Diet	Horse # / GH (ng/mL)								
Time	18	19	26	93	114	119	**Mean**	SEM	Median
0	0.42	1.52	4.12	0.61	0.44	2.75	**1.67**	0.75	0.61
19	1.00	6.01	3.72	0.25	7.39	1.33	**2.74**	1.30	1.33
30	0.62	5.70	3.33	0.35	11.10	0.78	**3.23**	2.04	0.78
60	1.80	3.27	4.34	3.14	8.21	5.97	**4.69**	1.12	4.34
90	0.74	1.48	5.61	9.03	6.58	1.96	**4.78**	1.52	5.61
120	0.47	1.77	3.87	5.33	2.98	0.40	**2.61**	0.96	2.98
150	1.46	2.34	5.79	1.40	1.07	0.25	**1.99**	0.97	1.40
180	0.75	2.22	1.50	0.71	0.39	6.77	**2.02**	1.20	0.75
210	0.56	2.76	0.58	0.65	0.87	1.87	**0.91**	0.25	0.65
240	5.58	4.08	0.53	4.83	8.03	0.35	**3.86**	1.49	4.83
270	11.52	1.07	0.38	6.03	2.00	0.21	**4.03**	2.15	2.00
300	3.47	3.79	0.87	4.01	0.46	3.21	**2.40**	0.72	3.21
330	0.83	3.23	0.89	2.34	0.35	0.46	**0.97**	0.36	0.83
360	0.47	3.03	2.73	0.40	0.67	0.21	**0.89**	0.46	0.47
Horse Mean	**2.02**	**2.94**	**2.61**	**2.65**	**3.40**	**1.79**	-	-	-
SEM	0.77	0.38	0.49	0.69	0.97	0.54	-	-	-
Basal GH	0.47	1.50	0.56	0.37	0.41	0.23	**0.41**	0.05	0.41
Max Peak	1.8	6.01	5.79	9.03	11.1	6.77	**6.90**	1.57	6.77
Time Max (0-180 min)	60	19	90	90	30	180	**90**	25	90
GH AUC (0-180 min)	90	258	667	522	871	347	**499**	134	522
GH AUC (0-360 min)	681	522	722	1001	1196	561	**832**	116	722

Table 9. Plasma growth hormone concentrations by time for individual weanlings adapted to the FF supplement. Times are relative to the administration of a glucose bolus (time = 0). Horse 19 was determined to be an outlier and is not included in calculations of means or medians. Data for Chapter 2.

SS Diet	Horse # / GH (ng/mL)								
Time (min)	12	97	110	128	129	134	**Mean**	SEM	Median
0	0.26	2.71	0.25	0.17	0.58	0.41	**0.73**	0.40	0.33
19	0.22	0.81	6.98	6.95	6.69	0.28	**3.65**	1.44	3.75
30	0.19	0.36	11.65	6.65	12.95	0.47	**5.38**	2.41	3.56
60	.	8.51	7.47	0.75	6.59	2.24	**5.11**	1.53	6.59
90	0.18	9.31	1.46	0.21	1.92	5.91	**3.17**	1.50	1.69
120	2.41	2.60	0.42	0.60	0.34	6.94	**2.22**	1.03	1.51
150	1.88	0.48	0.22	1.91	0.34	1.44	**1.04**	0.32	0.96
180	0.33	0.34	7.20	1.16	0.40	1.67	**1.85**	1.09	0.78
210	0.26	9.55	4.69	0.42	0.16	0.87	**2.66**	1.55	0.64
240	4.59	7.74	0.69	0.19	0.35	0.42	**2.33**	1.28	0.56
270	2.55	0.43	0.28	0.21	0.99	0.23	**0.78**	0.37	0.35
300	0.41	0.46	0.19	0.17	0.26	0.18	**0.28**	0.05	0.22
330	0.18	4.12	5.05	0.14	0.68	0.50	**1.78**	0.90	0.59
360	0.16	2.76	1.39	2.19	0.67	0.79	**1.33**	0.40	1.09
Horse Mean	**0.99**	**3.38**	**3.22**	**1.46**	**2.22**	**1.52**	-	-	-
SEM	0.36	0.93	0.95	0.58	0.95	0.54	-	-	-
Basal GH	0.18	0.39	0.24	0.17	0.30	0.28	**0.26**	0.04	0.26
Max Peak	2.41	9.31	11.65	6.95	12.95	6.94	**8.37**	1.55	8.13
Time Max (0-180 min)	120	90	30	19	30	120	**68**	19	60
GH AUC (0-180 min)	122	623	670	318	623	489	**474**	88	556
GH AUC (0-360 min)	337	1284	1055	356	682	543	**710**	157	613

