Glucose disposal and insulin sensitivity at rest and during exercise in trained horses adapted to different dietary energy sources and in association with laminitis in ponies

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Keywords: minimal model, endurance exercise, insulin sensitivity, glycemic dietetics, single injection tracer kinetics compartmental model, laminitis, proxy, horse

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ABSTRACT

Glucose is a fundamental energy source, the utilization and regulation of which impacts exercise performance and health. These studies have used modeling techniques to evaluate glucose kinetics and dynamics in equids and developed tests to evaluate the status of glucose metabolism. In Part I, 12 exercise-trained Arabians underwent insulin-modified FSIGT (with minimal model analysis) and single-injection glucose tracer (with compartmental analysis) studies at rest and during exercise to evaluate the effects of exercise on glucose kinetics and dynamics. These geldings were maintained on pasture, but adapted for 4 months to twice-daily feeding of feeds rich in sugar and starch (SS, n=6: NSC 45%, Fat 3%, NDF 24%) or fat and fiber (FF, n=6: NSC13%, Fat 11%, NDF 45%). Exercise increased insulin sensitivity ($P = 0.070$) and glucose transport ($P \leq 0.038$). Although variables were not different between FF and SS horses at rest, during exercise SS horses tended to have lower ($P = 0.085$) insulin sensitivity and increased ($P = 0.043$) glucose utilization compared to FF horses. In Part II, satisfactory proxies for minimal model parameters were developed to facilitate the evaluation of insulin sensitivity in larger populations. These proxies were applied to a population of 163 ponies and used to characterize metabolic differences between ponies predisposed to pasture laminitis (PL) from ponies not predisposed (NL). A subset of 14 ponies (7 PL, 7 NL) also underwent the FSIGT for minimal model analysis. Ponies predisposed to laminitis were found to have lower insulin sensitivity ($P \leq 0.007$) and higher insulin secretory response ($P \leq 0.045$) by both the minimal model and

proxies, and higher ($P < 0.001$) circulating triglycerides and body condition score. Cut-point analysis for these variables was used to define a pre-laminitic metabolic syndrome with total predictive power of 78% to identifity ponies at risk for developing pasture laminitis. Increased insulin resistance and prevalence of lamintis were associated with increased non-structural carbohydrates in spring pasture. These studies demonstrate the importance of glucose regulation for exercise and animal health. When glucose regulation is altered in the case of insulin resistance, performance could be impacted and diseases such as laminitis may occur. Insulin resistance may be moderated by exercise or by avoiding sugar and starch in feeds and pasture.

Keywords: insulin resistance, glycemic dietetics, minimal model, endurance exercise, single injection tracer kinetics, compartmental model, horse, laminitis, proxy

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Abbreviations

ACCB	acetyl-CoA carboxylase-β
AIRg	minimal model acute insulin response to glucose
BW	Body weight
CPT-1	carnitine palmitoyl transferase-1
DI	minimal model disposition index
EGP	endogenous glucose production
FSIGT	frequent sampling intravenous glucose tolerance test
GTT	glucose tolerance test
IMTG	intramyocellular triglyceride
IRS	insulin receptor substrate
ITT	insulin tolerance test
MAPK	mitogen activated protein kinase
MTT	meal tolerance test
NEFA	non-esterified fatty acids
NSC	non-structural carbohydrates
PI-3kinase	phosphatidylinositol 3-kinase
PKC	protein kinase C
RER	respiratory exchange ratio
Sg	minimal model glucose effectiveness
SI	minimal model insulin sensitivity index

INTRODUCTION

The horse evolved as a grazing species, utilizing pasture to fulfill the energy demands of survival, reproduction and performance. This nutritional strategy necessitates constant low-intensity activity (grazing and traveling) for approximately 65% of the day (Marlow et al., 1992). In addition, bursts of power-sprinting are required to escape predators. Humans have harnessed these abilities to elicit awesome feats of exercise performance from domesticated horses, from 100-mile endurance races to track sprinting at speeds over 40 mph. In order to accomplish such feats optimization of energy availability and utilization is paramount. Horses are fed a variety of supplements and feeds, with almost 60% of concentrates based on grain and molasses, which are high in hydrolyzable carbohydrates (sugars and starch) (USDA, 1998). While such diets provide concentrated, readily-available energy, they may oppose the horse's digestive and metabolic adaptations to perform optimally on a steady intake of high fiber forages.

Grazing species such as the horse have also evolved to persist during periods such as winter or dry seasons when digestible energy (DE) is restricted and sources of exogenous glucose scarce. These populations have adapted certain mechanisms to conserve energy and glucose. Collectively, these adaptations comprise a 'thrifty genotype' generally associated with insulin resistance. Insulin resistance promotes glucose conservation and the utilization of alternative energy sources, namely stored fat (Colagiuri and Brand Miller, 2002). When thrifty individuals are exposed to nutritional signals which deviate from traditional nutritional pressures (e.g. excess DE and high-glycemic meals) the metabolic response may be inappropriate, contributing to metabolic dysfunction. These dysfunctions may be analogous to the 'metabolic syndrome' in humans which is associated with insulin resistance and the high-energy, high-carbohydrate diets of the Western world (Adult Treatment Panel III, 2002; Brand-Miller and Colagirui, 1999; WHO, 1999) and possibly associated with equine pasture laminitis.

Laminitis is a systemic disease which manifest in the hooves of horses and especially ponies. In laminitis, failure of the inner wall of the hoof causes separation of the pedal bone from the hoofwall (Pass et al., 1998) leading to acute and sometimes

irreversible lameness, commonly justifying euthanasia. As with metabolic syndrome in humans, pasture laminitis has been associated with thriftiness and insulin resistance and may represent a mismatch between a physiological predisposition and animal nutrition and management (Coffman and Colles, 1983; Field and Jeffcott, 1989).

Efficient interaction between metabolic signals and responses is critical for maintaining animal health and ensuring optimal exercise performance as well. The increased energy demands of exercise require flexibility in the mobilization, uptake and utilization of carbohydrate and fat substrate from circulation, adipose tissue, and muscle stores. In particular, the ability to tap and efficiently utilize energy from its primary storage as triglyceride in adipose tissue may allow for prolonged endurance exercise. Glucose energy, meanwhile is necessary for periods of high-intensity work. Metabolic dysfunction associated with inappropriate nutrition could contribute to disrupted glucose and/or fat metabolism in working muscle, impacting exercise performance and increasing risk of breakdown (Kelley and Mandarino, 2000; Randle et al., 1963; Sidossis and Wolfe, 1996).

We have been developing an alternative concentrate supplement, substituting fat in place of soluble carbohydrates as a source of concentrated energy while incorporating fiber to complement the horse's adapted intake and digestion (Hoffman, 1999). Combined, the fat and fiber constitute a feed digested and metabolized similar to forage, providing recognizable signals to avoid digestive or metabolic upset while supplying increased energy to the performance horse.

The present work evaluates glucose regulation in the horse with respect to diet adaptation, exercise performance and disease risk by applying dynamic and kinetic models and proxy tests.

PART I - GLUCOSE, INSULIN AND EXERCISE

CHAPTER 1:LITERATURE REVIEW

Supplemental energy

The low-energy density of forage is a limiting factor in the daily amount of energy a grazing animal such as the horse can intake. For horses to meet the increased demands of performance - reproduction, growth, exercise - imposed by humans, more dietary energy is required. As a solution, we supply a more concentrated energy source. Traditionally this energy source comprises grains which are rapidly digested and provide ready energy in the form of glucose from soluble carbohydrates. However these diets poorly match the digestive and metabolic adaptations of the horse.

The horse's digestive tract is adapted to handle fibrous pasture and includes a relatively limited small intestine for the digestion and absorption of hydrolyzable carbohydrates. When excess carbohydrate overloads 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). The subsequent decrease in hindgut pH impacts the microbial environment, altering bacterial populations and prompting the release of endotoxins, exotoxins or amines - a chain reaction which can lead to colic or laminitis (Bailey et al., 2004a; Clarke et al., 1990).

Metabolically, the horse has adapted to survive under conditions where the availability of hydrolyzable carbohydrate varied considerably. Such persistence requires glucose sparing mechanisms which channel the limited glucose to essential glucose-dependent systems such as the central nervous system or the developing fetus. Insulin resistance represents a glucose-sparing mechanism. In the insulin resistant state, glucose storage and metabolism in peripheral tissues (skeletal muscle and adipose) decreases, conserving glucose for essential tissues. Meanwhile, stored fat may provide an alternative energy-source to peripheral tissues. Examples of this strategy include the 'thrifty phenotype' in which the fetus develops insulin resistance in response to nutrient restriction (Flanagan et al., 2000; Ozanne and Hales, 2002), gestational insulin resistance to cope with energy (particularly glucose) demands of the fetus, and the 'thrifty genotype'

which describes the evolutionary pressure towards insulin resistance in carbohydrate-restricted populations (Colagiuri and Brand Miller, 2002; Neel, 1962). The latter may apply to non-selective herbivorous species, carnivores, and populations in extreme climates such as the desert (Kaske et al., 2001).

Strategic insulin resistance may be confounded by metabolic signals following high-glycemic meals, when glucose increases abruptly and stimulates insulin hyper-secretion (Williams et al., 2001). Counter-regulatory mechanisms, including counter-regulatory hormones such as glucagon, cortisol and growth-hormone, may be stimulated both by postprandial hyperinsulinemia and by the abrupt fall in circulating glucose following the peak glycemic response (Forhead and Dobson, 1997; Roth et al., 1963). Such counter-regulatory mechanisms oppose insulin action, compounding insulin resistance (Smith et al., 1997). At the same time, hyperglycemia or hyperinsulinemia may down-regulate mechanisms of glucose and insulin signaling (Gavin et al., 1974; Saltiel and Kahn, 2001). This mismatch between glucose-conserving insulin resistance and metabolic signals following high-glycemic meals potentiates a breakdown in the cascade of hormonal and metabolite signals which regulate energy. The outcome is the appearance of familiar disease states such as obesity, dyslipidemia, diabetes and possibly laminitis in the horse.

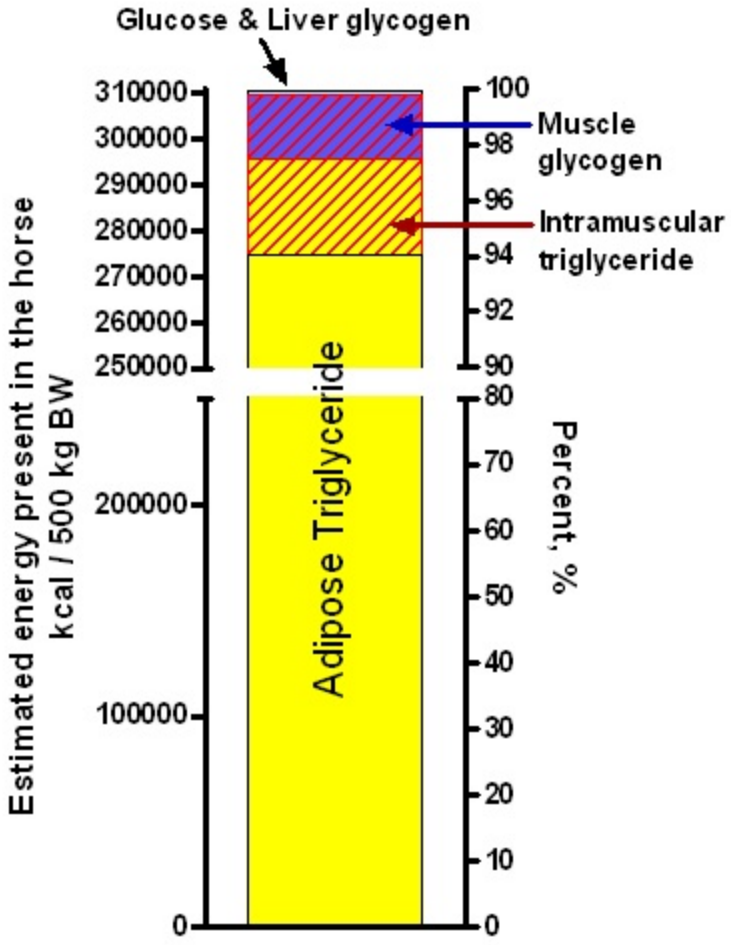
Feeds providing fat as an energy source present an alternative to high-glycemic grain concentrates. Fat provides 9 kcal/g, more than twice the energy density of carbohydrate (4 kcal/g). Fat digestion stimulates digestive hormones to slow the passage of digesta, avoiding abrupt changes in metabolite flux which could prompt insulin secretion (Welch et al., 1987). Fat-adaptation may also upregulate fat metabolism, improving the flexibility of the cell to use both fat and carbohydrate more efficiently (Dunnett et al., 2002).

Dietary fiber complements fat by adding bulk and further slowing digestion as well as intake. Fiber mimics the forage diet to which the horse has adapted and stimulates proliferation of a healthy microbial population in the hind-gut (Jenkins and Jenkins, 1985). Fiber has been suggested to decrease the risk of insulin resistance and

obesity in several species, however this property appears to relate mostly to fiber's ability to reduce post-prandial glycemic-response either by slowing digestion or replacing hydrolyzable carbohydrate (Davy and Melby, 2003; Jenkins and Jenkins, 1985; Nelson et al., 2000).

The fat and fiber meal used in the present studies has been demonstrated to induce post-prandial glucose and insulin responses resembling the responses to forage and significantly lower than the responses following an isocaloric typical grain meal (Williams et al., 2001). Fat and fiber feeds may therefore provide digestive and metabolic signals which better match the evolutionary adaptations of the horse, avoiding health risks associated with the introduction of foreign diets.

Figure 1.1 Energy substrate and storage in the horse (Harris, 1997).



Energy during exercise

Exercise performance is optimized when energy substrate is available and efficiently utilized to provide energy to working muscles. Endogenous sources of energy substrate include circulating glucose mobilized or generated primarily from the liver, circulating non-esterified fatty acids (NEFA) and triglycerides mobilized from adipose tissue, muscle glycogen, and intramyocellular triglyceride (IMTG). In a 500 kg horse, potential energy could be roughly estimated to be 350

kcal circulating glucose, 400-1000 kcal liver glycogen, 9500-18,000 kcal muscle glycogen, 14,000-28,000 kcal IMTG, and 150,000-400,000 kcal triglyceride in adipose tissue (Harris, 1997) (Figure 1.1). Relative energy expenditure of these sources in the exercising horse is illustrated in Figure 1.2 and discussed below.

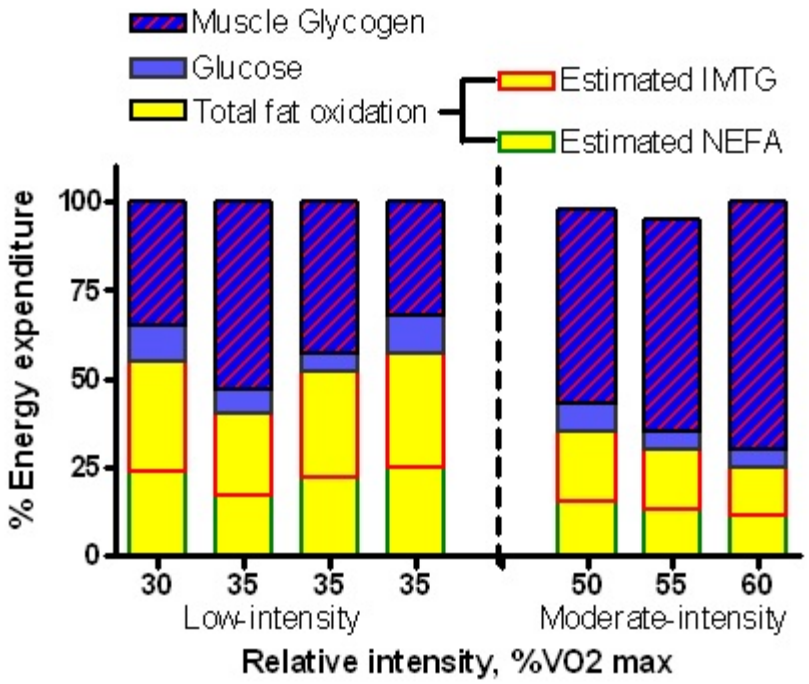


Figure 1.2. Proportion of total energy expenditure derived from energy sources in horses exercising at low and moderate intensity. Data adapted from Geor et al., 2000abc; Pagan et al., 2001; Jose-Cunilleras et al., 2002

Circulating glucose is maintained by exogenous provision (absorption from the gut) and endogenous glucose production primarily from the liver. Endogenous glucose production (EGP) includes gluconeogenesis (i.e. the production of glucose from non-carbohydrate sources (e.g. amino acids, glycerol, lactate (Gustavson et al., 2003)) and the mobilization of glucose from the breakdown of liver glycogen. Endogenous glucose production is stimulated by glucagon (Gustavson et al., 2003), and suppressed by hyperglycemia and insulin (Rossetti et al., 1993).

Glucose is transported into the intracellular space via a variety of transporters of which GLUT-1, GLUT-2 and GLUT-3 are the major insulin-independent glucose transporters expressed in various insulin-independent tissues (Scheepers et al., 2004), while GLUT-4 is expressed at the surface of skeletal muscle cells and adipocytes in

response to insulin (and muscle contraction) (Ploug et al., 1990; Wallberg-Henriksson et al., 1988). Glucose disposal represents the maximum glucose available for oxidation. Generally this glucose uptake is assumed synonymous with glucose utilization as studies have shown that most glucose lost from circulation is oxidized (Jeukendrup et al., 1999). Glucose uptake constitutes a small proportion (<10%) of total energy expenditure during low and moderate exercise (35-55% VO_{2max}) in horses (Geor et al., 2000a; Geor et al., 2000c; Jose-Cunilleras et al., 2002).

Muscle glycogen is the storage form of glucose within skeletal muscle. In the horse, muscle glycogen (and lactate) provides 35-50% of energy expended during low-intensity exercise and 55-70% of energy expended during moderate-intensity exercise (Geor et al., 2000a; Geor et al., 2000c; Jose-Cunilleras et al., 2002). Muscle glycogen is considered a limiting factor in exercise performance as depletion of muscle glycogen is observed and associated with fatigue (Hargreaves, 2004). However fatigue can occur for many reasons other than glycogen depletion, particularly in the horse which has large glycogen stores which are difficult to deplete (Davie et al., 1999). Extensive research has evaluated strategies for increasing muscle glycogen mass (e.g. pre-exercise carbohydrate loading) or decreasing muscle glycogenolysis during exercise (e.g. via fat adaptation).

Circulating NEFA are mobilized by hormone sensitive lipase from adipose tissue. Similar to EGP, this lipolysis is stimulated by epinephrine and glucagon, and supressed by insulin. In fact, insulin has been shown to be a more potent suppressor of lipolysis than promoter of glucose uptake (Nurjhan et al., 1986). Circulating NEFA leave the plasma via transporters such a fatty acid translocase and plasma membrane fatty acid binding protein (Bonen et al., 2004; Cameron-Smith et al., 2003). The liver also converts NEFA to triglyceride (Lewis et al., 2002), a process suppressed by insulin (Lewis et al., 1995). Circulating triglycerides represent another potential source of circulating fat energy (Schrauwen et al., 2000). Non-esterified fatty acids are released from triglyceride complexes by lipoprotein lipase and taken up by adipose tissue for storage. The contribution of circulating NEFA to energy expenditure during exercise has not

been evaluated in the horse. In humans, NEFA account for ~40-65% of total fat oxidized (Coyle et al., 2001; Helge et al., 2001; Watt et al., 2002).

Besides NEFA mobilized from adipose tissue, circulating triglycerides and intramyocellular triglyceride stores contribute to total fat oxidation during exercise. Although once disregarded as an energy component during exercise, recent studies in humans have demonstrated that triglyceride may constitute an important source of energy substrate, accounting for ~35-60% of total fat oxidized and ~20% of total energy expenditure (Helge et al., 2001; Schrauwen et al., 2000; Watt et al., 2002). Unfortunately current measurement techniques for IMTG are imprecise, confounded by inter-myocellular deposits, muscle fiber type, and rapid turnover (Watt et al., 2002).