Table 10. Plasma growth hormone concentrations by time for individual weanlings adapted to the SS supplement. Times are relative to the administration of a glucose bolus (time = 0). Horse 19 was determined to be an outlier and is not included in calculations of means or medians. Data for Chapter 2.

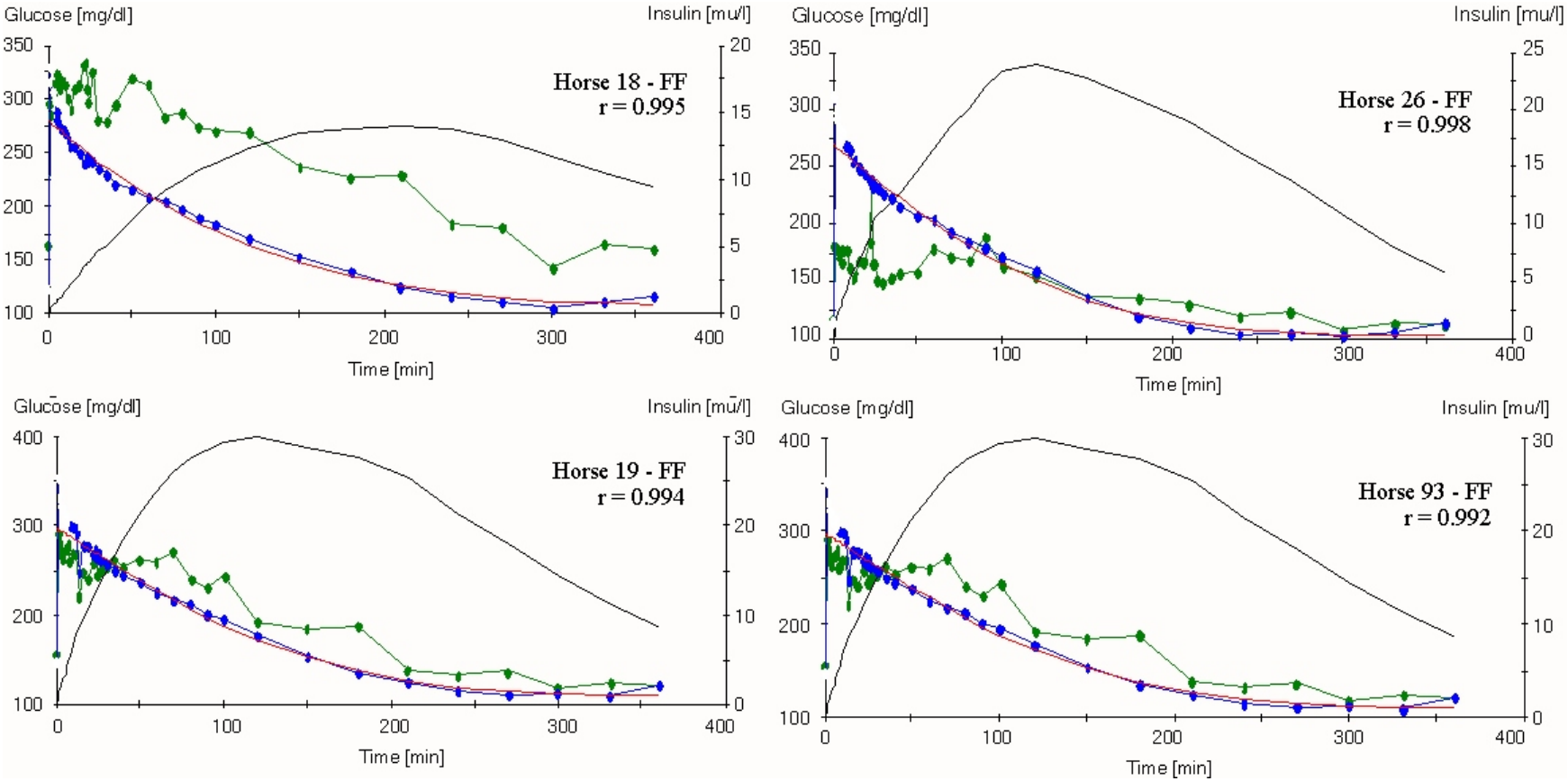

Figure 1. Experimental glucose values (-•-) fit by the minimal model (–) for FF adapted weanlings 18, 19, 26 and 93. Insulin values (-•-) and estimated insulin secretion (–) are also shown.

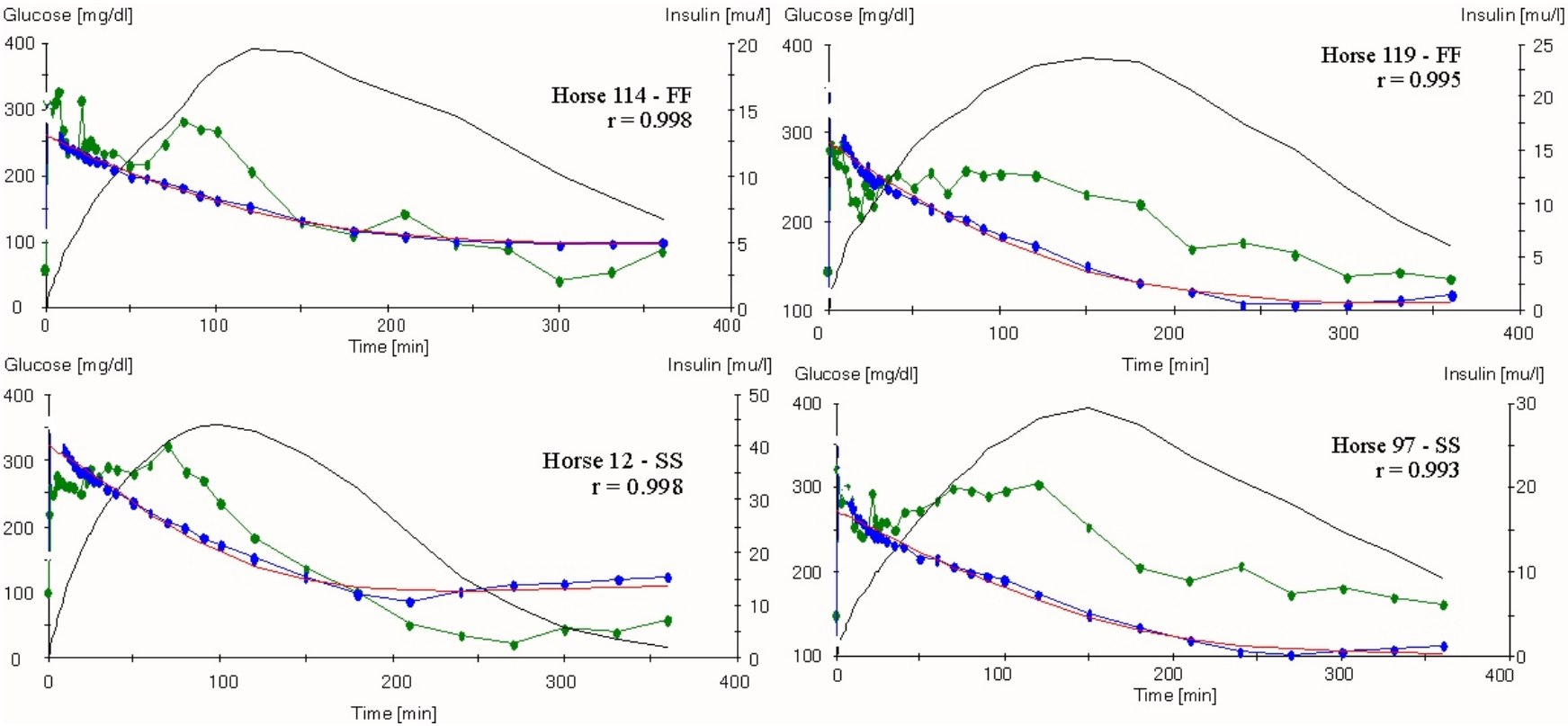

Figure 2. Experimental glucose values (-•-) fit by the minimal model (–) for FF adapted weanlings 114 and 119 ans SS adapted weanlings 12 and 97. Insulin values (-•-) and estimated insulin secretion (–) are also shown.