Few studies in the horse have considered individual components (triglyceride, IMTG, NEFA) of total fat oxidation (Orme et al., 1997). Total fat oxidation in the horse accounts for approximately 30% of energy expended during moderate-intensity exercise and 40-55% of energy expended during low-intensity exercise (Geor et al., 2000b; Jose-Cunilleras et al., 2002; Pagan et al., 2002). These values are comparable to those observed in humans during exercise (Burke et al., 2000; Jeukendrup et al., 1999; van Loon et al., 2001). Assuming similar proportions to humans, NEFA utilization by the horse can be estimated to contribute ~15-35% of energy expended during exercise and triglycerides (in adipose tissue, circulation, and IMTG) an additional ~15-30%.

Insulin regulates the mobilization and uptake of all energy substrate. Insulin is secreted in to circulation by the β-cells of the pancreas (Holley and Evans, 1979) and binds insulin receptors at the cell membrane, with one result being the stimulation of GLUT-4 insulin-dependant glucose transporters to aggregate at the plasma membrane (Bessesen, 2001; Farese, 2001). Insulin also increases the glucose-shuttling activity of GLUT-4 transporters (Michelle Furtado et al., 2003). Additionally, insulin alters the availability of circulating NEFA and glucose by suppressing lipolysis and the mobilization of NEFA (Nurjhan et al., 1986; Wasserman et al., 1989) and inhibiting hepatic glucose production (Jensen, 2003).

During exercise plasma insulin concentrations are significantly reduced (Jose-Cunilleras et al., 2002; Nout et al., 2003; O'Connor et al., 2004). This decrease is

probably due to the suppression of pancreatic insulin secretion by the exercise-induced rise catecholamines which increase during exercise (Aarnio et al., 2001; Geor et al., 2000b; Urano et al., 2004). Lower plasma insulin concentrations de-suppress lipolysis and heptic glucose production, increasing circulating substrate available for working tissue (Jensen, 2003; Wasserman et al., 1989). Insulin also influences intracellular metabolism of glucose and fat (Saltiel and Kahn, 2001). Thus changes in insulin signaling (e.g. hyperinsulinemia or insulin resistance) can critically impact energy dynamics and exercise performance.

Energy metabolism in skeletal muscle

The pathways of glucose and fat metabolism intertwine, with both metabolites oxidized to acetyl-CoA for entry into the TCA cycle the products of which drive oxidative phosphorylation to produce ATP, the fundamental energy providing unit for muscle contraction (Figure 1.3). Several theories exist to explain the balance of metabolism between fat and glucose oxidation from the perspective that glucose and fat 'compete' for oxidation. As with insulin resistance, however, the mechanisms regulating energy metabolism have evolved to optimize performance (which ultimately determines survival and reproduction of the species). Therefore the goal is rather to understand the conditions under which 'competition' between energy sources becomes limiting rather than adaptive.

The metabolism of glucose and fat in skeletal muscle is regulated by hormonal signals, concentrations of circulating metabolites, tissue responsiveness (sensitivity) and tissue metabolic capacity (e.g. muscle fiber type recruited) (Brooks and Mercier, 1994). Insulin and glucagon concentrations are altered during exercise, promoting glucose and NEFA mobilization and utilization (Avogaro et al., 1996; Geor et al., 2000a; Gyntelberg et al., 1977). The cells recognize and respond to hormonal regulatory signals according to their sensitivity, with the oxidative or glycolytic capacity of the cell determining the ultimate response capacity (Saltiel and Kahn, 2001).

Exercise-induced catecholamine secretion is proportional to exercise intensity. Intensity can be defined in terms of absolute work (e.g. moving a 500 kg horse 1 km

a set time, i.e. mass*distance*acceleration). A finite amount of energy is necessary to generate the movement force. If the horse performs this work over a long time-period (i.e. at a walk), the intensity is low. During low-intensity exercise, fat energy is preferentially utilized, taking advantage of the greater efficiency of fat metabolism (i.e. less heat generated compared to glucose metabolism) and its considerable storage capacity which ensures a sustained substrate supply (Kronfeld et al., 2004). If the horse performs the same work in 2 min (i.e. at a high intensity gallop), carbohydrate energy will be preferentially utilized, because glucose is more readily available for rapid oxidation in the skeletal muscle.

Because glucose and fat metabolism are regulated on multiple levels (e.g. substrate availability, hormone signaling, transport, enzyme activity) factors other than workload, such as training state and diet, can alter fuel selection during exercise. Therefore exercise may also be described in terms of *relative intensity* which scales intensity to the individual's exercise capacity. Relative intensity may be measured by heart rate (%HR_{max}), oxygen consumption (%VO_{2max}), lactate accumulation, respiratory exchange ratio (RER), or perceived difficulty. Relative intensity corresponds to the physiological response of the individual to exercise.

In 1963, Randle proposed the glucose-fatty acid cycle to explain the interaction between glucose and fatty acid metabolism (Randle et al., 1963). The glucose-fatty acid cycle described how fat metabolism determines fuel selection by inhibiting glucose metabolism (Figure 1.3). According to this theory, fat oxidation and the subsequent accumulation of acetyl-CoA in the mitochondria inhibits pyruvate dehydrogenase, the enzyme responsible for oxidation of pyruvate at the end of glycolysis. At the same time, accumulation of citrate from the TCA cycle inhibits phosphofructokinase, halting glycolysis and resulting in accumulation of glucose-6-phosphate which subsequently inhibits hexokinase and even glucose uptake.

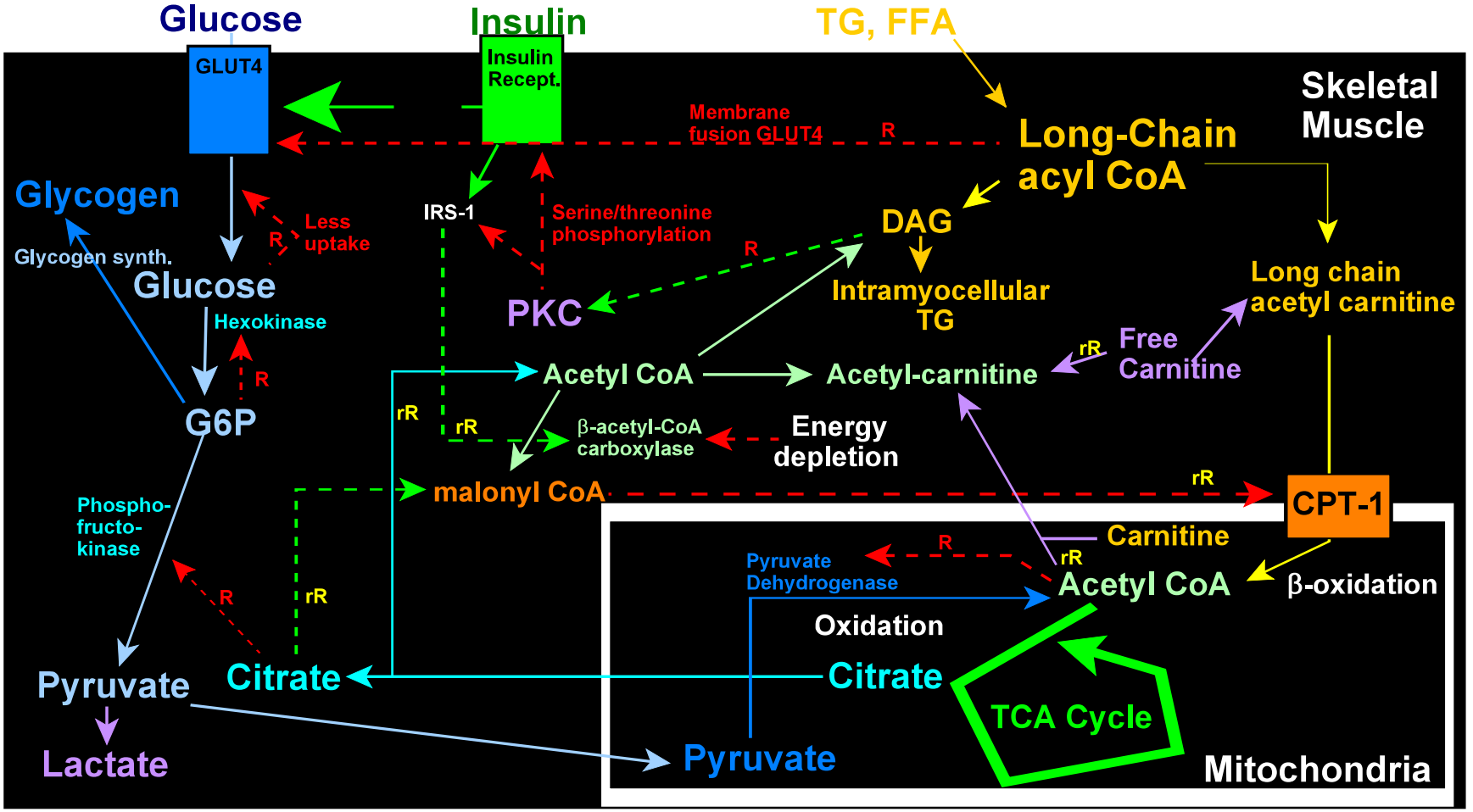


Figure 1.3. Glucose and fat metabolism. Broken red lines represent inhibition. Broken green lines represent stimulation. Mechanisms are shown which suppress glucose metabolism (red R) or fat metabolism (yellow rR). Abbreviations: IRS-1, insulin-receptor substrate-1; DAG, diacylglycerol; TG, triglyceride; FFA, non-esterified fatty acids,G6P, glucose-6-phosphate; PKC, protein kinase C; CPT-1, carnitine palmitoyl transferase-1

Recently inconsistent support of Randle's theory in skeletal muscle has prompted new hypotheses implicating glucose oxidation as the determining factor in fuel selection (Kelley and Mandarino, 2000; Sidossis and Wolfe, 1996) (Figure 1.3). These hypotheses are based on observations that hyperglycemia/hyperinsulinemia suppress fatty acid oxidation but not uptake (Sidossis et al., 1999) and that the decrease in fatty acid oxidation is due to a decrease in long-chain (but not medium-chain) fatty acid entry in the mitochondria (Sidossis et al., 1996). The distinction between fatty acid chain lengths implicates carnitine palmitoyltransferase-1 (CPT-1) , the enzyme required to transport carnitine-bound long-chain fatty acids into the mitochondria for oxidation.

The first hypothesis proposes that accumulation of malonyl-CoA inhibits CPT-1. Citrate shuttles acetyl-CoA from the mitochondria to the cytosol where acetyl-CoA is converted to malonyl-CoA by acetyl-CoA-carboxylase-β (ACCB). This ACCB is stimulated by insulin, but inhibited during energy depletion by AMP-activated kinase (AMPK) and decreased in glycogen depleted subjects. Malonyl-CoA and ACCB have therefore been shown to decrease proportionally to exercise intensity (van Loon et al., 2001). The above suggest a greater role for malonyl-CoA interference in fat oxidation in patients with abnormal insulin signaling rather than exercising subjects. Accordingly, exercise studies have demonstrated limited support for the role of malonyl-CoA (van Loon et al., 2001) compared to studies in insulin resistant subjects (Kelley and Mandarino, 2000; Kelley and Simoneau, 1994).

Another hypothesis implicating glucose oxidation as the driving force behind fuel selection involves carnitine, which must bind long-chain fatty acids before they can be transported by CPT-1. Reduced free carnitine could therefore down-regulate CPT-1 activity. Carnitine binds to acetyl-CoA from oxidation in the mitochondria, providing a potential sink to prevent inhibition of glucose-oxidizing pyruvate dehydrogenase (van Loon et al., 2001). In humans, acetyl-carnitine was shown to increase proportional to exercise intensity with an equivalent decrease in free carnitine, but the effect on CPT-1 activity was not evaluated (van Loon et al., 2001). In the horse, acetyl-carnitine also increased with exercise intensity but this increase was shown to be proportional to acetyl-CoA ($r = 0.89$), suggesting that carnitine was not limiting (Carlin et al., 1990).

All of these hypotheses and the studies illuminating their cellular mechanisms support the interrelationship between glucose and fat metabolism. This complex balance maintains homeostasis and ensures energy delivery during times of demand. The ability for hormones, metabolites, intermediaries and enzymes to signal changes in energy supply-and-demand and respond effectively to provide the appropriate energy for exercise performance has been coined ‘metabolic flexibility’ (Kelley and Mandarino, 2000). This concept was proposed to describe the reduced capacity for insulin resistant subjects to alter fuel selection and accommodate exercise (Kelley and Mandarino, 2000). Such metabolic inflexibility during exercise - perhaps in association with reduced insulin sensitivity - could limit exercise performance, for example by failing to provide sustained energy from fat oxidation during endurance exercise or by failing to “crossover” to higher glucose oxidation during intense exercise.

Exercise training

Exercise training describes the performance-improving adaptation to frequent exercise. Exercise training ensures survival of both prey (flight) and predator (fight) and may represent the biological ‘norm’ as opposed to the sedentary state of modern homo sapiens (Stannard and Johnson, 2004). Major exercise-training adaptations increase energy availability and the efficiency of energy utilization.

Increased blood flow to frequently exercised tissue increases the arrival of regulatory hormones and substrates to receptors and transporters (Sakamoto et al., 1999). At the same time, glucoregulatory hormones (e.g. glucagon, catecholamines and insulin), demonstrate blunted responses in exercise trained subjects (Geor et al., 2002; Gyntelberg et al., 1977; Sakamoto et al., 1999), potentially deinhibiting insulin action. Increased capacity for exercise-trained skeletal muscle to oxidize fat further spares limiting glucose. This improvement is mediated by increased mitochondria and oxidative enzymes and a shift towards the contractile properties of type I oxidative muscle fibers (Brooks and Mercier, 1994; Gondret et al., 2005; Manetta et al., 2002). Meanwhile, up-regulation of gluconeogenic enzymes increases lactate turnover and replenishes

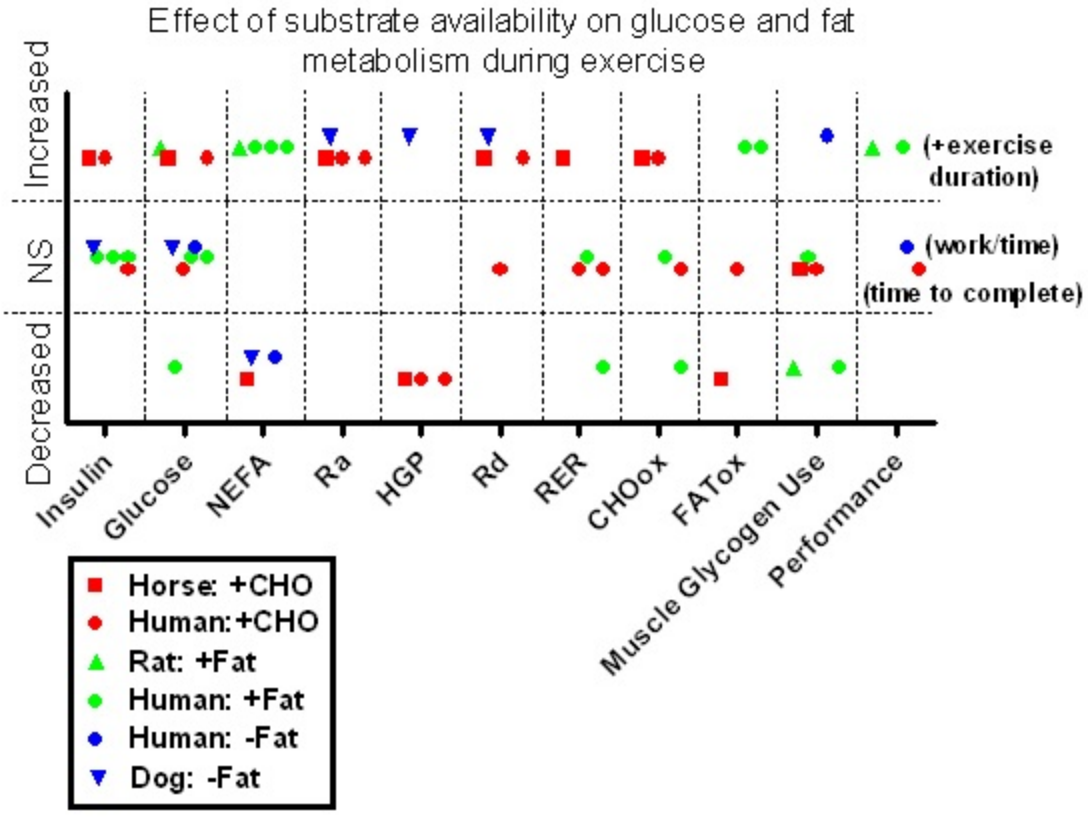
plasma glucose concentrations (Donovan and Sumida, 1990; Turcotte and Brooks, 1990).

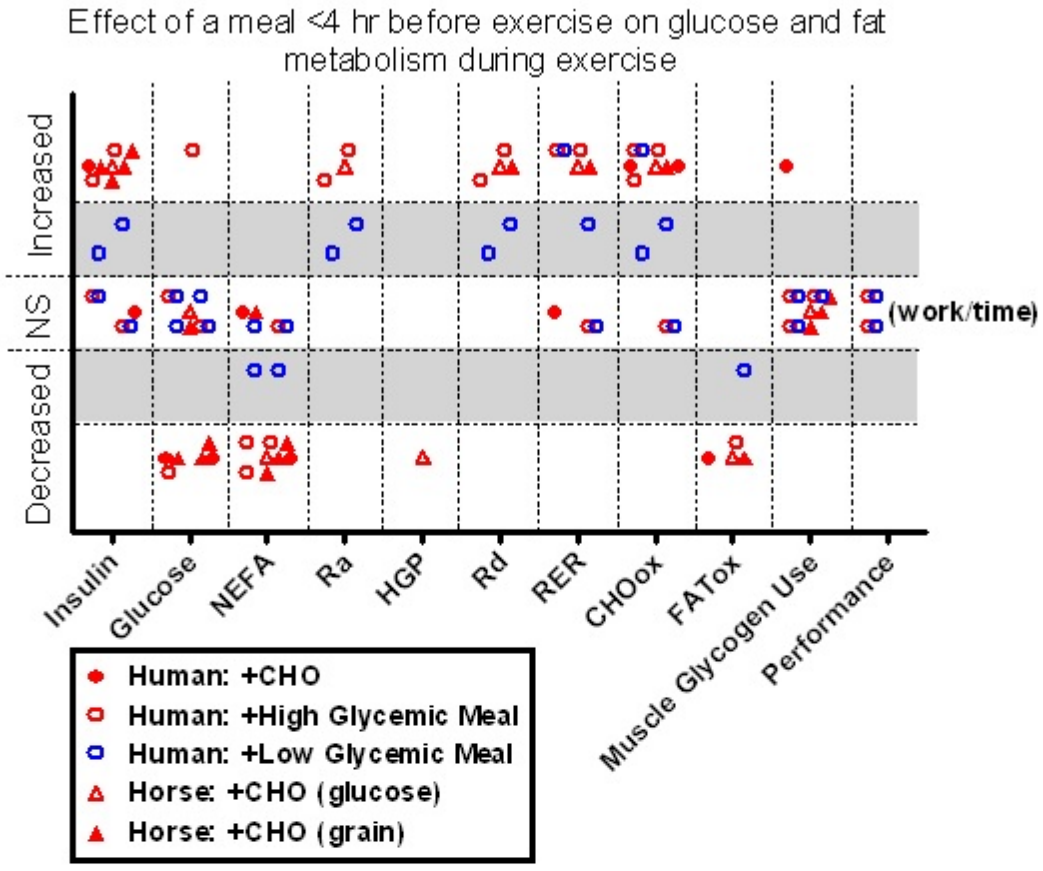
Training also increases the capacity of skeletal muscle to metabolize glucose, up-regulating enzymes such as hexokinase and succinate dehydrogenase (Donovan and Sumida, 1990; Tokuyama et al., 1993). This stream-lining of intracellular glucose metabolism may promote more efficient glucose uptake and insulin sensitivity (Saltiel and Kahn, 2001). Decreased glycogenolysis could also de-inhibit glucose uptake into cells (Donovan and Sumida, 1990). Perhaps the most potent exercise-training effect on the V_{max} of glucose uptake is up-regulation of insulin-independent GLUT-1 and insulin-responsive GLUT-4 glucose transporters (Ploug et al., 1990). These transporters increase glucose-stimulated, insulin-stimulated, and contraction-stimulated glucose uptake into trained muscle (Ploug et al., 1990).