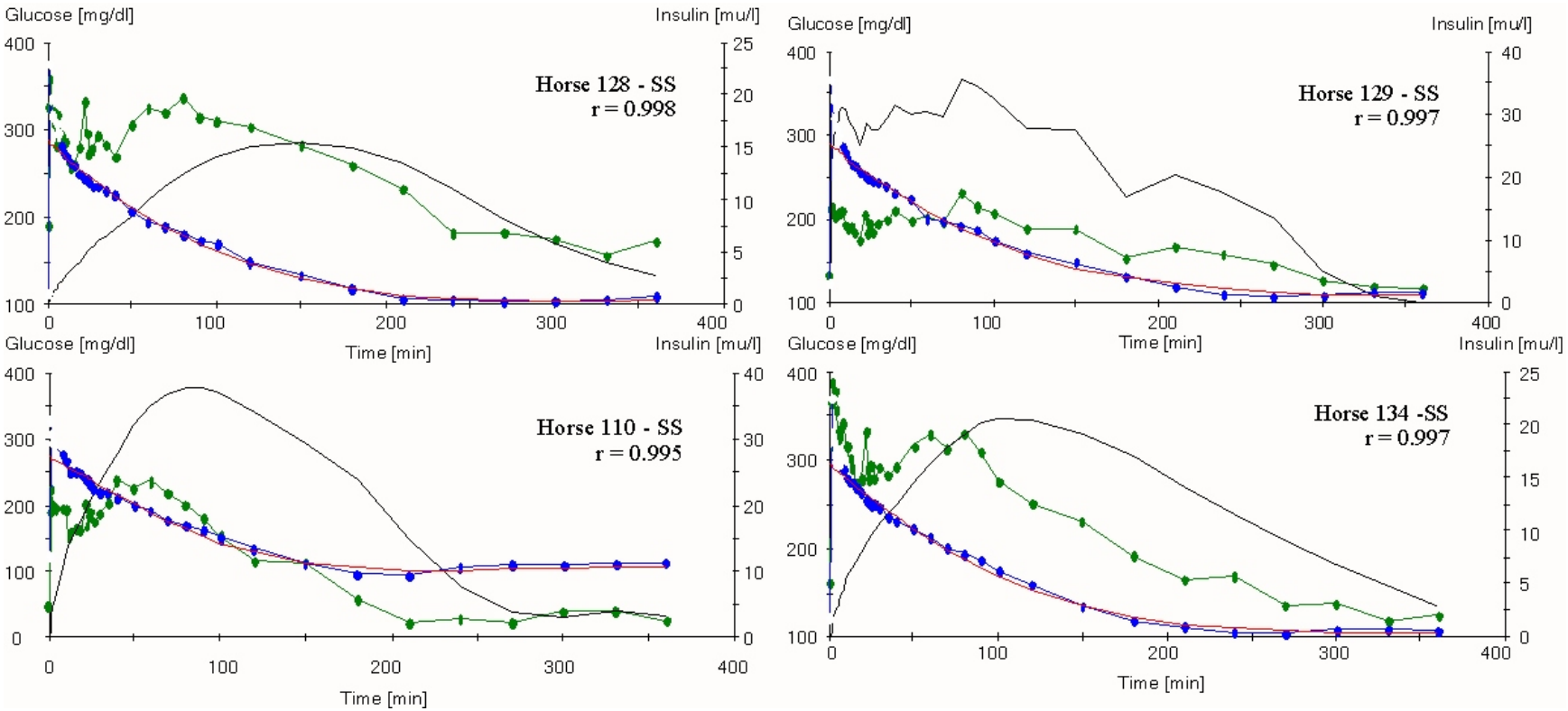

Figure 3. Experimental glucose values (-•-) fit by the minimal model (–) for SS adapted weanlings 128, 129, 110, and 134. Insulin values (-•-) and estimated insulin secretion (–) are also shown.

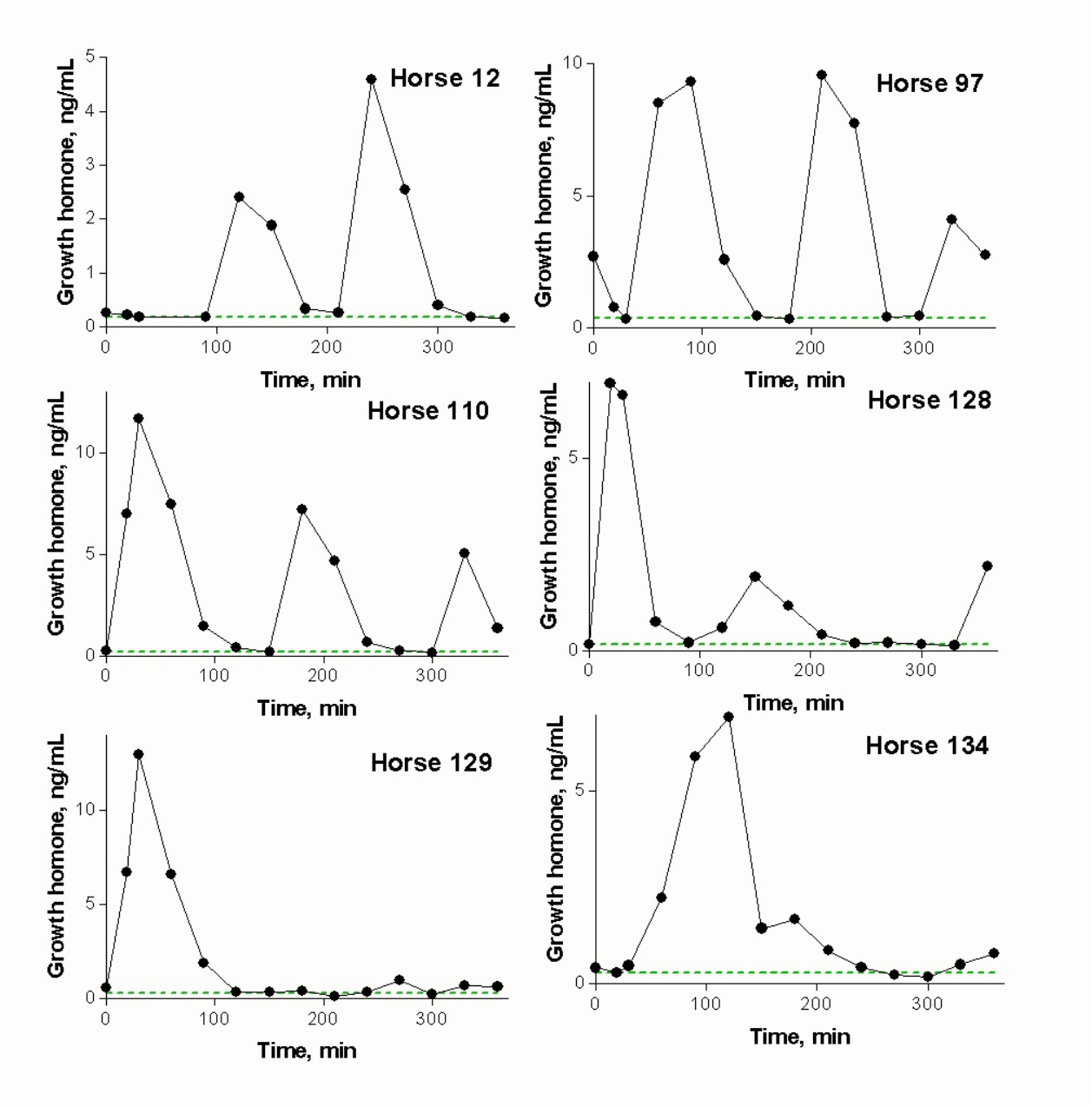

Figure 4. Growth hormone patterns and estimated basal levels (- -) for individual weanlings adapted to the SS supplement.