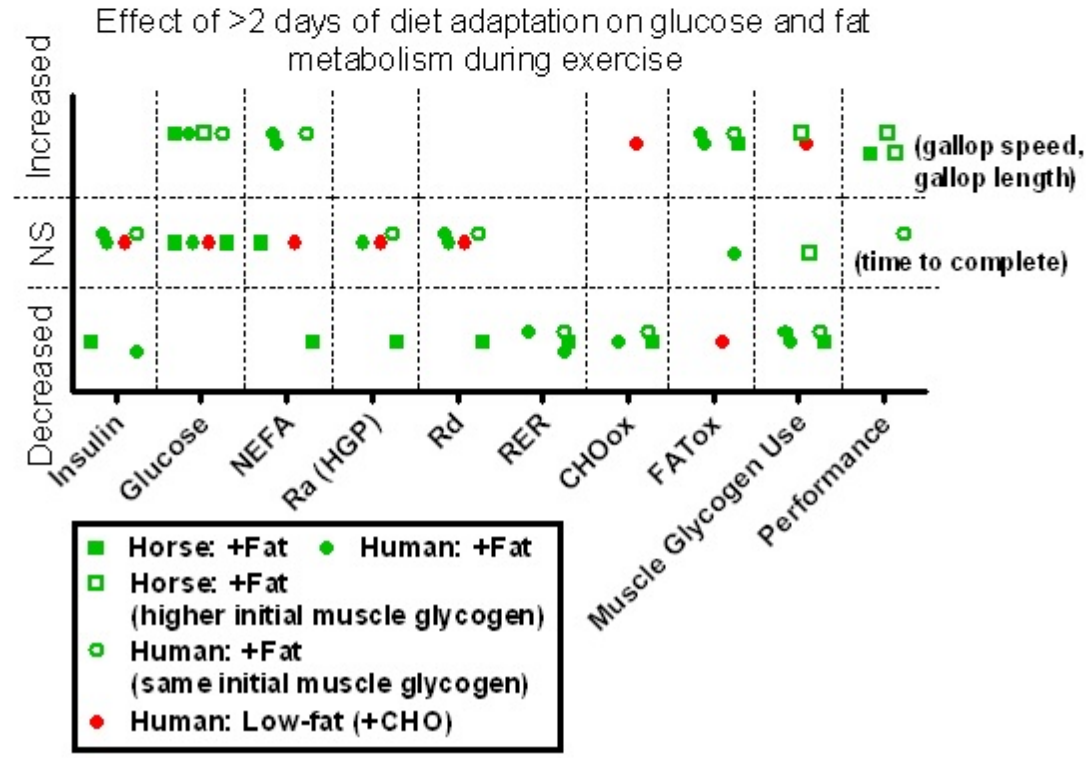
Exercise and diet

A feeding regime to maximize exercise performance has been long sought for human and animal athletes. This regime would promote the most efficient use of energy by optimizing energy storage, accessibility and replenishment. Key factors in this optimization are the availability of suitable substrate (glucose, NEFA) and signaling by energy regulating hormones (e.g. insulin, glucagon) conducive to mobilization and utilization of substrate. Timing represents another essential factor as the dynamics of energy metabolites and regulators influence the capacity for recruitment of energy by contracting skeletal muscle. Therefore the effect of various feed formulations, applied at different times relative to exercise has been evaluated in terms of energy availability and utilization and, ultimately, exercise performance. Results from studies in humans and animals are summarized in Figure 1.4.

Figure 1.4. Responses of metabolic parameters to manipulations of energy substrate during (A), immediately prior to (B) and repeatedly (C) prior to exercise. Administration of carbohydrate (+CHO) includes glucose infusion, oral glucose bolus, or high-carbohydrate meal. Administration of fat (+Fat) includes lipid infusion, heparin-stimulated fatty acid release, or fat-supplemented meal. Suppression of fat (-Fat) refers to nicotinic acid treatment. Significant differences for treatments are indicated as increased or decreased from controls. Abbreviations: NS, non-significant; NEFA, nonesterified fatty acids; Ra and Rd rate of glucose appearance or disappearance (mg/min); HGP, hepatic glucose production; RER, respiratory exchange ratio; CHOox, carbohydrate oxidation; FATox, fat oxidation. Data adapted from Bergstrom et al., 1969; Costill et al.,1977; Oldham et al., 1990; Hargreaves et al., 1991; Montain et al., 1991; Harkins et al., 1992; Custalow et al., 1993; Lawrence et al., 1993; Bracy et al., 1995; Eaton et al., 1995; Lawrence et al., 1995; Bosch et al., 1996; Febbraio and Stewart, 1996; Burke et al., 1998; Duren et al., 1999; Jeukendrup et al., 1999; Pitsiladis et al., 1999; Burke et al., 2000; Febbraio et al., 2000; Geor et al., 2000ac; Schrauwen et al., 2000; Coyle et al., 2001; Helge et al., 2001; Pagan et al., 2001; Carter et al., 2004 and Zderic et al., 2004.




Effect of a meal <4 hr before exercise on glucose and fat metabolism during exercise
Increased
NS
Decreased
(work/time)
Insulin
Glucose
NEFA
Ra
HGP
Rd
RER
CHOox
FATox
Muscle Glycogen Use
Performance
Human: +CHO
Human: +High Glycemic Meal
Human: +Low Glycemic Meal
Horse: +CHO (glucose)
Horse: +CHO (grain)


Effect of >2 days of diet adaptation on glucose and fat metabolism during exercise
Increased
NS
Decreased
(gallop speed, gallop length)
(time to complete)
Insulin
Glucose
NEFA
Ra (HGP)
Rd
RER
CHOox
FATox
Muscle Glycogen Use
Performance
Horse: +Fat
Human: +Fat
Horse: +Fat (higher initial muscle glycogen)
Human: +Fat (same initial muscle glycogen)
Human: Low-fat (+CHO)

Provision of exogenous energy during exercise has been hypothesized to spare endogenous stores and maintain circulating metabolites to prolong exercise. At the same time, changes in circulating metabolites may stimulate regulatory hormones or result in an accumulation of metabolic intermediates, potentially limiting substrate mobilization, uptake and utilization and reducing metabolic flexibility.

In the cycling human and running horse, ingestion of carbohydrates during exercise has been shown to increase circulating glucose and insulin and the rate of glucose arrival in the plasma but suppress EGP (Bosch et al., 1996; Carter et al., 2004; Geor et al., 2000c). Some but not all of these studies reported increases in rate of glucose disappearance from the plasma, RER, and carbohydrate oxidation, but a decrease in circulating NEFA and fat oxidation. No change in muscle glycogen utilization or performance (as measured by the time required to complete a set amount of work) were observed. These results suggest an increase in the use of the readily available exogenous carbohydrate, compensated for by a decrease in fat utilization.

In rats and humans, a heparin-induced increase in circulating NEFA has been associated with increased fat oxidation and decreased carbohydrate oxidation including decreased oxidation of muscle glycogen (Costill et al., 1977; Hargreaves et al., 1991; Hickson et al., 1977; Pitsiladis et al., 1999). Conversely, increased carbohydrate oxidation and muscle glycogenolysis were observed in exercising humans and dogs treated with nicotinic acid to inhibit lipolysis and therefore fat oxidation (Bergstrom et al., 1969; Bracy et al., 1995). Prolonged exercise before exhaustion has been demonstrated in heparin-treated rats and humans (Hickson et al., 1977; Pitsiladis et al., 1999). Manipulations of NEFA availability during exercise therefore appear to impact the utilization of both circulating glucose and muscle glycogen.

Another strategy for manipulating available energy substrate is to provide fat or carbohydrates (usually as a meal) prior to beginning exercise. Both the timing and composition of the meal influence metabolism during exercise. In humans, 2 g carbohydrate/kg BW ingested 2 or 4 h prior to exercise resulted in decreased circulating

glucose and NEFA, and increased carbohydrate oxidation during cycling exercise compared to ingestion 8 or 12 h prior to exercise, with 6 h proving intermediate (Montain et al., 1991). Similarly, horses fed a meal 3 h prior to exercise resulted in decreased circulating glucose and increased insulin during exercise compared to horses fed 8 h or 12 h prior to exercise (Duren et al., 1999). One difficulty in performing such tests is the fact that meals or exercise must occur at different times of day for each treatment, with diurnal patterns potentially affecting hormone signaling and meal response.

The effect of pre-exercise meals on metabolism during exercise is attributable to post-prandial fluctuations in metabolites and hormones. Generally these meals contain primarily carbohydrate as carbohydrate is considered the limiting fuel source for exercise performance. The meals are generally high-glycemic, promoting postprandial increases in glucose and insulin. This increase in insulin may carry over into exercise despite the exercise-induced drop in insulin. At the same time, glucose disposal rate tends to increase, probably attributable to increased carbohydrate oxidation and insulin action, with glucose sometimes dropping below the levels maintained by fasted subjects, perhaps also due to insulin-suppression of EGP which has been demonstrated in the horse (Geor et al., 2000a). Lipolysis suppression by insulin may explain the observed decrease in circulating NEFA and fat oxidation during exercise following a carbohydrate meal. Despite these changes in circulating fuel sources, only one study in humans observed increased muscle glycogen use in subjects ingesting carbohydrates prior to exercise (Costill et al., 1977), whereas a number of studies reported no difference (Febbraio and Stewart, 1996; Jose-Cunilleras et al., 2002). These results indicate an increase in the utilization of plasma glucose becomes available from the pre-exercise meal and compensating for the suppressed utilization of fat energy sources.

Studies in humans comparing the influence of high- and low-glycemic meals prior to exercise observed that subjects who ingested low-glycemic meals demonstrated metabolic changes intermediary between subjects who ingested high-glycemic meals and fasted subjects (Burke et al., 1998; Febbraio et al., 2000; Jeukendrup et al., 1999). Neither high- or low-glycemic meals were shown to influence the amount of work (cycling) each subject was capable of completing in 15 or 30 min (Febbraio et al., 2000;

Febbraio and Stewart, 1996). However these studies observed only a transient drop in circulating glucose in the meal-fed groups and this disappeared before the performance test, therefore the influence of post-prandial exercise hypoglycemia observed in other studies (including 3 in the horse) was not addressed in terms of performance.

Longer term adaptation to specific diets may prove more beneficial to exercise performance by influencing the functional capacity of skeletal muscle cells similar to the effects of training. Alterations in membranes, transporters, enzyme function, and sensitivity to hormonal signaling are examples of mechanisms responsive to persistent dietary signals. High carbohydrate diets are typical for athletes (human and horse) and may be used to increase muscle glycogen content, with the perceived benefit of increasing exercise endurance (Bosch et al., 1996). However recent attention had fallen upon fat-supplemented diets on the premise that increased fat oxidation during exercise could spare limited carbohydrate fuel. This 'sparing' could result from increased oxidative enzymes such as citrate synthase and acyl-CoA dehydrogenase (Dunnett et al., 2002) and increased IMTG providing a ready source of lipid fuel (Stannard and Johnson, 2004). In the horse, fat-supplementation up to ~12% DE has actually been shown to increase muscle glycogen storage, perhaps by reducing turnover (Hambleton et al., 1980; Harkins et al., 1992; Oldham et al., 1990).

Studies in fat-adapted humans and horses have demonstrated a tendency for decreased insulin during exercise, increased circulating glucose, increased circulating NEFA (in humans) but potentially decreased NEFA in horses (Pagan et al., 2002), a reduction in respiratory exchange ratio and carbohydrate oxidation compensated for by increased fat oxidation (Coyle et al., 2001; Duren et al., 1999; Schrauwen et al., 2000). Several studies have also demonstrated a sparing effect on muscle glycogen (Helge et al., 2001; Pagan et al., 2002; Zderic et al., 2004). This 'sparing' effect may be attributable to lower resting muscle glycogen stores and indeed one study in horses where muscle glycogen increased with fat-adaptation, muscle glycogen use was also higher (Oldham et al., 1990). However in another study in humans, fat supplemented humans were given a carbohydrate meal prior to exercise which normalized resting muscle glycogen concentrations to controls (Burke et al., 2000). Compared to controls,

these fat-adapted subject still demonstrated increased fat oxidation during cycling while using 30% less muscle glycogen, although there was no significance performance difference (time to complete set work) between diet groups.

Fat supplemented horses have demonstrated improved exercise performance in several studies. Horses supplemented with fat have shown prolonged run times during incremental speed tests compared to controls (Eaton et al., 1995). Fat supplemented Arabians have also demonstrated lower heart rates and less acidosis during repeated sprints (Duren et al., 1999), and lactate breakpoints at higher speeds (Custalow et al., 1993). These changes reflect decreased contribution by carbohydrate energy sources and perhaps improved lactate turnover (Turcotte and Brooks, 1990). Glucose oxidation in fat-adapted horses could be inhibited by intermediates of fat metabolism (Randle et al., 1963). However, more rapid increases in lactate following the breakpoint have been observed in fat-adapted horses and attributed to more efficient regulation of glycolysis (Kronfeld et al., 2000; Taylor et al., 1995). Fat-adapted horses have also demonstrated faster gallop times over 600 and 1600 m (Harkins et al., 1992; Oldham et al., 1990). These findings suggest increased metabolic flexibility to adapt fuel utilization to demand in fat-adapted horses and may explain improved performance during high- as well as low- intensity exercise (Kelley and Mandarino, 2000). This metabolic flexibility may be potentiated by decreased circulating insulin in fat-adapted horses (Duren et al., 1999) and improved insulin sensitivity.

Differences in the effects of fat-adaptation observed in humans and horses may arise from differences in diet composition. Fat-supplemented diets in humans are 30 to >60% fat, whereas as recommended fat-supplementation in horses is ~10% DE (Harris and Kronfeld, 2002). Thus in humans a greater direct influence of fat on metabolism might be expected, whereas in the horse, fat-supplementation may exert its effect by moderating the effects of a high carbohydrate diet (e.g. reliance on carbohydrates and insulin resistance) which limit the ability for cells to switch between fuel sources. This could explain why horses supplemented with dietary fat tend to display increased resting muscle glycogen, whereas fat-supplemented humans show decreased muscle glycogen (Burke et al., 2000; Oldham et al., 1990), and why fat-supplemented horses

(but not humans) display improved performance during high-intensity exercise tests (Harkins et al., 1992; Kronfeld et al., 2000).

Glucose kinetics

Glucose kinetics describe the distribution and flow of glucose through circulation, providing information about glucose availability, with a compartmental representation of the physiological system. Each compartment describes a population of glucose units moving in the same manner with a space. This space likely represents a physiological compartment through which glucose distributes and from which it exits according to a specific mechanism. However if physiological spaces exchange very rapidly (e.g. plasma and erythrocytes) or very slowly sampling schemes may not permit differentiation of these spaces, therefore observed compartments often represent composites of several physiological compartments. Conversely if multiple mechanisms of loss exist for a physiological space then units within that space may demonstrate differing kinetics therefore multiple compartments would describe this space.

Linear models describe systems in which the response to individual input components sum to determine the observed response. In other words, the transport of units from a compartment is proportional to the mass of that compartment (Carson et al., 1983). During steady-state (i.e. when the mass leaving a compartment equals the mass entering, thus the compartment mass is constant) the conditions for linearity are met. Also tracer doses resulting small or negligible perturbation of this steady-state must therefore describe a linear system (Shipley and Clark, 1972). By measuring output from linear, steady-state system, tracer studies allow for algebraic solving of unknown parameters to describe the compartmental system.

Intrinsically, the glucose system is unlikely to be linear due to the complex nature of the glucose transport system which includes circulation and distribution variability and dozens of transporter types each with unique properties (Ader et al., 1997; Scheepers et al., 2004; Zierler, 1999). Michaelis-Menten mechanics have been touted as a more appropriate representation as it describes the saturatability of the system (Zierler, 1999), although this presents a hardly less radical simplification. It has been stated that linear

assumptions are invalid when saturatable (i.e.Michaelis-Menten) processes exist (Gottesman et al., 1983), but this claim needs to bequalified. In the event that Km (Michaelis constant, proportional to the binding affinity) is much smaller or much larger than the concentration of circulating glucose, Michaelis-Menten kinetics reduce to a linear or constant model.

The major insulin-independent glucose transporters include GLUT-1, GLUT-2 and GLUT-3, which are expressed in various tissues with GLUT-1 predominating (Scheepers et al. 2004). GLUT-3 has the lowest influx Km compared to basal circulating glucose concentrations and therefore may be assumed to be saturated, representing a constant component of glucose uptake (Zierler, 1999). GLUT-1's Km is just above basal glucose concentrations, suggesting that this transporter will be saturated during hyperglycemia (Gould and Holman, 1993) although as glucose concentrations return towards baseline GLUT-1 may transport more glucose relative to the circulating concentration. GLUT-2 has received increasing focus for its role in regulating glucose homeostasis both because it may provide glucose sensing to the pancreatic B-cells and because of its very high Km, which renders this transporter virtually unsaturatable (Gould and Holman, 1993). Thus GLUT-2 glucose transport is proportional to glucose concentrations and may determine the linear patterns observedin glucose disposal (Ader et al., 1997).

Another property of the system of glucose disposal is the fact that transporter capacity and Km are influenced by intracellular metabolism. Enzymes of glucose metabolism may present rate-limitations or enhancements to glucose uptake (Perriott et al., 2001). Most likely, glucose metabolism is upregulated during hyperglycemia and therefore Km values would be increased, allowing for proportional glucose uptake and further explaining the linear trends observed in clamp studies (Ader et al., 1997). In addition, glucose has been shown to stimulate translocation of 'insulin-dependent' GLUT-4 transporters to the cell surface (Bandyopadhyay et al., 2001), further increasing the proportionality of glucose transport to glucose concentration and decreasing the argument for saturation. These adaptations ensure glucose arrival as an energy source for functioning tissue.

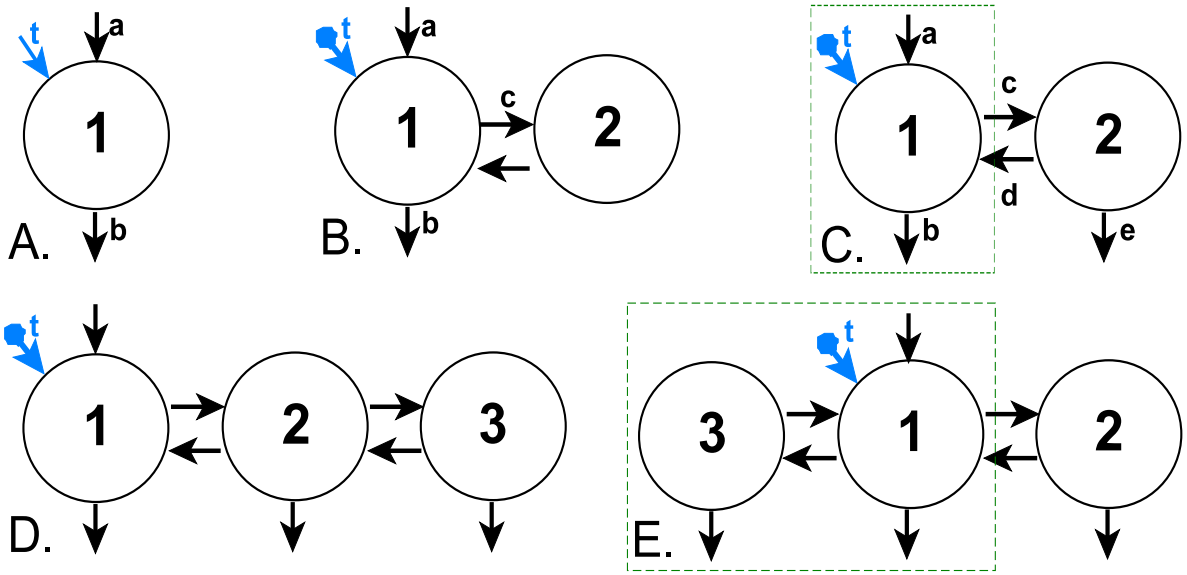


Figure 1.5. Compartmental models of glucose kinetics. A) 'Noncompartmental' model of Steele used to evaluate tracer infusions. where t is the tracer infusion rate, a is the rate of appearance (Ra, mg/min), and b is the rate of disappearance (Rd, mg/min). B) Two-compartmental model used in early single-injection tracer studies where a+c is the total entry rate, b is the irreversible loss, and c is the recycling. C) Full, unidentifiable two-compartmental model. D) Catenary 3-compartment model. E) Mammillary 3-compartment model. Boxes with broken lines indicate how the primary compartment in model C is a simplification of the primary and the rapidly exchanging peripheral compartment of model E.