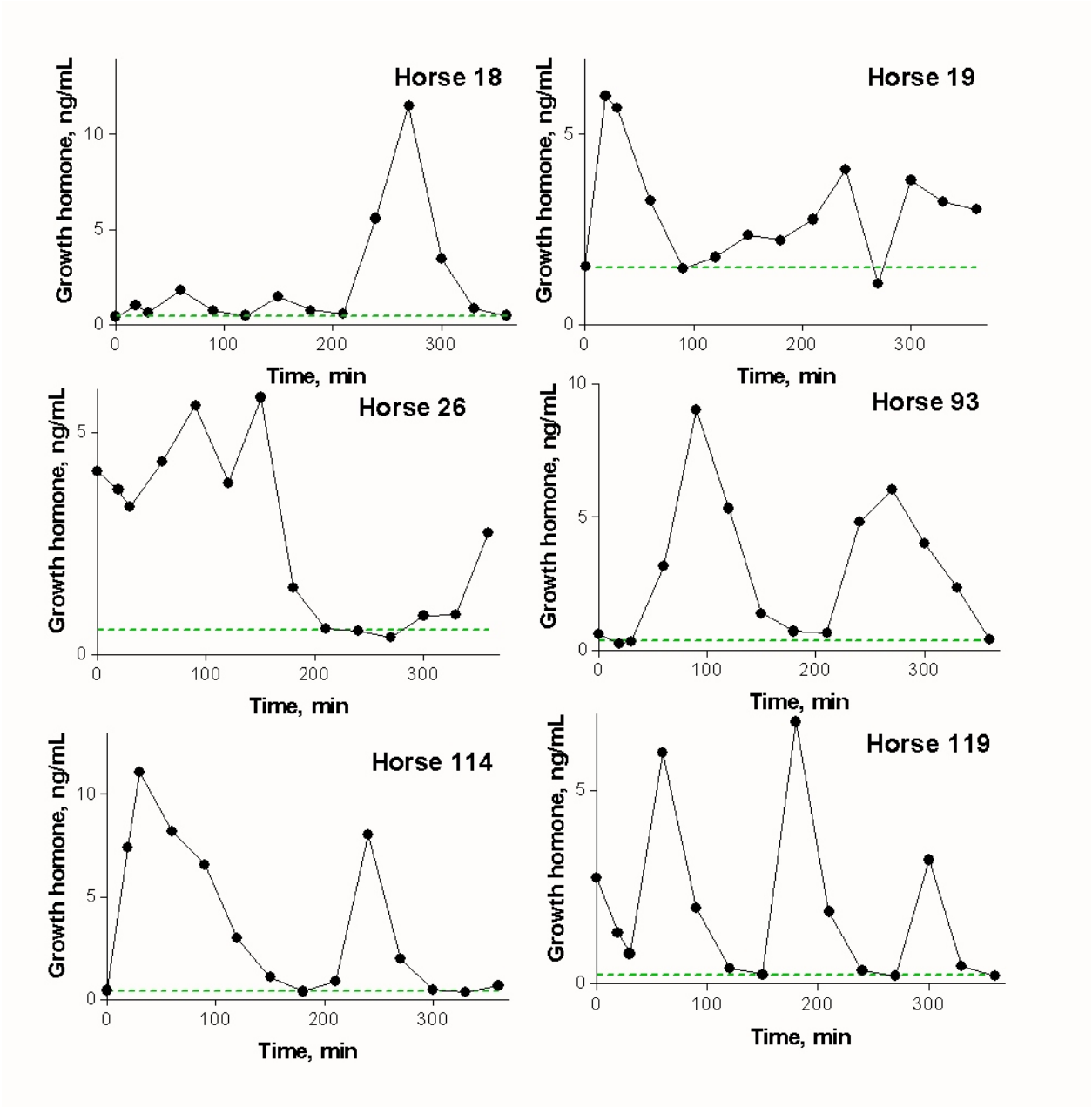

Figure 5. Growth hormone patterns and estimated basal levels (- -) for individual weanlings adapted to the FF supplement.

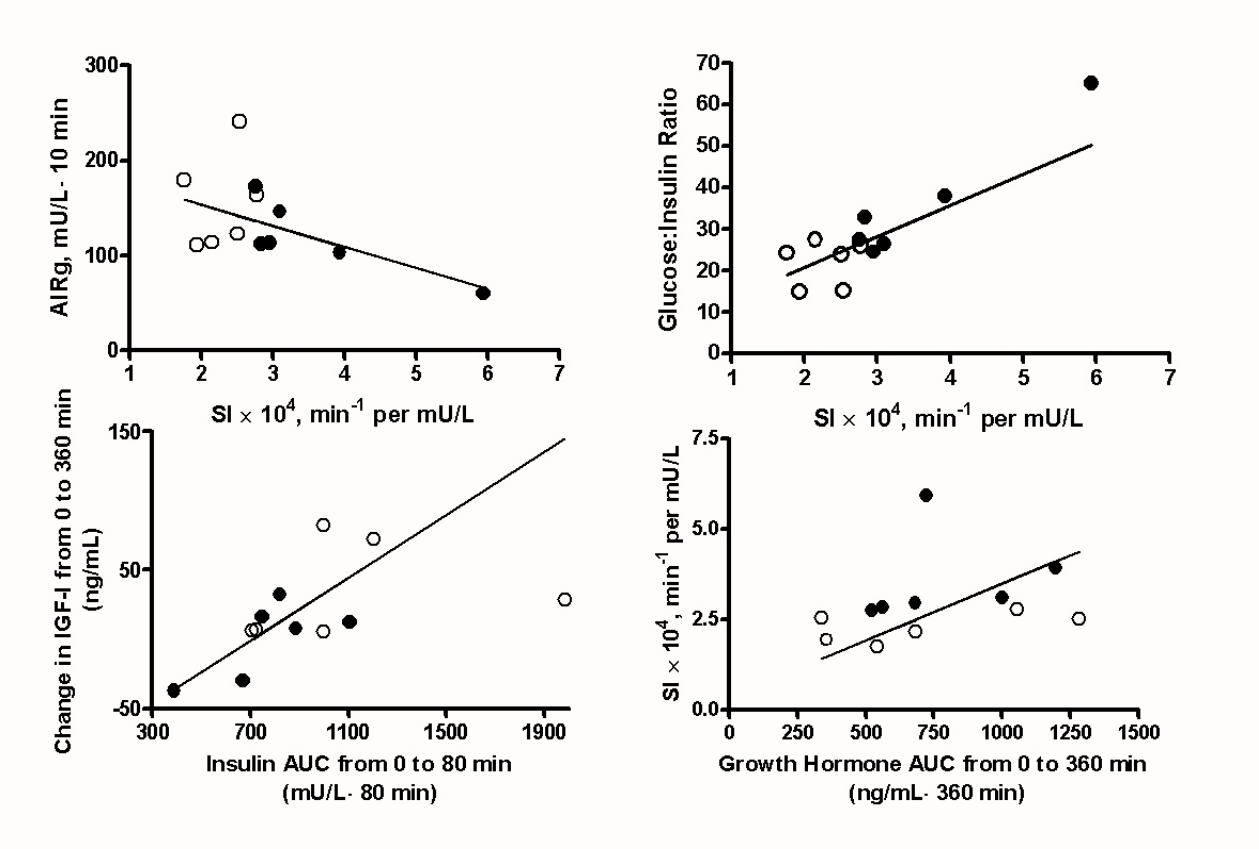

Figure 6. Robust linear regressions for Minimal Model parameters and hormone concentrations of Thoroughbred weanlings during an FSIGT. Horses were adapted to a supplement rich in hydrolyzable carbohydrates (SS, ○) or one resembling forage and containing fat and fiber (FF, ●)