Constant and primed infusions of tracer glucose are used extensively to estimate glucose loss from the compartment containing plasma (where infusions and sampling occur). Infusion studies assume that all glucose enters and leaves from one compartment (Steele et al., 1956) (Figure 1.5A). The disposal rate of glucose from the compartment is determined by achieving tracer steady-state (i.e. matching glucose loss with the infusion rate) and observing the proportion of tracer within the compartment. Glucose primed-infusion kinetics have been applied to many species and conditions (Carter et al., 2004; Chandrasena et al., 1979; Ford and Evans, 1982).

Single-injection tracer glucose kinetics is a method used predominantly in the 1970's. This method involves introducing a tracer bolus into the glucose systems and observing its rate of loss from the sampled compartment. Because the initial tracer mass is known (e.g. 1000 units) and assuming these units are instantaneously distributed throughout the compartment, the inital tracer concentration can be used to calculate the volume of the compartment into which the tracer is injected.

The curve describing the disposal of tracer from the system involves a sum of linear disposal equations with each term describing a unique route of loss from the sampled compartment. This curve is fit by a multi-term exponential decay (the sum of the linear loss terms). The observational parameters of this fit describe the complex system of sampled and nonaccessible glucose compartments. Examples of compartmental models suggested and modified for the glucose system are shown in Figure 1.5.

Single-injection tracer studies were performed in horses and ponies in the 1970s. These studies compared glucose transport from and the size of the sampled glucose pool in controls to animals that were fasted (Anwer et al., 1976; Argenzio and Hintz, 1970), exercise-trained, gestating or lactating (Evans, 1971). Only one study in equines considered glucose disposal from a secondary non-accessible compartment (Argenzio and Hintz, 1972). Single-injection tracer kinetics have been performed in sheep to characterize glucose disposal during pregnancy (Bergman, 1963; Steel and Leng, 1973) and a three-compartment mammillary model was used to estimate glucose in the thoracic lymph duct (i.e. interstitial fluid) (Gastaldelli et al., 1997). Glucose kinetics in cattle have also been characterized with a catenary three-compartment model of glucose kinetics during rest, fasting, insulin-treatment, and ketosis (Kronfeld, 1977; Kronfeld et al., 1971). Glucose kinetics have been modeled from the single-injection method in the rat (Baker et al., 1959), human (Ferrannini et al., 1985; Insel et al., 1975), and rabbit (Atkins, 1980b).

Infusion and single-injection techniques for tracer studies have been compared theoretically and experimentally. Experimentally, estimates of glucose turnover from single-injection and infusion methods in piglets have revealed no significant difference between techniques (Pegorier et al., 1984; Wootton et al., 1987). Similar estimates were also found from a comparison of methods in cows (Kronfeld et al., 1971). The infusion method is touted for the simplicity of requiring only the establishment of a plateau and as opposed to the single-injection technique which requires a complete and well-defined curve. However establishment of an asymptotic plateau may not be as straightforward as claimed due to recycling and the dynamic nature of the glucose system (Geor et al., 2000c; Kronfeld and Ramberg, 1981). In the event that a plateau is not established, a non-steady state model had been applied to infusion data (Steele, 1959). More complex dynamic modeling of the the single-injection technique is also possible. Single-injection studies can provide a more detailed desciption of the glucose system compared to infusions (Cobelli et al., 1992). These estimates rely in part on assumptions about the construction of the glucose system (i.e. number of compartments and their interaction). However such assumptions are founded on biophysical principles and physiological hypotheses which are tested by the fit of the data and by logical physiological interpretation of the results (DiStefano and Landaw, 1984).

Compartmental analysis of glucose kinetics

Compartmental analysis of stable isotope glucose tracer kinetics begins with fitting an exponential decay equation to the disposal curve of the tracer in the form of :

$$y(t) = A_1 \cdot e^{-a_1 \cdot t} + A_2 \cdot e^{-a_2 \cdot t} + \dots A_n \cdot e^{-a_n \cdot t} \qquad (1)$$

where y(t) is the fraction of the tracer dose per volume (e.g. [tracer mg / dose mg]·L^{-1}) at time = t. The number of terms (n) reveals the number of unique exchanges from the sampled compartment, one representing irreversible loss and each additional term

a compartment exchanging with the injection site (Shipley and Clark, 1972; Wastney et al., 1999). Therefore n is the number of compartments in the system. From the eigenvectors (A_n) and eigenvalues (a_n), several details of the system can be calculated. Firstly the intercept of the curve can be fitted:

$$y(0) = A_1 \cdot e^{-a_1 \cdot 0} + A_2 \cdot e^{-a_2 \cdot 0} + \dots A_n \cdot e^{-a_n \cdot 0} = A_1 + A_2 + \dots A_n \quad (2)$$

Because this point represents the initial dilution of the tracer in the sampled compartment, the volume of the sampled compartment (V1) can be determined:

$$y(0) = \left(100\% \cdot \frac{tracer_0}{tracerdose} \right) \Big/ V1 = \frac{1}{V1} \quad (3)$$

Where $tracer_0$ is the mass of tracer at time = 0, which is equivalent to the initial tracer dose. Therefore from equations 2 and 3:

$$V1 = \frac{1}{A_1 + A_2 + \dots A_n} \quad (4)$$

From the simultaneous steady-state plasma glucose concentration, the mass of glucose in compartment 1 (Q1) can be determined:

$$Q1 = G(t) \cdot V1 \quad (5)$$

where G(t) is the steady-state glucose concentration in the sampled compartment.

The parameters for A_n and a_n can also be used to derive turnover rate constants (kii, min^{-1}) which describe the total rate of glucose loss from compartment i:

$$kii = -\sum_j kji \quad (6)$$

where k is the rate constant of glucose travel, and j comprises the compartments interchanging with compartment i so that kji is the rate of glucose moving from compartment i to compartment j. Also derivable are the products of the flow rate from

compartment i to i+1 and the flow rate back from i+1 to i (ki,i+1·ki+1,i; e.g. k12·k21),. However it is not possible to solve for rate constants (kij) independently unless certain assumptions are made. For example: in model B of Figure 1.5 the rate of irreversible flow from compartment 2 (i.e. k02) is assumed to equal zero. Applying equation 9 to model B:

$$k22 = -(k02 + k12) \tag{7}$$

For this model k02 is zero, therefore k22 and -k12 are equivalent. Subsequently by knowing k11 and k12·k21 and applying equation 9 to the primary compartment of model B:

$$k11 = -(k01 + k21) \tag{8}$$

all rate constants from model B can be identified. Model B is thus considered to be uniquely identifiable.

When unique values for all rate constants cannot be determined (e.g. model C, Figure 1.5) the model is unidentifiable. In this case, minimum and maximum bound for the unidentifiable rate constants can be calculated by systematically setting each output to its minimum value (i.e. zero) (Chen et al., 1985; DiStefano, 1983; Landaw et al., 1984). These determinations are detailed in Part I, Chapter 3: Supplement 1.

From the determined or bounded rate constants and the known mass of compartment 1 (Q1), glucose transport flow rates (Rij, mg/min) are determined:

$$Rij = kij \cdot Qj \tag{9}$$

By definition, in the steady state the mass of glucose arriving in each compartment is equivalent to the mass leaving. From the above knowledge, bounds or unique solutions for the mass of nonaccessible compartments can be determined and from these, bounds or solutions for the compartment's volume and flow rates (DiStefano et al.,

1988; Vajda et al., 1989; Vicini et al., 2000). In addition, the total transport of glucose from the system is determined by setting all losses (R0i) save one equal to zero. During steady-state, transport of glucose from the system must equal the flow of glucose into the system (i.e. EGP).

Compartmental volumes provide indications of the size of spaces where glucose exchanges within the body. The total volume of the glucose space has been shown in numerous species to approximate extracellular fluid volume of 20-25% bodyweight (BW) (i.e. vascular volume and interstitial fluid) (Evans, 1971; Insel et al., 1975; Katz et al., 1974). Generally the sampled compartment includes circulation (plasma and erythrocytes which rapidly equilibrate). If irreversible loss (i.e. glucose entering tissue for utilization) from this compartment the sampled compartment probably includes interstitial fluid in rapid exchange with plasma (Jacquez, 1992). This hypothesis is supported by studies which estimate the volume of the sampled compartment close to or just surpassing expected vascular volume (Atkins, 1980b; Kronfeld and Ramberg, 1981). A second compartment demonstrating irreversible loss is taken to represent the remaining slowly exchanging component of interstitial fluid, possibly representing the glucose being cleared by insulin-mediated means (Cobelli et al., 1984).

Several three compartment models have been suggested for glucose kinetics. Primarily, a mamillary model has been considered, where the sampled compartments exchanges with two peripheral compartments, one rapidly exchanging and one slowly exchanging (Cobelli et al., 1984) (Figure 1.5E). This model essentially separates the plasma component of the sampled compartment of the two compartment model (Figure 1.5C) from the rapidly exchanging erythrocytes and interstitial fluid. A catenary three compartment model of glucose kinetics has also been explored, where the sampled compartments exchanges with an intermediary compartment which in turn exchanges with a peripheral compartment (Figure 1.5D). This model has been interpreted as representing spaces similar to those in the two compartment model, with the most peripheral pool comprised of glucose precursors or derivatives (Kronfeld, 1977; Kronfeld et al., 1971).

Glucose kinetics and diet

Glucose kinetics have been evaluated in a number of species under different nutritional treatments to determine the influence of diet on glucose transport. The majority of these studies compare the fed and fasting states, demonstrating decreased transport of glucose from the plasma compartment in unfed animals (Anwer et al., 1976; Argenzio and Hintz, 1972; Chandrasena et al., 1979; Ford and Evans, 1982; Kronfeld et al., 1971; Pegorier et al., 1984; Steel and Leng, 1973). No change in glucose distribution space was observed with fasting. Interestingly irreversible glucose loss from the plasma compartment in these studies were similar across species (sheep, cows, ponies, horses and camels) under fed (approximately 1.3 to 2.5 mg/min per kg) or fasting conditions (approximately 0.7 to 1.1 mg/min per kg). The reduction in glucose flow during fasting is attributable to decreased circulating glucose concentrations, utilization of alternative energy sources (stored fat), or increased insulin resistance (a possible thrifty adaptation) which is observed during fasting (Forhead and Dobson, 1997).

Diets which differ in the delivery of glucose to the circulation have also been studied to determine their effect on glucose flow. Consistent with the effect of fasting on glucose kinetics, restriction of dietary energy has been shown to decrease the transport of glucose from the plasma (Ortigues-Marty et al., 2003; Steel and Leng, 1973). This relationship between energy intake and glucose kinetics is a complicating factor in studies comparing diet compositions.

Low-roughage/increased concentrate diets have been shown to increase the transport of plasma glucose in ruminants (Evans and Buchanan-Smith, 1975). This effect in ruminants has been demonstrated primarily by studies in which corn is a major portion of the concentrate (Ortigues-Marty et al., 2003). Corn is rich in hydrolyzable carbohydrates which contribute substantially to glucose delivery to the circulation. In addition to increasing glucose transport, high-concentrate diets interact with increased dietary energy to further impact glucose kinetics in animals (Evans and Buchanan-Smith, 1975; Ortigues-Marty et al., 2003). Similarly increasing the proportion of dietary

carbohydrate for humans has been suggested to increase the rate of glucose transport. However studies of humans on high-carbohydrate diets have not found differences in resting glucose transport. Rather, carbohydrate oxidation is increased by higher carbohydrate diets, suggesting increased utilization of muscle glycogen rather than circulating glucose (Coyle et al., 2001; Koutsari and Sidossis, 2003; Zderic et al., 2004). The different results for ruminants and humans suggests a physiological difference in the handling of readily available circulating glucose or the difference in diets studied (e.g. high-fiber roughage vs fat supplementation).

Only one study has explored the effect of diet adaptation on glucose kinetics in equines (Argenzio and Hintz, 1972). Using tracer glucose infusion, this study demonstrated increased glucose turnover and utilization in 4 ponies when adapted to a diet comprising oats and with higher soluble-carbohydrate content compared to an alfalfa and beet-pulp diet. This study also applied the single-injection tracer method and a 2-compartment model to the same ponies, but the results are inconclusive due to application to only 2 ponies per diet group and undisclosed model assumptions.

Dietary protein could also play a role in glucose kinetics as protein is a substrate for gluconeogenesis. Infusing casein into the abomasum of sheep on various rations increased the rate of glucose transport (Lindsay and Williams, 1971). Unfortunately this study was confounded by the increase in metabolizable energy from the added protein.

Glucose kinetics and insulin

Insulin is the primary regulator of glucose homeostasis and is therefore expected to significantly influence glucose kinetics. In cows glucose transport was not altered by 10 d treatment with intramuscular insulin injections and concommitant hypoglycemia (Kronfeld et al., 1971). Rather, insulin treatment significantly increased the glucose space possibly indicating increased cell penetration and permeability by glucose resulting in glucose exchange with the intracellular space. A similar effect on the glucose distribution volume has been observed in humans (Ferrannini et al., 1985). In this latter study, euglycemia was maintained during an insulin infusion to eliminate confoundment by hypoglycemia. Under euglycemic conditions, insulin significantly

increased glucose tranport from the plasma and suppressed EGP (Ferrannini et al., 1985). Further it has been hypothesized that the flow of glucose from the slowly exchanging compartment represents insulin-dependent glucose uptake (Cobelli et al., 1984). Therefore the change in the rate of glucose disposal from the slowly exchanging compartment per unit change in insulin has been suggested as an index of insulin sensitivity (Insel et al., 1975).

Glucose kinetics and exercise

In general, characterization of the glucose system during exercise has used the tracer glucose primed-infusion method and single-compartment model of Steele which determines the rate of glucose appearance (Ra, mg/min) from the dilution of equilibrated tracer (corrected for the infused glucose) in the sampled compartment (Steele et al., 1956). This method requires a separate determination of the compartment volume. Because kinetics experiments involving exercise commonly stray from the assumption of steady-state, 'non-steady state' estimates for Ra and glucose disappearance (Rd) are determined incrementally, with Rd calculated from the difference between Ra and the change in the glucose concentration (Radziuk et al., 1978).

In trained horses, Ra was 1.5 ± 0.1 mg/min per kg at rest, 5.5 ± 0.7 mg/min per kg after 30 min of exercise at 30% VO_{2max}, and 9.9 ± 0.8 mg/min per kg after a subsequent 30 min exercise at 60% VO_{2max} (Geor et al., 2000b). Because the glucose infusion was a negligible portion in this study, these values for Ra largely reflect EGP. The Rd, identical to Ra during the steady-state rest period also increased during exercise to 5.0 ± 0.4 mg/min per kg after 30 min exercise at 30% VO_{2max} and 6.8 ± 0.5 mg/min per kg after 30 min exercise at 60% VO_{2max}. Similar increases in Ra and Rd during exercise at 30% VO_{2max} describe the lack of change in circulating glucose concentration, while the disparity between Ra and Rd at higher intensity reflects the steadily increasing plasma glucose concentrations observed. Similarly 60 min of exercise at 55% VO_{2max} resulted in an increase in Ra from approximately 1.5 mg/min per kg to a plateau after about 30 min at approximately 7.6 mg/min per kg, while Rd

increased to approximately 6.0 mg/min per kg again reflecting the increase in plasma glucose concentration (Geor et al., 2000b).

Untrained dogs demonstrated at increase in Ra from ~2.7 mg/min per kg at rest to ~9.1 mg/min per kg after 30 min of running at 100 m/min and 12% incline (Finegood et al., 1992). Hepatic glucose production in sheep was also reported to increase from ~1.3 mg/min per kg at rest to ~2.8 mg/min per kg during exercise (treadmill walking) (Harman and Pethick, 1986). In humans, 30 min of cycling at 55% VO_{2max} increased Ra and Rd from ~1.8 mg/min per kg to ~4.5 mg/min per kg (Kjaer et al., 1991). This increase was only about a third of the increase observed in horses at the same relative intensity (Geor et al., 2000a). Another study in humans showed an Ra of 8.7 ± 0.9 mg/kg per min after approximately 45 min of cycling at 75% VO_{2max} , however no resting reference was reported (Carter et al., 2004).

During exercise, fuel selection and consequently glucose kinetics are influenced by epinephrine which is secreted by the adrenal glands proportional to exercise intensity and perhaps exercising muscle mass (Figure 1.6) (Kjaer et al., 1991). Epinephrine has been shown to increase EGP (hence, Ra) via direct stimulation primarily of gluconeogenesis and indirectly via insulin suppression, which desupresses EGP, and subsequently increases glucagon to stimulate liver glycogenolysis (Gustavson et al., 2003; Sumida et al., 2003; Wasserman and McIlroy, 1964). Epinephrine has also been shown to reduce plasma glucose utilization (Rd) and promote compensatory muscle glycogenolysis in the horse (Geor et al., 2000a). Exercise-induced epinephrine secretion and insulin suppression also promote lipolysis, increasing circulating NEFA which may be oxidized in place of plasma glucose (Geor et al., 2000b). Thus epinephrine spares circulating glucose by increasing gluconeogenesis, glycogenolysis and lipolysis, perhaps to conserve plasma glucose to fuel the simultaneously epinephrine-stimulated central nervous system and maximize performance as during “flight or fight” response.

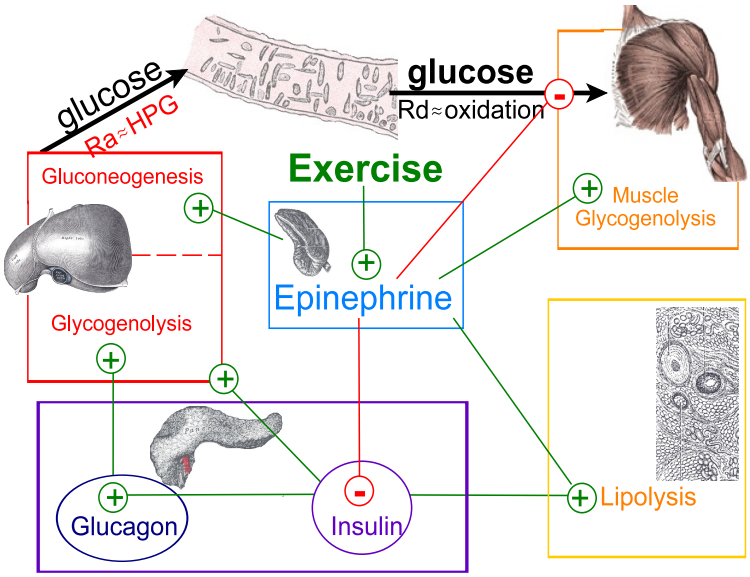


Figure 1.6. Influence of epinephrine on glucose and fat mobilization and utilization.

The effects of epinephrine on fuel selection and glucose kinetics during exercise have been demonstrated in the horse by epinephrine infusion and administration of a β-adrenergic blocker (propranolol) to prevent some of the actions of epinephrine (Geor et al., 2000a; Geor et al., 2000b). Infusion of epinephrine in horses exercising 1 hour after receiving 2g/kg oral glucose was shown to de-suppresses circulating glycerol (a marker of lipolysis) and NEFA and completely suppress the increase in exercising Rd associated with the pre-exercise glucose dose (Geor et al., 2000a). At the same time, epinephrine infusion resulted in a 75% greater decrease in muscle glycogen compared to glucose-treated and control horses. Because no change in RER was observed with epinephrine treatment, increased muscle glycogenolysis must have compensated for epinephrine suppression of plasma glucose oxidation. Blocking the β-adrenergic effects of epinephrine in exercising horses increased glucose Rd and oxidation, suppressed lypolysis and fat oxidation, and did not alter muscle glycogenolysis (Geor et al., 2000b). These studies emphasize the role of epinephrine as a regulator of circulating glucose kinetics during exercise. Unfortunately these studies failed to demonstrate the role of epinephrine on EPG in the horse. Epinephrine-infused horses demonstrated an unexpected decrease in Ra, confounded by marked hyperglycemia and possible glucose absorption from the gut (Geor et al., 2000a), while β-blockade resulted in increased Ra, perhaps due to the fact that epinephrine secretion was higher in β-

blocked horses and epinephrine continued to act on glucagon and insulin via un-blocked α-receptors (Geor et al., 2000b).