Appendix B

An example of the WinSAAM (Ver. 3.0.1, Greif and Boston, 1997) program adapted from Wastney et al., 1999 to fit the glucose and insulin data from each foal to the minimal model:

```
A SAAM31                        FOAL93
2      21
H PAR
  P(01)    6.152100E-03  7.799707E-04   7.799707E-02
  P(02)    9.992300E-03  5.060000E-03   5.060000E-01
C   P(03)    3.098000E-06  2.110000E-06   2.110000E-04
  P(7)     3.1004E-04    3.1004E-05     3.1004E-03
  P(4)     1.211050E+02
  P(5)     2.152000E+00
  P(6)     2.962500E+02
  P(9)     8.000000E+01
  P(11)    3.000000E+02
C   P(7)=P(3)/P(2)*10000
  P(8)=P(11)*P(9)/P(6)
  P(16)=P(1)-P(7)*P(5)/10000
  P(24)=20* P(5)/(P(4)/18-3.5)
  P(25)=P(4)*P(5)/(18*22.5)
  L(0,7)=P(2)
  UF(7)    1                              8G07
  IC(6)=P(6)
  IC(9)=-10*P(5)
H DAT
X UF(6)=-(P(1)+F(7))*F(6)+P(1)*P(4)
X FF(8)= F(8)
X UF(9) = FF(8)
X G(7)=P(7)*P(2)*(FF(8) - P(5))
X G(8)=F(7)*100/(P(1)+F(7))
X G(9)=P(7)*F(9)
110
```

```
  P(1)
  P(2)
C   P(3)
  P(4)
  P(5)
  P(6)
  P(7)
  P(8)
  P(9)
  P(11)
  P(16)
  P(24)
  P(25)
109 F(9)
        10
108QL
        0.      5.55
        1.      19.136
        2.      17.972
        3.      16.939
        4.      16.35
        5.      17.416
        6.      17.325
        7.      18.108
        8.      16.05
        10.     17.063
        12.     17.036
        14.     12.09
        16.     14.782
        19.     13.997
        22.     15.791
        23.     16.162
        24.     16.432
        25.     14.509
        27.     15.175
        30.     15.336
        35.     16.308
```

40. 15.436
50. 16.26
60. 15.911
70. 17.121
80. 14.072
90. 12.971
100. 14.321
120. 9.305
150. 8.517
180. 8.838
210. 3.93
240. 3.283
270. 3.729
300. 1.863
330. 2.396
360. 2.152

107 G(8)

0.
1.
2.
3.
4.
5.
6.
7.
8.
10.
12.
14.
16.
19.
22.
23.
24.
25.
27.
30.

```
      35.
      40.
      50.
      60.
      70.
      80.
      90.
      100.
      120.
      150.
      180.
      210.
      240.
      270.
      300.
      330.
      360.
106                   WT=0.0
      0.      147.3
106                   WT=0.0
      1.      353.15
106                   WT=0.0
      2.      332.31
106                   WT=0.0
      3.      323.
106                   WT=0.0
      4.      318.155
106                   WT=0.0
      5.      312.94
106                   WT=0.0
      6.      309.27
106                   WT=0.0
      7.      304.865
106                   FSD=0.02
      8.      300.36
      10.      298.575
      12.      290.94
```

```
        14.     247.42
        16.     278.325
        19.     275.825
        22.     269.055
        23.     273.5
        24.     271.365
        25.     265.81
        27.     262.915
        30.     258.81
        35.     251.325
        40.     244.605
        50.     236.685
        60.     225.815
        70.     217.665
        80.     211.92
        90.     202.185
        100.    195.52
        120.    177.43
        150.    154.
        180.    135.38
        210.    124.09
        240.    114.91
        270.    110.405
        300.    112.36
        330.    108.815
        360.    121.105
105 G(9)
        10
```

Wastney, M. E., B. H. Patterson, O. A. Linares, P. C. Greif and R. C. Boston. 1999. Investigating biological systems using modeling: Strategies and software. Academic Press, Boston.

Vitae

Kibby Hoffer Treiber graduated from the North Carolina School of Science and Mathematics and received her B.S. in Biology and B.A. in Ancient/Medieval History from the University of North Carolina at Chapel Hill. She has competed at the national level in gymnastics and fencing (women's sabre) and has co-authored five unpublished (as yet) novels with her identical twin sister: *Children of Gaia*, *Bloodright*, *The Gordian Knot*, *Fate Map* and *Shadow of the Bear*. Her future plans are to live in a castle and continue writing novels, with horses as a primary source for inspiration.