Glucose kinetics, exercise and diet adaptation

Several studies in humans have evaluated the effects of short-term diet adaptation on glucose kinetics during cycling exercise in humans using tracer infusion following an overnight fast. Trained male cyclists consumed high fat [DE: 68% fat, 19% carbohydrate] or high-carbohydrate (DE: 13% fat, 74% carbohydrate) meals for 5 days, then ingested high-carbohydrate meals for 1 day (to normalize resting muscle glycogen) before undergoing a cycling test (Burke et al., 2000). Plasma glucose concentrations were lower in the high-carbohydrate fed group from 80 min to the end of exercise (120 min) and total carbohydrate oxidation was lower in subjects adapted to high-fat meals, but diet did not significantly affect circulating glucose Ra or Rd during exercise. Thus in these subjects, diet adaptation produced a greater effect on muscle glycogenolysis than on circulating glucose utilization. Another study provided cyclists with high-fat (DE: 60% fat, 24% carbohydrate) or control (DE: 22% fat, 65% carbohydrate) for 2 d, with the final meal on the evening before exercise controlled between subjects (Zderic et al., 2004). No difference between plasma glucose concentration or circulating glucose Ra or Rd was observed, although Carbohydrate oxidation was again greater in the control group. When the same subjects were treated with a lipolysis inhibitor (depriving them of the energy source of circulating NEFA), control-fed subjects compensated by increasing glucose utilization as indicated by a 20% increase in glucose Ra and Rd, whereas high-fat fed subjects showed no change in glucose kinetics. Trained male cyclists consumed either a fat restricted diet (DE: 2% fat, 88% Carbohydrate) or a moderate fat diet (DE: 22% fat, 68% Carbohydrate) for one week, then ingested a high carbohydrate snack 9-10 h prior to exercise (Coyle et al., 2001). Plasma glucose concentrations and glucose Ra and Rd did not differ between diet groups, despite greater Carbohydrate oxidation in the fat-restricted group.

These studies indicate that increasing the fat content of meals ingested for 2 to 7 days prior to exercise increases fat oxidation during subsequent exercise independent

of the effect of the last meal and that this decrease in the relative contribution by Carbohydrate to total energy expenditure is not associated with a change in plasma glucose kinetics. Both studies which compared glucose kinetics at rest also found no differences between diet groups. These results differ from those in resting animals, from which increased circulating glucose Ra and Rd would be expected in humans adapted to high-Carbohydrate diets providing more exogenous glucose. The difference may be attributable to species, or an overwhelming effect of exercise on metabolism shifting focus to energy stores rather than circulating metabolites. Perhaps more likely factors are the short adaptation periods and the difference in diet composition for humans (relatively high-fat and high hydrolyzable-carbohydrate) compared to roughage and grain diets fed animals. Another important factor may be the effect of the final meal before the evaluating glucose kinetics, as pre-exercise meals can influence glucose kinetics (Febbraio et al., 2000; Jose-Cunilleras et al., 2002), although meals fed 8 or more hours prior to exercise do not appear to affect other aspects of metabolism during exercise (Duren et al., 1999; Montain et al., 1991).

One study in horses evaluated the effect of diet adaption on glucose kinetics during exercise by tracer primed-infusion method (Pagan et al., 2002). Horses supplemented with fat [DE: 29% fat, 53% carbohydrate (32% NSC)] demonstrated decreased glucose Ra and Rd during exercise at 35% VO_{2max} after 5 and 10 weeks of training compared to horses fed a control diet [DE: 7% fat, 74% carbohydrate (50% NSC)]. This decrease in plasma glucose utilization coincided with a decrease in muscle glycogen utilization and an increase in fat utilization. These results are consistent with metabolic adaptations associated with fat adaptation in studies which did not evaluate glucose kinetics (Custalow et al., 1993; Eaton et al., 1995; Harkins et al., 1992) and indicate that longer-term adaptation to dietary fat can increase fat utilization during exercise, and spare potentially limiting carbohydrate.

Insulin resistance

Insulin impacts not only performance, by animal and human health. Insulin resistance is a condition in which normal concentrations of insulin inadequately

stimulate insulin-dependent mechanisms (e.g. glucose uptake and possibly suppression of lipolysis). This resistance may results from changes in receptor density (possibly in response to hyperinsulinemia) (Gavin et al., 1974), but is more likely attributable to a reduction in signal transduction from the insulin receptor to the cell. Insulin binding causes autophosphorylation of the receptor tyrosine kinase which in turn activates insulin receptor substrates (IRS) (Lamothe et al., 1998) (Figure 1.7). Subsequently the mitogen-activated protein kinase (MAPK)/Ras pathway is stimulated, influencing gene expression and also, via p38 MAPK, activating GLUT-4 transporters at the membrane (Furtado et al., 2002). The phosphatidylinositol 3- kinase (PI 3-kinase) pathway is also activated contributing to further changes in gene expression as well as initiating the translocation of intracellular GLUT-4 to the cell membrane where it can influence glucose uptake (Furtado et al., 2002; Lamothe et al., 1998). These pathways may be disrupted by changes in the concentrations of these intermediates or their activity due to signals from intracellular processes, namely metabolism (Saltiel and Kahn, 2001). In particular, stimulation of protein kinase C (PKC) may deactivate the receptor tyrosine kinase or downstream kinases via serine phosphorylation (Saltiel and Kahn, 2001; Shafrir and Ziv, 1998).

The onset of insulin resistance is marked by increases in pancreatic insulin secretion and possibly decreased insulin clearance which compensate for the hormone's inefficiency (Kahn, 1978; Yoshii et al., 2006). Persistently increased insulin concentrations may contribute to down-regulation of insulin-responsive mechanisms or provide inappropriate regulatory signals that could contribute to diseases such as developmental orthopaedic disease, hyperlipidemia and laminitis (Jeffcott and Field, 1985; Ralston, 1996). In some species, including humans, the insulin producing β-cells may fail, resulting in declining insulin concentrations and increasing blood glucose - a condition known as type 2 diabetes (Gavin, 1997; Shafrir and Ziv, 1998). This condition rarely occurs in the horse without accessorial complication (McCoy, 1986).

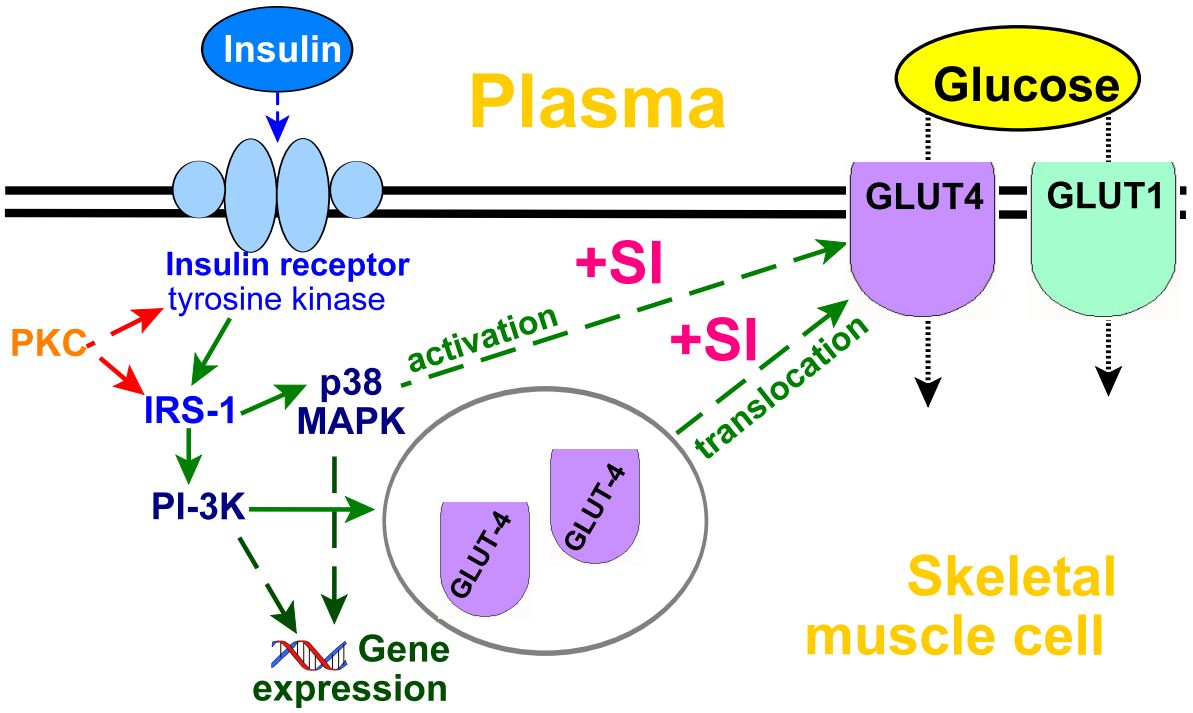


Figure 1.7. Insulin signaling at rest. Binding of insulin to its receptor promotes the translocation of glucose transporters (GLUT) to the cell membrane. Abbreviations: PKC, protein kinase C; IRS-1, insulin-receptor substrate-1; MAPK, mitogen activated protein kinase; PI-3K, phosphatidylinositol-3 kinase; SI, insulin sensitivity.

As mentioned previously, insulin resistance may be an adaptive mechanism for periods of restricted nutrition, particularly of glucose (Colagiuri and Brand Miller, 2002; Kaske et al., 2001; Rijnen and van der Kolk, 2003). This ‘thrifty’ adaptation, however, is no longer adaptive when energy and glucose become abundant, as has occurred in the domesticated horse as well as westernized human cultures (Cohen et al., 1988). The result is metabolic dysfunction which has been extensively (though perhaps not conclusively) characterized in human populations - the “metabolic syndrome” (Adult Treatment Panel III, 2002; WHO, 1999).

An equine metabolic syndrome has been suggested for the horse based on analogy (Johnson, 2002), but the true similarities have only begun to be specifically evaluated (Treiber et al., 2006). Similarities between horses with metabolic dysfunction and humans with metabolic syndrome include insulin resistance, hyperinsulinemia and obesity, which facilitate each other. Storage, mobilization and oxidation of NEFA may all be altered potentiating increased adiposity (Kelley and Mandarino, 2000). Adipose tissue, meanwhile secretes adipocytokines which influence tissue insulin sensitivity (Chaldakov et al., 2003). Furthermore, when insulin resistance results in a failure of insulin to suppress lipolysis, excess circulating triglycerides may result - an observation in human metabolic syndrome and a risk factor for dyslipidemia in equines (Jeffcott and Field, 1985; Jeffcott et al., 1986). Insulin resistance may also contribute to laminitis, with hoof separation resulting from or exacerbated by restricted glucose metabolism and changes in vascular function (see Part II) (Bailey et al., 2004a; French and Pollitt, 2004; Pass et al., 1998). Insulin resistance may affect growth as well, through its interactions with growth hormone and the somatotropic axis (Treiber et al., 2004). Osteochondrotic lesions may be more likely to develop in foals with exagerrated insulin responses (Ralston, 1996), particularly those maintained on high-energy diets (Glade and Belling, 1984; Kronfeld et al., 1990; Savage et al., 1993).

Measuring insulin resistance

A number of techniques have been used to identify and characterize insulin resistance, ranging from one-sample tests, to meal response test, to hormone and metabolite response tests, to complex mathematical models.

One-sample tests generally involve fasting levels of glucose and insulin. Baseline samples may provide useful representations of the glucose/insulin system and predictors of disease because they describe the chronic unperturbed state of the

subject. Baseline insulin has long been used as an indicator of insulin resistance, as insulin concentrations increase to ensure euglycemia, resulting in compensatory hyperinsulinemia. However, low baseline insulin concentrations can be ambiguous, indicating either a small insulin requirement or failed insulin secretion. Baseline glucose concentrations complete the picture by indicating the functionality of circulating insulin levels to maintain euglycemia. Several tests for insulin resistance, such as the HOMA (homeostasis model assessment) and QUICKI (quantitative insulin sensitivity check index), combine baseline glucose and insulin values to predict insulin sensitivity in humans (Katz et al., 2000; Matthews et al., 1985).

Tolerance tests involve observing the interactions of circulating glucose and insulin - by creating a perturbation and observing the subject's response. The most basic tolerance test is the meal tolerance test (MTT) - similar to a glycemic index test - which observes postprandial changes in glucose and insulin following a controlled meal (Caumo et al., 2000; Williams et al., 2001). Although the MTT is useful in comparing individual response to existing foods, it does not separate individual variation in the rate of intake, digestion and absorption. Glucose tolerance tests (GTT) provide some additional control by standardizing and simplifying these parameters, especially when the glucose is introduced directly into the plasma (Jeffcott et al., 1986; Roberts and Hill, 1973). Neither the MTT or GTT specifically evaluate variations in the insulin response which may impart variation on glucose disposal. The insulin tolerance test (ITT) involves an intravenous dose of insulin which can be used to show the direct effect of insulin on glucose disposal from the plasma (Forhead and Dobson, 1997). However, the ITT does not provide information on the individual's ability to secrete insulin and can result in hypoglycemia.

The euglycemic-hyperinsulinemic clamp was the first comprehensive quantitative method widely accepted for determining insulin sensitivity (Andres et al., 1966). As such, the clamp method has been regarded by some as the gold-standard. The clamp involves simultaneous glucose and insulin infusions, with insulin maintained at a hyperinsulinemic value and glucose infused until a euglycemic steady state is reached. At this point the infusion rate of glucose is equivalent to the rate of plasma glucose

disposal. Despite its considerable contribution to the study of glucose and insulin dynamics, the clamp has been criticized for its non-physiological experimental conditions (such as arbitrarily constrained hyperinsulinemia) (Ferrannini and Mari, 1998; Martinez et al., 1993).

An alternative quantitative test for insulin senstivity based on a dynamic mathematical model has been introduced (Bergman et al.,1979). This model, known as the minimal model, analyzes the glucose and insulin responses to the intravenous glucose tolerance test to evaluate the interaction between glucose and insulin in glucose disposal.

Minimal Model

The Minimal Model is a two-compartment representation of the dynamic glucose/insulin system (Bergman et al., 1979) (Figure 1.8). The primary compartment of the minimal model represents the intermixing plasma and interstitial glucose pool (G). Glucose is presumed by the minimal model to be lost from G via two distinct pathways. The first path is non-insulin mediated (or 'glucose-mediated') glucose disposal (i.e. glucose disposal at basal insulin). Glucose from the plasma and interstitial fluid enters cells primarily 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 linear (i.e. proportional to the glucose concentration gradient) (for more detail see Part I, Chapter 1: Glucose kinetics).

The second route of plasma glucose disposal is via insulin mediation. Insulin mediation is described by compartment X of the model, known as the 'remote insulin' or 'insulin action' compartment and comprising those insulin units engaging receptors at the cell membrane and stimulating translocation and activation of GLUT-4 glucose transporters (Furtado et al., 2002).

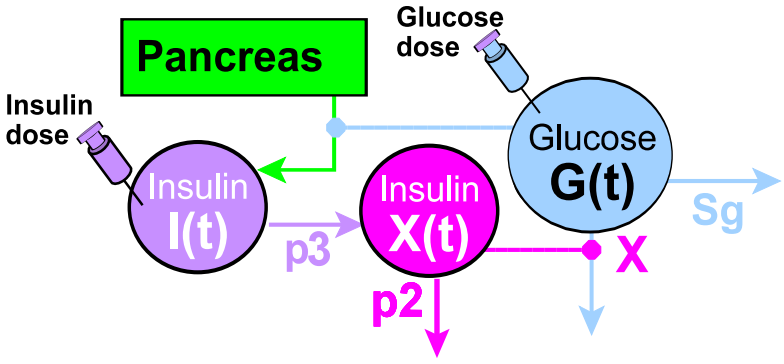


Figure 1.8. Compartmental interpretation of glucose and insulin dynamics as represented by the minimal model (adapted from Bergman, 1979) in which G(t) represents plasma glucose at time=t, I(t) is plasma insulin and X is remote insulin. Glucose-mediated glucose disposal rate is Sg (min^{-1}) and insulin-mediated glucose clearance rate X. The fractional rate of insulin disposal from the remote compartment is defined as p_2. The contribution of plasma insulin to the remote compartment is p_3.

The minimal model is applied to data from a frequently sampled intravenous glucose tolerance test (FSIGT) in which glucose and insulin system are manipulated by administering a rapid intravenous glucose dose in a subject at metabolic baseline, typically following an overnight fast in humans, or during a basal grazing state in horses (Bergman, 1997; Hoffman et al., 2003a). Clearance of the exogenous glucose from circulation is observed for 20 min via frequent sampling. An insulin bolus – large enough to affect glucose disposal but small enough to avoid hypoglycemia – is then administered rapidly into the bloodstream. This insulin dose is intended to calibrate the insulin response and increase the sensitivity of the test (Pacini et al., 1998). Frequent venous blood sampling is continued for several hours to observe glucose regulation until glucose and insulin return to baseline.

The disposal 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):

$$G'(t) = -G(t) \cdot \left[Sg + X(t)\right] + Sg \cdot G_b \qquad (10)$$
$$G(0) = Gb$$

where G'(t) is the rate (mg/dl·min) of glucose loss from the glucose compartment

- i.e. the rate of uptake and maximum rate of utilization of glucose by the cells. The plasma glucose concentration (mg/dL) at time = t is G(t). Glucose effectiveness (Sg) describes the glucose-mediated glucose disposal rate (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 disposal rate. This component is further described by:

$$X'(t) = p_3 \cdot \left[I(t) \cdot I_b \right] - p_2 \cdot X(t) \qquad (11)$$
$$X(0)=0$$

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:

$$SI = p_3 / p_2 \qquad (12)$$

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; tissue which has a lower insulin sensitivity requires more insulin to properly clear glucose.

Minimal Model in application

The minimal model was developed to aid the study of insulin resistance and type 2 diabetes in humans by quantifying and separating insulin sensitivity (SI) and glucose effectiveness (Sg) (Bergman et al., 1979). Studies of diabetic subjects have shown insulin sensitivity to be approximately 75% lower in diabetic humans or cats compared

to controls (Feldhahn et al., 1999; Ward et al., 1991; Welch et al., 1990) with a concomittant decrease in Sg for diabetics of both species.

The has been 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 and increased health risks (Bergman, 1989) (Table 1.1). The dynamic and physiological nature of the minimal model ensures a robust test applicable under many conditions. For example, the model can be applied to glucose and insulin results from oral or meal glucose tolerance test in addition to the FSIGT (Caumo et al., 2000), with various doses (Pacini et al., 1998) and with flexible sampling schedules (Cutfield et al., 1990).

Table 1.1. Glucose effectiveness (Sg) and insulin sensitivity (SI) values determined by the minimal model for humans and other species.

	Sg × 10^2 (min^{-1})	**SI × 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
Rhesus Monkeys			
Normal (n=12)	3.09 ± 0.48	3.04 ± 0.63	Gresl et al., 2003
Diet restricted (n=12)	4.13 ± 0.40	5.90 ± 0.92	
Rats			
Normal (n=9)	5.43 ± 0.76	6.17 ± 1.68	Natalucci et al., 2000
Hypertensive (n=10)	6.23 ± 0.93	5.66 ± 0.86	
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	
Normal (n=5)	2.2 ± 1.6	12.4 ± 6.9	Petrus et al., 1998

	$Sg \times 10^2$ (min^{-1})	$SI \times 10^4$ (min^{-1} per mU/L)	Reference
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 (fat & fiber feed) (n=6)	0.756 ± 0.32	3.57 ± 0.46	Treiber et al., 2005
Weanlings (sugar & starch feed) (n=6)	0.758 ± 0.56	2.30 ± 0.15	
Normal (n=16)		2.93	Pratt et al., 2005
Quarter horse mares (n=8)	1.37	1.00	
Quarter horse mares after 8 wk of levothyroxine (n=8)	2.28	2.48	Frank et al., 2005

The minimal model has been compared to the euglycemic-hyperinsulinemic clamp in several species, with values for SI shown to closely correlate to clamp-derived insulin sensitivity with r = 0.84 for humans (Beard et al., 1986), r = 0.82 for dogs (Finegood et al., 1984), r = 0.91 for cats (Petrus et al., 1998)), and r = 0.85 for horses (Pratt et al., 2005). However, insulin sensitivity from the minimal model and clamp are represented by different units (Finegood et al., 1984) that may not represent the same physiological process. The minimal model has several benefits over the clamp, including more physiological experimental conditions, a model which allows for flexibility in studying changes in several physiological compartments and parameters, and a more detailed characterization of the dynamic glucose and insulin system - for example, the differentiation of glucose-mediated and insulin-mediated components of glucose clearance. The minimal model may therefore provide a new ‘gold-standard’ for the determination of insulin resistance and changes in glucose metabolism.

Minimal Model and diet

Diet studies applying the minimal model in humans are often inconclusive due to confounding lifestyle characteristics such as dietary energy, smoking, ethnicity, gender and complex diets. Higher grain intake has been associated with increased SI (Liese et al., 2003; Lovejoy and DiGirolamo, 1992) while high fat diets (~50% energy as fat) have been associated with decreased SI (Chen et al., 1988; Lovejoy and DiGirolamo, 1992) or with no change in SI (Swinburn et al., 1991; Thomsen et al., 1999; Wolever and Mehling, 2002).

Diet studies in animals have been more informative as experimental conditions and diets can be better controlled. In the horse, the minimal model has demonstrated lower insulin sensitivity in geldings and weanlings adapted to a grain feed as compared to a fibrous, fat-supplemented feed (Hoffman et al., 2003a; Treiber et al., 2005a). 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 might 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. In miniature pigs, the minimal model has shown the effect of different fat sources on insulin sensitivity (Behme, 1996) whereas related studies in humans have not shown a difference (Lovejoy et al., 2002). Mild diet restriction has also be associated with improved insulin sensitivity in Rhesus monkeys (Gresl et al., 2003; Gresl et al., 2001). All of these studies have implications to both animal and human health.

Minimal Model and exercise

Studies on exercise using the minimal model have been performed primarily in humans. These studies can be divided into two categories: 1) studies evaluating the effect of training on glucose and insulin dynamics and 2) studies evaluating the effect of a single bout of exercise on glucose and insulin dynamics.

Exercise trained humans demonstrate increased SI and Sg compared to sedentary humans. Increased SI is attributed to increased insulin-mediated GLUT-4 glucose transporters in the muscle (Cox et al., 1999) but may also be associated with changes in insulin-like binding protein-I and insulin-like growth factor-I (Manetta et al., 2003a; Manetta et al., 2003b). Similarly, increased Sg is probably due to increased non-insulin mediated GLUT-1 glucose transporters in the muscle (Ploug et al., 1990).

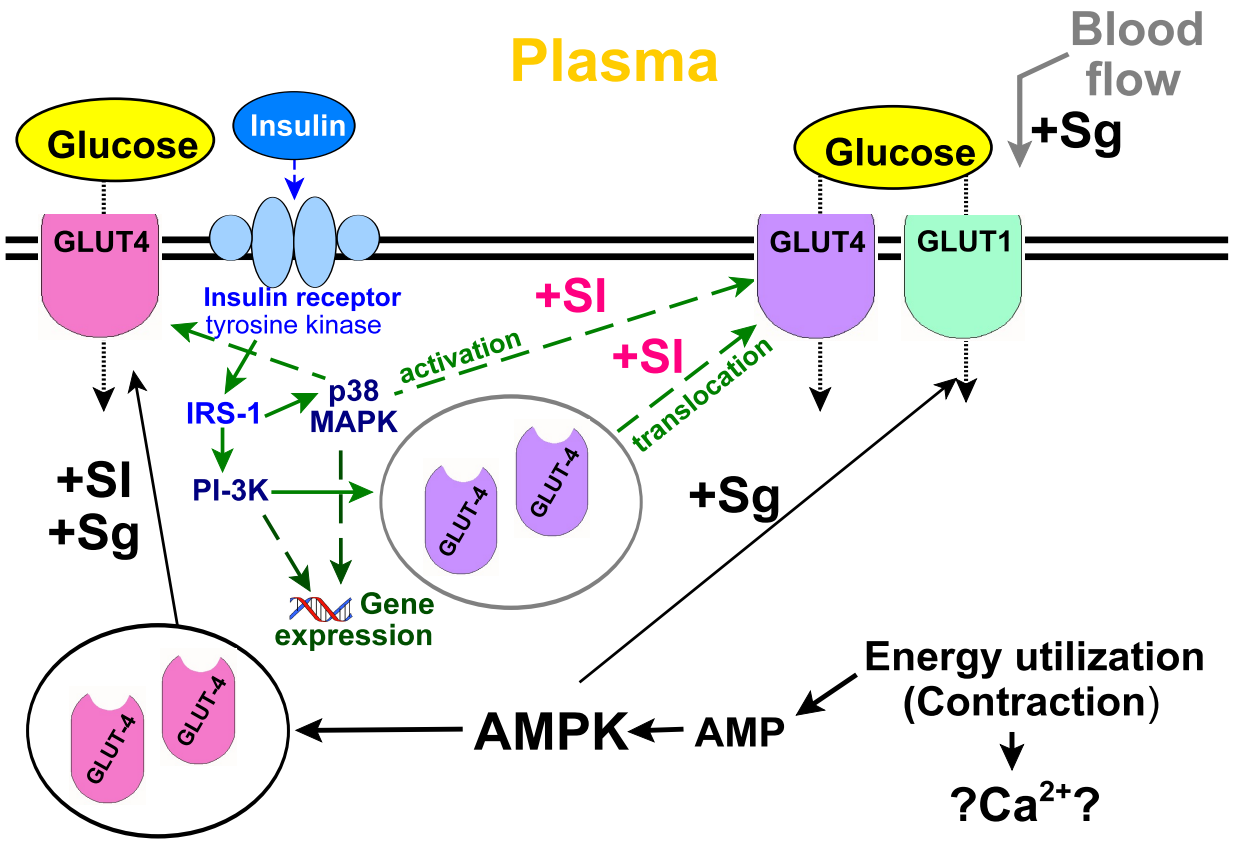
Exercise training has also been shown by the minimal model to reduce the difference in SI between older and young subjects, indicating that muscle retains its adaptability despite aging (Cox et al., 1999; Manetta et al., 2003a). However, increasing training intensity does not appear to further increase SI or Sg (Houmard et al., 2004; Manetta et al., 2003b). This limit may be a protective mechanism to avoid hypoglycemia (Manetta et al., 2000). No changes in SI or Sg were observed between 16 h post-exercise to 1 wk post-exercise in trained subjects (Nishida et al., 2001; Tokuyama et al., 1993), indicating persistant exercise effects on glucose and insulin dynamics.

Transient changes in SI and Sg have also been reported in post-exercise trials. Both SI and Sg are increased 25 min after intense exercise bouts of as little as 15 min (Brun et al., 1995), but only Sg was shown to increase 25 min after 25 min of mild exercise (Sakamoto et al., 1999). Eleven hours post-exercise, the increase in SI, but no change in Sg, was apparent only in subjects exercised to exhaustion (Higaki et al., 1996).

Increased blood flow during exercise may increase glucose avaliability to GLUT transporters and insulin availability to insulin receptors, promoting both Sg and SI (Sakamoto et al., 1999). Muscle contraction and calcium signaling may recruit additional GLUT-4 transporters responsible for the observed increases in SI just following exercise (Richter et al., 2004; Wallberg-Henriksson et al., 1988). Contractile affects on SI and Sg may be due to increases in intracellular AMPK in exercising skeletal muscle. During exercise increased energy utilization increases intracellular AMP, stimulating AMPK which stimulates energy-provisioning, including increasing both GLUT-4 and GLUT-1 transporters through non-insulin mediated pathways (Fryer et al., 2002; Musi et al., 2001). Effects of exercise on Sg and SI are illustrated in Figure 1.9.

These post-exercise studies may approximate changes occurring during exercise which remain apparent during recovery. However, recovery and exercise states are very different in terms of metabolic signaling and energy demand. To the author's knowledge, the studies reported here include the first application of the minimal model during an exercise bout in any species.

Figure 1.9. Insulin signaling and glucose uptake in skeletal muscle during exercise. Glucose transport is facilitated by insulin- and contraction-mediated pathways. Abbreviations: PKC, protein kinase C; IRS-1, insulin-receptor substrate-1; MAPK, mitogen activated protein kinase; PI-3K, phosphatidylinositol-3 kinase; AMPK, AMP kinase; SI, insulin sensitivity; Sg, glucose effectiveness.



Conclusions

Glucose regulation plays a principle role in energy provision during exercise. Glucose availability to tissues is regulated by insulin and influenced by exercise and diet Understanding these influences and their interactions can aid in the improvement of nutritionandmanagement to optimize glucose utilization for health and exercise performance.

The objectives of the following studies were to model glucose kinetics and glucose and insulin dynamics in horses at rest and during exercise. The affect ofadaptation to different dietary energy sources was also evaluated

CHAPTER 2:

Dietary energy sources affect Minimal Model parameters in trained Arabian geldings during endurance exercise

Treiber, KH, TM Hess, DS Kronfeld, RC Boston, RJ Geor, M Friere, AMGB Silva, and PA Harris. 2006. Dietary energy sources affect Minimal Model parameters in trained Arabian geldings during endurance exercise. Equine Vet J (In Press).

Abstract/Summary:

Objectives: To quantify the effects of exercise and diet on insulin sensitivity (SI), glucose effectiveness (Sg), acute insulin response to glucose (AIRg) and disposition index (DI) in horses.

Methods: This study applied the minimal model of glucose and insulin dynamics to exercise-trained Arabian geldings during rest or constant moderate-intensity exercise after 8 weeks adaptation to feeds high in sugar and starch (SS, n=6) or fat and fiber (FF, n=6). Horses underwent two frequently sampled intravenous glucose tolerance tests (FSIGT). For both tests, a resting basal sample was collected, followed by an i.v. dose of 600 mg/kg BW glucose defining 0 min of the test. Insulin (0.01 IU/kg BW) was administered 20 min post-glucose for each test. Resting horses were sampled for 240 min. The exercise FSIGT began after each horse had warmed up for 25 min on the treadmill at which point they had reached the speed representing 57% of their predetermined lactate breakpoint which was maintained for the rest of the FSIGT. Exercising horses were sampled identically to rest, but for only 150 min post-glucose.

Results: Exercise increased ($P < 0.008$) SI, Sg, and DI and decreased AIRg in all horses. Overall, horses adapted to FF tended to have higher SI ($P = 0.070$) and DI ($P = 0.058$). During exercise, FF horses tended to have higher ($P \leq 0.085$) SI and DI, than SS horses and these variables tended to be increased more ($P \leq 0.075$) by exercise in FF horses than SS horses.

Conclusions: Insulin and glucose dynamics adjust during exercise, increasing plasma glucose uptake, presumably to meet demand by contracting skeletal muscle. Trained horses adapted to a high fat diet showed greater metabolic adjustment during exercise than trained horses adapted to a high starch and sugar diet, potentially allowing them to better meet energy demands.

Keywords: minimal model, endurance exercise, insulin sensitivity, glycemic dietetics, glucose metabolism

Introduction

Dietary energy source has been suggested to alter glucose and fatty acid metabolism during exercise in horses (Duren et al., 1999; Graham-Thiers et al., 2001). Glucose-infusions have been used to study glucose kinetics during exercise and have shown decreased glucose production and utilization in horses adapated to high fat diets (Pagan et al., 2002). However, dynamic regulatory models have not been applied to explore changes in metabolite and hormone interactions occurring during exercise and after fat-adaptation in the horse. Such models could evaluate the system under more physiological conditions as well as the capacity of the system to respond to changes in metabolite and hormone signaling.

This study applied the minimal model of glucose and insulin dynamics during rest and then constant low-intensity exercise in 12 trained Arabian geldings adapted to feeds rich in starch and sugar (SS) or fat and fiber (FF). Diet was expected to affect glucose disposal during exercise.

Materials and Methods

Twelve Arabian geldings were paired by body condition score, weight and age. Eight weeks prior to any test, one horse from each pair was assigned to each diet group. Horses were maintained on mixed grass/legume pasture. Diet groups were kept separate with pastures rotated monthly so that all subjects were exposed to the same pasture conditions. Diet groups were fed collectively but in individual pans, with each

pan containing a single portion (1 kg feed). Horses were supplemented twice daily, which fulfilled approximately 30% of the recommended DE for a 450 kg horse undergoing light work (NRC, 1989). Horses were weighed and scored for body condition (BC) before beginning feed adaptation and just prior to testing (Henneke et al., 1983). The study was approved by the Institution's Animal Care and Use Committee.

The supplements were formulated to be isocaloric and isonitrogenous with vitamin and mineral contents designed to complement the pasture and fulfill present recommendations (Hoffman et al., 2001; NRC, 1989). Feed compositions were reported previously (Hoffman et al., 2003b). Pasture and supplements were sampled monthly and submitted to a commercial laboratory[1] for proximate analysis (Table 2.1).

Table 2.1. Partial proximate analysis of SS and FF supplements. Pasture was not different for diet groups for any variable (P > 0.26) and results for all pasture samples were combined. Results are reported on a dry matter basis as means ± sem.

	SS (2 kg/d, n=6)	FF (2 kg/d, n=6)	Pasture (n=12)
Dry matter, %	87.4 ± 0.2[a]	91.1 ± 0.5[b]	24.0 ± 2.2
Crude Protein, %	15.4 ± 0.8	14.0 ± 0.5	18.3 ± 0.9
ADF, %	14.7 ± 0.8[a]	32.6 ± 0.6[b]	35.0 ± 1.0
NDF, %	24.2 ± 1.2[a]	45.1 ± 1.1[b]	62.4 ± 1.2
NFC, %	52.2 ± 2.1[a]	26.4 ± 1.0[b]	11.6 ± 1.1
NSC, %	44.7 ± 2.1[a]	13.1 ± 0.8[b]	10.2 ± 0.9
Crude Fat, %	3.3 ± 0.1[a]	10.6 ± 0.8[b]	3.3 ± 0.3
Ash, %	7.2 ± 0.6[c]	8.4 ± 0.1[d]	10.5 ± 0.7
DE[1], Mcal/d	5.30 ± 0.09	5.19 ± 0.09	N/A

[1]DE per kg feed calculated according to Kronfeld and Harris (2000)
[a,b] Supplements differ (P < 0.001)
[c,d] Supplements tend to differ (P = 0.060)

Previous studies had demonstrated a two-fold higher glycemic and 6-fold insulinemic effects of the SS feed compared to the FF feed (Williams et al., 2001); accordingly the SS feed has been assigned a high glycemic index and the FF feed a

moderate glycemic index (Kronfeld et al., 2004).

All horses had been conditioned to running on a treadmill for 8 months before the current studies were undertaken. Each horse was exercised for approximately 1 hour, 3 times per week. Exercise consisted primarily of treadmill work (at least once per week) and work in an automatic walker (walk, trot). Horses were also ridden (light trail riding) or lunged (walk, trot, canter) when the treadmill and walker were not available. Horses were weighed on an electric scale just prior to beginning diets and on the day of each test.

Each horse underwent 3 testing protocols: firstly a lactate threshold test (Kronfeld et al., 2000), then an FSIGT at rest and an FSIGT performed during exercise. Generally, one exercise and one resting test were performed each day, and one FF and one SS horse were tested. The following day, the diet groups represented for each test were switched. Three horses from each diet group shared the same sequence of exercise or rest tests. For all tests, horses were maintained on pasture until the morning of the test and given no concentrate for at least 12 hours prior to the test. All procedures and test were performed in the same open barn. Environmental conditions were recorded every 5 min using a heat-stess monitor[2]. For the exercise and lactate tests, four fans were positioned on the front and sides of the treadmill. A catheter was inserted into the jugular vein the morning of each test and horses allowed at least 30 min to rest following the procedure. Basal samples were then taken, followed by initiation of the test. Horses had at least 8 days to recuperate between tests and missed one training session before and one training session after their exercise test. Three lactate threshold tests were held each morning between 7 and 11am; all FSIGTs were begun between 8 and 10 am.

Lactate threshold

Horses were warmed up on the treadmill for 10 min at 1.8 m/s and 0% slope. A blood sample was taken and then the speed was increased to 2.7 m/s and the incline raised to a 6% slope. The horses exercised under these conditions for 2 minutes after which a blood sample was taken and then the speed increased by 0.45 m/s. The test

continued with sampling every 2 minutes followed by an increased speed until the horse could no longer maintain its position on the treadmill. Two FF horses and 1 SS horse performed for 3 minutes at each interval but this was deemed too strenuous as horses became hyperthermic and exhibited nasal bleeding, so the intervals was reduced to 2 minutes for the remaining horses.

FSIGT at rest

Horses had access to grass hay and water ad libitum throughout this test. Following baseline samples, an i.v. glucose dose (600 mg glucose/kg BW, Dextrose Solution 50%)[3] was administered rapidly (less than 2 min) through the catheter. Blood samples were collected at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 min following the glucose dose. At 20 minutes, 0.01 IU insulin/kg BW (Humulin R)[4] was administered through the catheter and sampling continued at 22, 23, 24, 26, 28, 30, 32, 35, 40, 45, 50, 60, 70, 80, 90, 100, 115, 130, 150, 180, and 240 min after the glucose injection.

FSIGT During Exercise

Following baseline samples each horse was warmed up by walking for 10 min at 1.8 m/s at a 0% slope and then trotting for 15 min at a 2% slope at 57% of its lactate threshold as determined by the lactate threshold test. Samples were collected at 10 and 20 min of warmup, followed by a sample at 25 min which constituted the 0 min sample for the FSIGT. The horse continued at the same speed (57% of its lactate threshold) for the remainder of the test. Immediately following the 0 min sample, an i.v. glucose dose (600 mg glucose/kg BW) was administered rapidly (less than 2 min) through the catheter. Blood samples were collected at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 min following the glucose dose. At 20 minutes, 0.01 IU insulin/kg BW was administered through the catheter and sampling continued at 22, 23, 24, 26, 28, 30, 32, 35, 40, 45, 50, 60, 70, 80, 90, 100, 115, 130, and 150 min after the glucose injection.

Blood Sample Handling

Blood was withdrawn into heparinized sample tubes (Vacutainer)[5] and placed in

ice water until centrifuged. Plasma was removed within 30 min of collection and frozen at -20º C until analysis.

Plasma glucose and lactate were analyzed by enzymatic assay (Beckman Instruments, Glucose Procedure #16-UV, SYNCHRON Lactate (LAC) Procedure)[6]. Insulin was determined using a radioimmunoassay (Coat-A-Count Insulin)[7] previously validated for equine insulin (Freestone et al., 1991). The intraassay CV of duplicate samples was <1% for glucose, 4% for lactate and 5% for insulin.

Statistics

Lactate threshold was defined as the speed when plasma lactate concentrations began to rapidly increase, as determined using a broken-line model (Kronfeld et al., 2000; Langfort et al., 2004). The breakpoint was defined for each subject as the intersection of the two regression lines fit to the lactate versus speed curve which resulted in the least residual squared error (Kronfeld et al., 2000). Minimal model parameters (SI, Sg, DI, and AIRg) were calculated using MinMod Millenium software (Ver. 5.10, BeBos Assoc., 2001) (Boston et al., 2003). Statistics were performed using Intercooled Stata Version 8. Effects of diet and exercise on minimal model parameters were determined using a two-way ANOVA. Areas under the curve (AUC) were calculated by trapezoidal approximation. Significance was assessed at $P < 0.05$ and a trend at $P < 0.10$.

Results

Populations

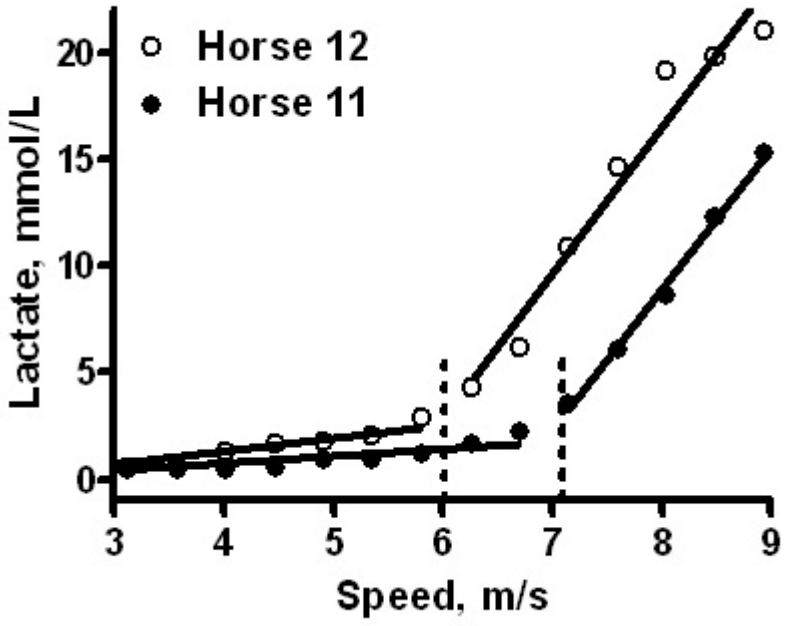
Prior to diet adaptation, diet groups did not differ by weight, BC, or age ($P = 0.27$, 0.54 and 0.62, respectively). Horses weighed 456 ± 13 kg, had BC scores of 5.3 ± 0.3 (range 4 to 7), and were 11 ± 1 yrs old (range 5 to 16). Body weight was increased following diet adaptation ($P = 0.013$), primarily due to an increase in the weight of SS horses (20 ± 5 kg) compared to FF horses (3 ± 3 kg) ($P = 0.014$). Body condition score was not associated ($P \geq 0.20$) with basal concentrations of glucose or insulin, or any minimal model parameters.

Across all minimal model tests, the temperature ranged from 6 to 24 ºC, and the relative humidity from 55 to 98%. Over the 3 hours of testing, temperature increased (P = 0.008) an average of 5.5 ± 1.6 ºC. The ambient temperature at the start of the exercising FSIGTs was slightly colder (P = 0.021) on average for FF horses (11.0 ± 1.4 ºC) than SS horses (15.9 ± 1.1 ºC). There was no difference (P > 0.20) in hydration status between diet groups at the beginning or completion of exercise as determined by hematocrit or plasma total protein (data not shown). There was also no difference (P > 0.35) between diet groups in heart rate before exercise (46 ± 3 bpm), after 25 min of warmup (122 ± 3 bpm), or after 150 min of exercise (110 ± 3 bpm). The SS horses had a slightly higher (P = 0.016) resting body temperature (37.6 ± 0.07 ºC) compared to FF horses (37.3 ± 0.08 ºC). Immediately following exercise, however, body temperatures were not different (38.4 ± 0.16 ºC; P = 0.61). All horses successfully completed all tests.

Lactate threshold

Results for the lactate test are shown in Figure 2.1.

Figure 2.1. Plasma lactate concentrations during an incremental exercise test for two Arabians. Vertical broken lines indicate the lactate breakpoint which was 6.0 m/s for Horse 12 and 7.1 m/s for Horse 11.



Peak heart-rates (at the end of the test) did not differ (P = 0.79) between diet groups and were 209 ± 4 bpm. Although lactate breakpoint did not differ between diet groups (FF: 6.95 ± 0.15 m/s, SS: 6.59 ± 0.35 m/s, P = 0.36), all horses within the lowest quartile of lactate breakpoint were in the SS group. Horses in the SS group also had significantly higher lactate at 4.5, 5.4 and 6.7 m/s (P < 0.05). The AUC of lactate from 1.8 to 8.5 m/s for the SS group (120.3 ± 18.7 mmol/L) tended to be greater (P = 0.094) than for the FF group (81.3 ± 9.8 mmol/L). Because lactate breakpoints were not different, there was no difference (P = 0.16) in the speed during the FSIGT for FF horses (3.99 ± 0.09 m/s) compared to SS horses (3.73 ± 0.14 m/s).

FSIGT and the Minimal Model

The minimal model successfully described the glucose curves for all 12 horses during both trials (Rsq = 0.987 ± 0.002). Glucose and insulin curves are shown in Figure 2.2. Results of minimal model parameters are shown in Figure 2.3. There were no differences in minimal model parameters between diet groups at rest. Exercise increased SI (P < 0.001), Sg (P = 0.001), and DI (P = 0.003) and decreased AIRg (P = 0.008). During exercise SI and DI tended to increase more (P = 0.075 and 0.069, respectively) in FF horses than SS horses, resulting in higher SI (P = 0.084) and DI (P = 0.074) in FF horses than SS horses during exercise.

Figure 2.2. Plasma concentrations of (A) glucose and (B) insulin during the FSIGT for FF horses at rest (solid square, dotted line) and exercise (solid circle, dotted line) and SS horses at rest (open square, solid line) and exercise (open circle, solid line). The first period (-35 to 0 min) represents the exercise warm-up. The second period (0 to 20 min) represents the first phase insulin response to the intravenous glucose dose at 0 min. The third period (20 to 180 min) represents the dispoal of glucose and insulin following the insulin dose at 20 min as well as the secondary insulin response.

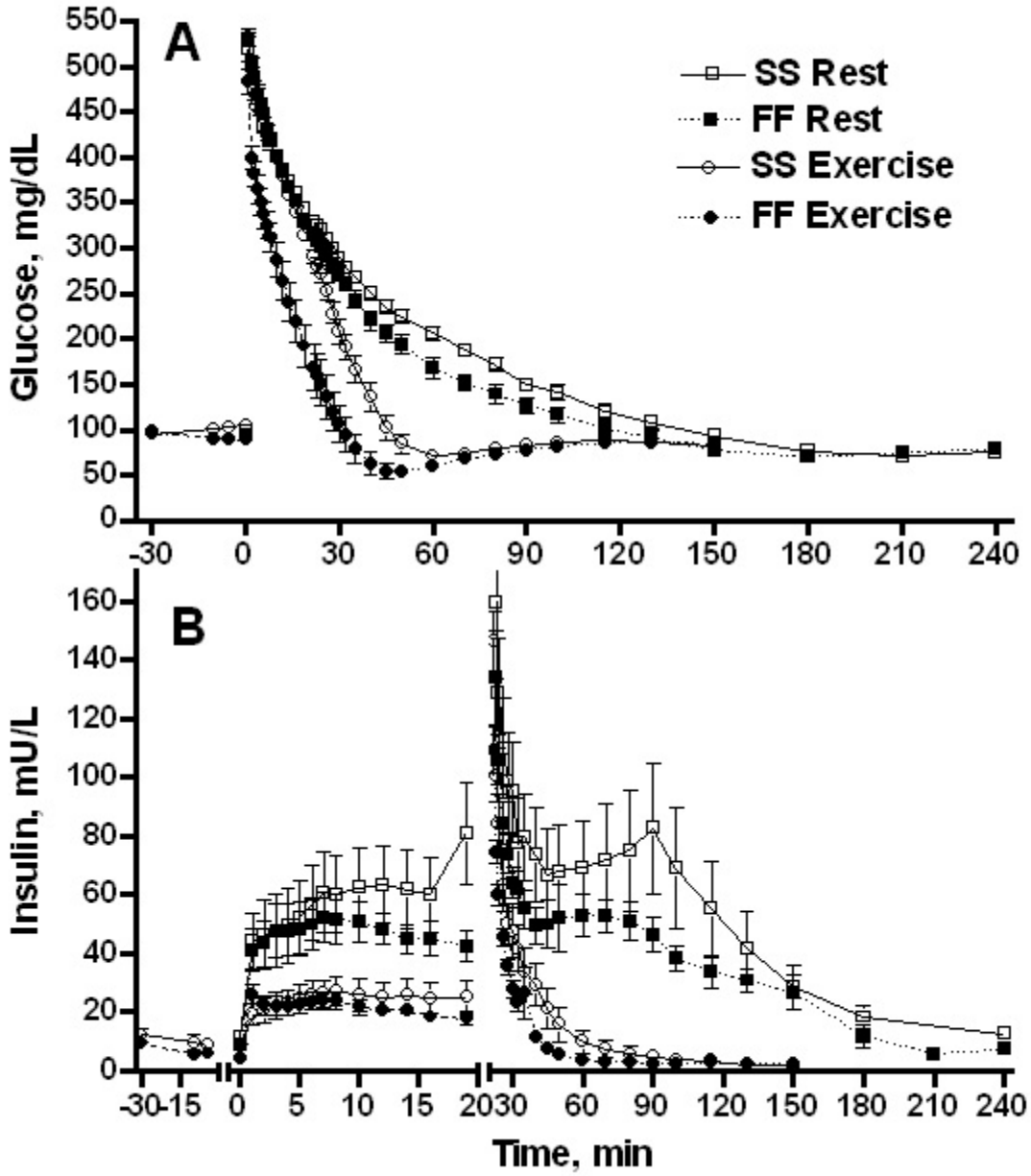
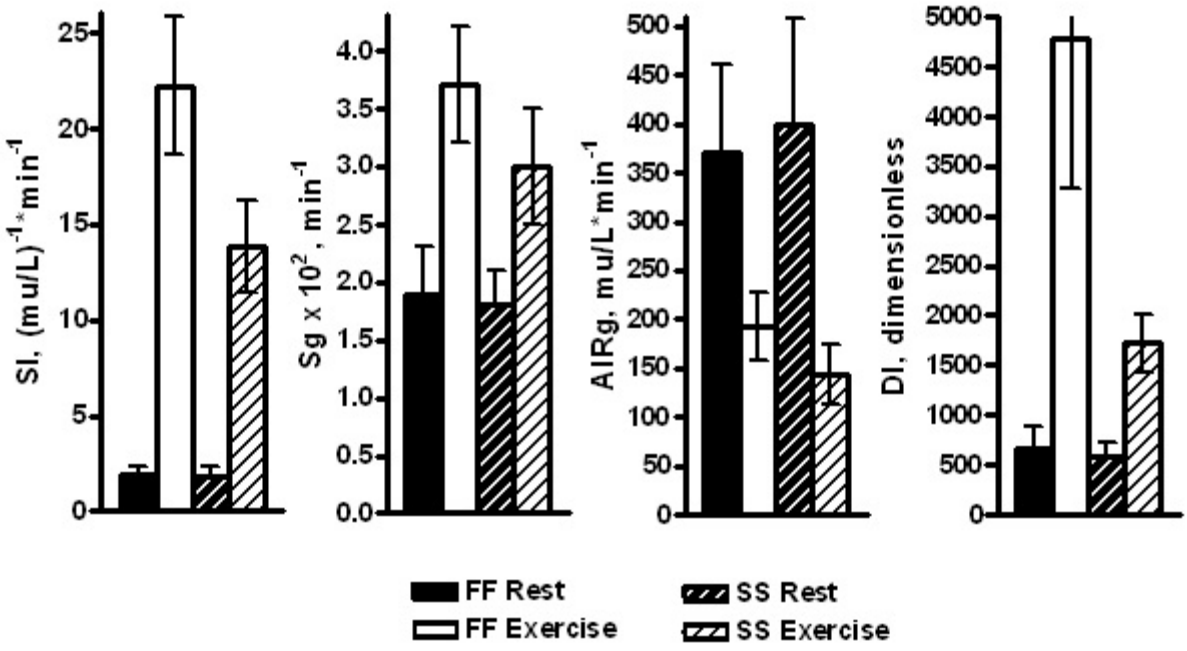


Figure 2.3. Effects of diet and exercise on minimal model parameters. Results are reported as means and standard errors. There was an exercise effect on all parameters ($P \leq 0.005$). There was a trend for SI and DI to be higher in FF horses during exercise ($P \leq 0.084$). There was a trend for a greater increase in SI and DI in FF adapted horses compared to SS adapted horses ($P \leq 0.075$).



Discussion

These results show that insulin and glucose dynamics adjust during exercise to increase glucose uptake. These changes likely increase energy metabolite availability to skeletal muscle cells while promoting metabolism to meet energy demands and maintain muscle function. Horses supplemented with fat rather than starch and sugar as a dietary energy source showed greater metabolic adjustment during exercise, potentially enhancing their ability to maintain performance.

Bodyweight

Adaptation to the SS diet was associated with a slight increase in body weight, but no significant change in body condition during the adaptation period. As the daily

energy provided by the FF and SS feeds did not differ, this difference in weight gain may reflect a metabolic effect of high-glycemic meals. Such meals have been associated with weight gain in humans (Gutierrez et al., 1998; McAuley et al., 2005; Samaha et al., 2003). This weight gain is probably associated with increased insulin resistance which has been observed in humans and horses adapted to high-glycemic diets (Garca-Estevez et al., 2004; Hoffman et al., 2003a; Powell et al., 2002). In this study, it is possible that weight gain in SS horses contributed to lower insulin sensitivity observed during exercise. However, throughout the study no significant relationship between body condition and minimal model parameters was found, perhaps attributable to the fact that 9 of 12 horses had moderate condition scores of 5 or 6 and all horses had undergone the same exercise training.

Temperature

Studies on the effect of cold temperatures (0 ºC) during exercise in humans have shown no difference in carbohydrate or fat metabolism compared to mild temperatures (20 ºC) (Layden et al., 2004). The slight differences in temperature in this study (5 ºC) and the overall mild/cool temperature was unlikely to have an effect on the metabolic profiles of the diet groups, particularly as there was no difference in hydration status, heart rate, or body temperature during exercise.

Lactate threshold

Higher lactate accumulation in the SS horses during strenuous exercise could indicate higher relative exercise intensity and up-regulated glycolysis (Brooks and Mercier, 1994; Coggan et al., 1992). Changes in lactate transport or recycling may also contribute to the observed difference between diets (Turcotte and Brooks, 1990). The result is potentially a difference in skeletal muscle cell metabolism when exercise intensity is defined by speed increment, although no significant difference was observed in this study in terms of lactate breakpoint.

A trend for SS horses to have greater lactate accumulation has been previously observed in fat-adapted Arabians performing an incremental test (Kronfeld et al., 2000)

and repeated sprints at 7 m/s (Graham-Thiers et al., 2001), but the opposite effect was found during repeated sprints at 10 m/s (Ferrante et al., 1994) and no effect was found for Thoroughbreds exercising up to 11 m/s (Duren et al., 1999). Different speeds may represent different metabolic demands and may therefore be modified differently by dietary energy source (Kronfeld et al., 2005b).

Lactate breakpoint did not differ significantly by diet in this study, although a trend towards increased breakpoint has been observed previously in FF adapted Arabians (Kronfeld et al., 2000) and in humans fed a low-carbohydrate for 3 days (Langfort et al., 2004). The higher lactate values observed in SS horses may be indicative of a similar pattern.

For this study, lactate breakpoint was used to determine the speed at which each individual would perform the FSIGT exercise test. The lactate breakpoint is generally considered to reflect the respiratory capacity or 'metabolic fitness' of the skeletal muscle and its physiological response to exercise; as such, the lactate breakpoint may define a more appropriate relationship between speed and metabolic activity than VO_{2max} (Coggan et al., 1992; Coyle, 1995). Defining the speed of exercise as a given percent of each individual's lactate breakpoint provides a control for the metabolic signaling occurring in skeletal muscle (e.g. the presence of metabolites and metabolic intermediates) and reduces variations in metabolic feedback which could affect insulin sensitivity (Saltiel and Kahn, 2001). Sixty percent of lactate breakpoint was used in the study as this represented a speed for each horse which could be comfortably maintained over the time course of the exercising FSIGT.

Minimal Model

This is the first application of the minimal model to any species during exercise, as far as we know. The procedure was modified slightly, with the initial glucose dose doubled to provide a longer period of decline and the insulin dose reduced to one third of the typical dose on the assumption that insulin sensitivity would be increased by exercise, as has been observed in other species (Brun et al., 1995; Donovan and

Sumida, 1990). The sampling schedule was also reduced to 150 min, to ensure that horses completed the test with minimal stress. The successful fitting of all curves its physiological, rather than empirical, nature.

One of the most important contributions of the minimal model to the field of exercise is its ability to separate insulin-mediated and non-insulin-mediated glucose clearance. A number of studies, particularly in isolated rat muscle, have attempted to differentiate the effects of contractile activity from that of insulin action on the uptake of glucose (Etgen et al., 1996; Henriksen et al., 1990; Yeh et al., 1995). Other studies have observed transient effects on insulin sensitivity and glucose uptake following exercise (Brun et al., 1995; Powell et al., 2002; Tokuyama et al., 1993). The minimal model, however, allows for an in vivo test during the exercising state which uses minimal perturbation of the system to effectively separate insulin's action on glucose uptake (SI) from insulin-independent actions (Sg).

Insulin sensitivity (SI) was not different between SS adapted horses and FF adapted horses in the trained state. Previous studies have shown lower insulin sensitivity in untrained, sedentary Thoroughbred geldings (Hoffman et al., 2003a) and Thoroughbred weanlings (Treiber et al., 2005a) when adapted to the SS compared to FF feed. Adaptation to high carbohydrate diets in humans has also been associated with decreased insulin sensitivity (Jenkins et al., 1987; Salmeron et al., 1997; Samaha et al., 2003) and may be due to changes in metabolic signaling both within and between cells (Kronfeld et al., 2005b; Saltiel and Kahn, 2001). Exercise training is known to increase insulin sensitivity (Donovan and Sumida, 1990; Powell et al., 2002) and may therefore partially reverse insulin resistance in SS adapted individuals. However, during exercise, SS horses appear less able to adjust to energy demands compared to FF horses as reflected by the lesser increase in SI. This decreased adjustment may represent 'metabolic inflexibility' in which upregulated glucose metabolism in SS adapted horses could contribute to insulin resistance and an inability for insulin signaling to regulate contributions of glucose or fatty acid metabolism in response to demands, as has been proposed in humans (Kelley and Mandarino, 2000; Saltiel and Kahn, 2001).

Additionally, the lesser increase in SI during exercise observed in SS horses may reflect differences in regulatory signaling from growth hormone, cortisol, epinephrine or plasma metabolite concentrations (Blackard and Hubbell, 1970; Thompson et al., 1992).

Insulin sensitivity increased dramatically with exercise, approximately 12-fold in FF horses and 7-fold in SS horses. Similarly, a greater increase in insulin sensitivity in lean, more insulin sensitive horses compared to obese, less insulin sensitive horses was observed 24 hr post exercise (Powell et al., 2002). Increased SI has been observed just following exercise in humans (Brun et al., 1995; Tokuyama et al., 1993). Increased insulin sensitivity post-exercise have also been observed using the glucose clamp in horses (Powell et al., 2002). During exercise, increased responsiveness to insulin has been shown in exercised rat muscle, possibly due to increased availability of insulin-responsive glucose transporters (GLUT-4) (Ploug et al., 1990; Wallberg-Henriksson et al., 1988), or via effects on glycogen synthesis in rat muscle and untrained humans (Mikines et al., 1988; Powell et al., 2002; Richter et al., 1982).

Glucose effectiveness (Sg) is a measure of the tissues' capacity to uptake glucose independent of insulin sensitivity (Bergman, 1997). There was no difference in Sg between diets, similar to results found in Thoroughbred geldings and weanlings (Hoffman et al., 2003a; Treiber et al., 2005a). Exercise had a dramatic effect on Sg in both diet groups, increasing this parameter almost 80%. A similar increase in Sg was observed in humans tested just after exercise (Brun et al., 1995) and in trained athletes 16 h and 1 wk post-exercise (Tokuyama et al., 1993). Higher Sg would be expected to increase the ability for the muscles to acquire glucose from circulation regardless of very low insulin secretion during exercise. This increase in Sg is most likely attributable to muscle contraction which has been shown in rat muscle to promote GLUT-4 glucose transporter proteins to the surface of the cell and increase glucose uptake independent of and additive to the effect of insulin (Etgen et al., 1996; Henriksen et al., 1990; Yeh et al., 1995). In addition, increased blood flow during exercise may increase Sg (Sakamoto et al., 1999). In the resting state, Sg has been assumed to be associated with insulin-independent GLUT-1 transporters (Flanagan et al., 2000). However, studies in isolated rat muscle have not shown a change in GLUT-1 during exercise (Douen et al., 1990),

although GLUT-1 was increased in fast-twitch muscle fibers of exercise-trained rats (Ploug et al., 1990).

Effects of exercise and muscle contraction on SI and Sg may be mediated by increases in intracellular AMP-activated protein kinase (AMPK) which promotes both GLUT-4 and GLUT-1 transporters through non-insulin mediated pathways (Fryer et al., 2002; Musi et al., 2001). Calcium signaling may also recruit additional GLUT-4 transporters to enhance glucose uptake (Richter et al., 2004; Wallberg-Henriksson et al., 1988).

The acute insulin response to glucose (AIRg) records the secretion of insulin for the first ten minutes following the intravenous glucose dose (Bergman, 1997). The AIRg was 55% lower during exercise when compared to rest. This mirrors the increases in SI and Sg, i.e. the decreased need for insulin to facilitate glucose disposal during exercise.

The disposition index (DI) is the product of SI and AIRg and is a measure of the ability of the combined effect of insulin secretion (AIRg) and insulin efficiency (SI) to prevent hyperglycemia. The DI was lower in SS adapted horses compared to FF adapted horses, indicating that SS horses had slower glucose disposal. Similarly, Thoroughbred geldings adapted to SS had a lower DI than those adapted to FF (Hoffman et al., 2003a). The difference in DI between diet groups in this study was even more pronounced during exercise, which increased DI 7-fold in FF horses and 3-fold in SS horses due to the increases in SI. Faster glucose disposal during exercise could be adaptive as it may reflect glucose taken up by muscle cells to meet the increased demand for immediately available glucose.

Exercise increased glucose uptake from the plasma with and without insulin-mediation. In addition, exercise decreased insulin secretion which could promote mobilization and utilization of fatty acids as an additional energy source. Adaptation to a diet with high-glycemic carbohydrates as the primary energy source tended to increase circulating lactate and attenuate the increase in insulin sensitivity in horses during exercise.

[1]Dairy One, Ithaca, New York, USA
[2]Casella CEL Ltd, Bedford, UK
[3]Phoenix Pharmaceutical, Inc., St. Joseph, Missouri, US
[4]Eli Lilly and Company, Indianapolis, Indiana, USA
[5]Fisher Health Care, Chicago, Illinois, USA
[6]Sigma Diagnostics, St. Louis, Missouri, USA
[7]Diagnostic Products, Los Angeles, California, USA

CHAPTER 2 Supplement:

Dietary energy sources affect insulin sensitivity and β-cell resposiveness of trained Arabian geldings during endurance exercise

Adapted from:

Treiber, K.H., Hess, T.M., Kronfeld, D.S., Boston, R.C., Geor, R.J. and Harris, P.A. (2005) Dietary energy sources affect insulin sensitivity and B-cell responsiveness of trained Arabian geldings during endurance exercise. *J Anim Phys Anim Nutr* **89**, 429.

Introduction

The amount of insulin secreted and the action of insulin on muscle and adipose tissue impact metabolism of energy substrates by skeletal muscle. Adaptation to diets high in carbohydrates have been shown to increase insulin secretion and reduce insulin sensitivity in resting horses. Such changes might limit the availability of glucose and/or non-esterified fatty acids to the muscle when substrate demand is increased during exercise.

Tests for insulin secretion and sensitivity are often complex and intensive. However, one-sample proxies RISQI and MIRG have been statistically validated and standardized in resting horses for minimal model parameters of insulin sensitivity (SI) and insulin response (AIRg), respectively (Treiber et al., 2005d). We compared these proxies in resting and exercising horses adapted to a feed rich in hydrolyzable carbohydrates (SS, NSC 45%, Crude Fat 3%) or a feed with carbohydrates replaced by fat (FF, NSC 13%, Crude Fat 11%).

Materials and Methods

Twelve Arabian geldings were kept on pasture and adapted for 8 weeks to twice daily meals of concentrate intended to provide 30% of their dietary energy. Six were adapted to the SS feed and six to the FF feed. All horses were exercise-trained

throughout feed adaptation. Horses were sampled between 0900 and 1000 h, either at rest or following 25 min of moderate intensity treadmill exercise reaching 60% of each horse's lactate threshold. Plasma was analyzed for glucose and insulin. The minimal model was also performed on all horses immediately following basal sampling (Treiber et al., 2005a). Affects of diet, exercise and interactions were tested by ANOVA. Proxies were calculated according to the following equations (Treiber et al., 2005d):

Insulin Sensitivity	B-cell insulin secretory response
$RISQI := \frac{1}{\sqrt{insulin}}$	$MIRG := \frac{800 - 0.30\,(insulin - 50)^2}{glucose - 30}$

Results

Exercise increased insulin sensitivity (RISQI) ($P = 0.020$) and decreased MIRG ($P = 0.012$). Horses adapted to the FF diet also had higher insulin sensitivity overall ($P = 0.054$). Diet had no significant effect on MIRG ($P = 0.19$). Exercise increased RISQI 56% in FF horses and 13% in SS horses (Figure 2S.1).

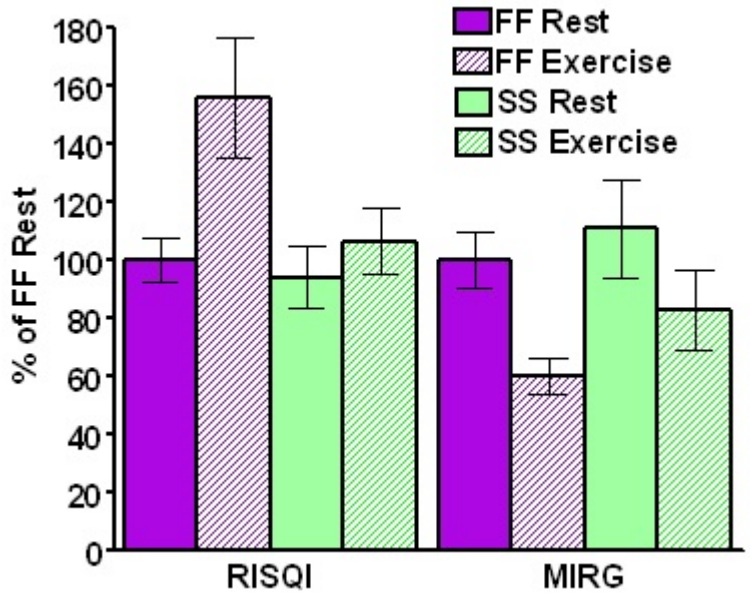


Figure 2S.1. Proxies for insulin sensitivity (RISQI) and insulin secretory response (MIRG) for horses adapted to feeds rich in sugar and starch (SS) or fat and fiber (FF) at rest and during exercise.

Proxies were plotted against the results from the Minimal Model and the regressions previously determined for resting horses. Diet appeared to have no affect on the relationship between proxies and minimal model parameters. Similarly, the regression for MIRG (Figure 2S.2A) was consistent at rest and during exercise. However, exercise dramatically decreased the slope of the regression between RISQI and minimal model insulin sensitivity (Figure 2S.2B).

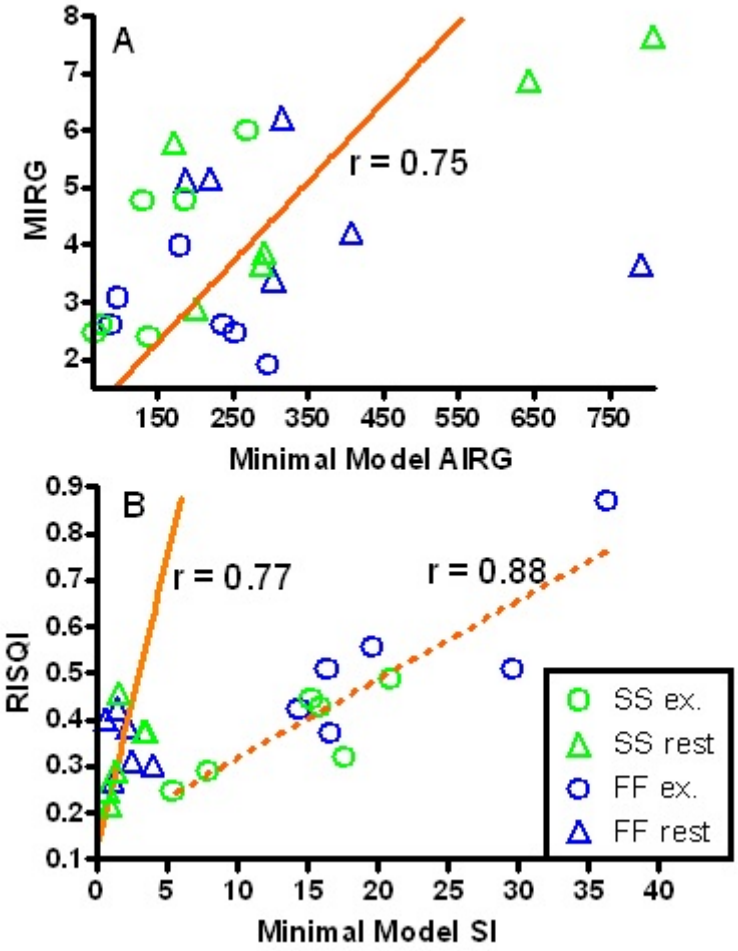


Figure 2S.2. Minimal model SI and AIRg plotted plotted against their respective proxies (RISQI and MIRG). Solid lines represent regressions previously determined for resting horses. The dotted line represents the regression between SI and RISQI during exercise.

Discussion

Insulin sensitivity is well-known to increase during exercise, possibly due to increased availability of insulin-responsive glucose transporters (GLUT-4) or changes in glucose storage as glycogen in skeletal muscle (Ploug et al., 1990; Richter et al., 1982).

The success of one-sample proxies to characterize this change demonstrates their usefulness in a broadening physiological context. The proxies also successfully documented the coinciding decrease in insulin secretory response which occurs during exercise in the horse, is attributed to supression by catecholamines, and has been considered an adaptation to promote fat mobilization as an additional or alternative energy source (Geor et al., 2000a).

The lower slope of RISQI versus SI during exercise may also be due to the suppression of circulating insulin. Also, while apparent SI may increase due to GLUT-4 transport and activation by both circulating insulin concentrations and muscle contraction (Ploug et al., 1990; Yeh et al., 1995), RISQI may reflect only the direct effects of insulin. Although speculative, this possibility provides an interesting perspecitve on muscle contraction, insulin sensitivity, and the physiological representation of the minimal model.

The effect of a diet rich in hydrolyzable carbohydrates such as the SS diet, on insulin sensitivity has been documented in horses and other species (Hoffman et al., 2003a; Samaha et al., 2003). Such meals result in exaggerated fluctuations of glucose followed by a cascade of hormonal regulation and counter-regulation beginning with insulin. Conflicting signals may contribute to metabolic dysfunctions such as insulin resistance (Treiber et al., 2005a). Exercise places additional demands on energy regulation and relies on effective hormonal signals. Therefore underlying metabolic dysfunction may become more apparent under exercising conditions, as evidenced by the lower increase of insulin sensitivity in SS horses compared to FF horses.

No difference between diet groups in terms of insulin secretory response indicates that the SS horses did not need to compensate for their lower insulin sensitivity in orer to maintain glucose homeostasis. Although compensation has been observed in SS adapted horses previously (Hoffman et al., 2003a; Treiber et al., 2005a) the horses in this study were exercise trained. Exercise training could be responsible for a decreased demand for insulin compensation.

CHAPTER 3:

Dietary energy sources and glucose kinetics in trained Arabian geldings during endurance exercise

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Abstract: Exercise and dietary energy sources affect the supply and demand of glucose, possibly stimulating adaptations in the distribution and utilization of glucose. This study modeled glucose kinetics during rest and exercise in trained Arabian geldings adapted to different dietary energy sources, to characterize glucose space and transport. Twelve Arabian geldings were adapted for 4 months to feeds high in sugar and starch (SS) or fat and fiber (FF). All horses were exercise-trained prior to and throughout feed adaptation. Horses underwent two tests. For both tests, horses received 100 μmol/kg BW [6,6-^{2}H]glucose through a venous catheter at 0 min of the test. At rest, the test began with a basal sample, followed by the glucose dose and sampling continued for 150 min. For the exercise test a basal resting sample was taken and then each horse was allowed 25 min to warm up on the treadmill at the end of which each horse was exercising at 60% of their predetermined lactate threshold which was maintained for the rest of the exercise test. The 0 min glucose dose was then applied and sampling continued identically to that of the resting test. Two and three term exponential decay curves were fit to the data and analyzed by compartmental analysis. Curves were compared using Aikaike's Information Criterion, the Durbin-Watson statistic for residual autocorrelation, and the fractional standard deviation of the parameter estimates. Microparameters of the selected compartmental models were compared by diet and exercise using two-way analysis of variance with horses nested in diet.

Results: The conservative, two compartment model was selected based on poor parameter estimation and the inconsistency of improvement in the fit when a third term

was added to the exponential equation. Exercise increased ($P \leq 0.038$) the maximum bounds of all rate constants (min^{-1}) between compartments by (approximately) 110%, all flow (mg/min) between compartments by 75%, and the total transport through the system by 170%. Total glucose entering and leaving the system increased more during exercise in SS horses (from 1.6 ± 0.1 to 4.8 ± 0.3 mg/min per kg) than FF horses (from 1.7 ± 0.1 to 4.1 ± 0.3 mg/min per kg) ($P = 0.043$). There was a tendency for exercise to decrease the minimum bounds for volume and mass of both the peripheral and sampled compartment ($P \leq 0.089$). Changes in the sampled compartment were attributable to FF adapted horses only.

Conclusions: Glucose transport increases during exercise enhancing glucose availability to working tissue. Trained horses adapted to a fat-supplemented diet appear to rely less on glucose as an energy substrate during low-intensity exercise, a potential benefit in maintaining energy supplies during endurance exercise.

Keywords: glucose kinetics, endurance exercise, glycemic dietetics, compartmental model, single injection tracer

Introduction

Kinetic models can be used to characterize the glucose system and allow for quantitative comparisons under controlled conditions. Recently, glucose kinetics have been applied to the horse via primed-infusion to evaluate the effects of exercise and energy substrate (Geor et al., 2000c; Jose-Cunilleras et al., 2002; Pagan et al., 2002). This technique allows for characterization of the directly sampled glucose pool but when non–steady state equations are applied, a priori knowledge (or assumption) of the volume of the glucose distribution space is required.

Single-injection kinetics modeling allows for a more detailed characterization of the glucose system including non-accessible compartments (e.g. slowly exhanging interstitial fluid). It has been over 30 years since this technique has been applied to the horse (Anwer et al., 1976; Argenzio and Hintz, 1972; Evans, 1971). Since then, technological advancement of stable-isotope tracers and improved mathematical

modeling have increased the ease with which kinetic studies can be performed and the detail provided by this important window into the glucose system.

The present study applied single-injection stable isotope tracer glucose kinetics and compartmental modeling to glucose disposal curves from 12 trained Arabian geldings adapted to feeds rich in starch and sugar (SS) or fat and fiber, during rest or constant, low-intensity exercise. Exercise was expected to promote glucose transport as was the higher glycemic sugar and starch feed compared to the feed rich in fat and fiber.

Materials and Methods

Twelve Arabian geldings were matched by body condition score (BC), weight, age, and training history. Horses were maintained on mixed grass/legume pasture and adapted to a feed rich in fat and fiber (FF, n=6) or sugar and starch (SS, n=6). Diet groups were kept separate with pastures rotated and monthly to ensure all subjects were exposed to the same pasture conditions. Diet groups were fed collectively but in individual pans, with each pan containing a single portion (1 kg feed). Feeding occurred twice daily and provided approximately 30% of the recommended DE for light work (NRC, 1989). Horses were weighed and scored for BC before beginning feed adaptation, after 2 and 3 months of feed adaption, and just prior to testing (Henneke et al., 1983). The study was approved by the Institution's Animal Care and Use Committee.

The supplements were formulated to be isocaloric and isonitrogenous with vitamin and mineral contents designed to complement the pasture and fulfill present recommendations (Hoffman et al., 2001; NRC, 1989). Feed compositions were reported previously (Hoffman et al., 2003b). Pasture and supplements were sampled monthly and submitted to a commercial laboratory (Dairy One, Ithaca, NY, USA) for proximate analysis (Table 3.1). Previous studies had demonstrated a 2-fold higher glycemic and 6-fold insulinemic effect of the SS feed compared to the FF feed (Williams et al., 2001); accordingly the SS feed has been assigned a high glycemic index and the FF feed a moderate glycemic index (Kronfeld et al., 2004).

Table 3.1. Partial proximate analysis of SS and FF supplements. Pasture was not different for diet groups for any variable (P > 0.26) and results for all pasture samples were combined. Results are reported on a dry matter basis as means ± sem.

	SS (2 kg/d, n=6)	FF (2 kg/d, n=6)	Pasture (n=12)
Drt matter %	87.4 ± 0.2[a]	91.1 ± 0.5[b]	24.0 ± 2.2
Crude Protein, %	15.4 ± 0.8	14.0 ± 0.5	18.3 ± 0.9
ADF, %	14.7 ± 0.8[a]	32.6 ± 0.6[b]	35.0 ± 1.0
NDF, %	24.2 ± 1.2[a]	45.1 ± 1.1[b]	62.4 ± 1.2
NFC, %	52.2 ± 2.1[a]	26.4 ± 1.0[b]	11.6 ± 1.1
NSC, %	44.7 ± 2.1[a]	13.1 ± 0.8[b]	10.2 ± 0.9
Crude Fat, %	3.3 ± 0.1[a]	10.6 ± 0.8[b]	3.3 ± 0.3
Ash, %	7.2 ± 0.6[c]	8.4 ± 0.1[d]	10.5 ± 0.7
DE[1], Mcal/d	5.30 ± 0.09	5.19 ± 0.09	N/A

[1]DE per kg feed calculated according to Kronfeld and Harris (2000)
[a,b] Supplements differ (P < 0.001)
[c,d] Supplements tend to differ (P = 0.060)

All horses had been conditioned for 10 months to running on a treadmill before the present tests were undertaken. Each horse was exercised for approximately 1 hour, 3 times per week. Exercise consisted primarily of treadmill work (at least once per week) and work in an automatic walker (walk, trot). Horses were also exercised an equivalent amount by riding or lunging when the treadmill and walker were not available.

Each horse underwent 2 similar tracers tests, one at rest and one during exercise. Horses were randomly assigned to tests days and 3 horses from each diet group shared the same test sequence. For all tests, horses were maintained on pasture until the morning of the test and given no concentrate for at least 12 hours prior to the test. A catheter was inserted into the jugular vein the morning of each test and horses allowed at least 30 min to rest following the procedure. Basal samples were then taken, followed by initiation of the test. Horses had at least 8 days to recuperate between tests and missed one training session before and one training session after their exercise test. All tests were initiated between 9 and 10 am.

Rest

Horses had access to grass hay and water ad libitum throughout this test. Following baseline samples, an intravenous glucose tracer dose (100 µmol/kg BW of [6,6-^{2}H] glucose, 98% enriched; Sigma-Aldrich, St. Louis, MO) in 0.9% saline solution was administered rapidly through the catheter. Blood samples were collected at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 23, 26, 30, 35, 40, 50, 60, 70, 90, 110, 130, 150, 180, and 210 (4 FF, 3 SS) or 240 (2 FF, 3 SS) min following the glucose injection.

Exercise

Following baseline samples each horse was warmed up by walking for 10 min at 1.8 m/s at a 0% slope and then trotting for 15 min at a 2% slope at 60% of its lactate threshold as determined by a lactate breakpoint test performed previously and described in Chapter 2. Samples were collected at 10 and 20 min of warm up, followed by a sample at 25 min which constituted the 0 min sample for the test. The horse continued at the same speed (60% of its lactate breakpoint) for the remainder of the test. Immediately following the 0 min sample, an intravenous glucose tracer dose (100 µmol/kg BW of [6,6-^{2}H] glucose, 98% enriched; Sigma-Aldrich, St. Louis, MO) in 0.9% saline solution was administered rapidly through the catheter. Blood samples were collected at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 23, 26, 30, 35, 40, 50, 60, 70, 90, 110, 130, and 150 min following the glucose injection.

Blood Sample Handling

Blood was withdrawn into sample tubes containing EDTA anticoagulant (Vacutainer evacuated blood collection tubes, Fisher Health Care, Chicago, IL, USA) and placed in ice water until centrifuged at 3000 g for 10 min. Plasma was removed within 30 min of collection and frozen at -20º C until analysis.

Plasma glucose was analyzed by enzymatic assay (Beckman Instruments, Glucose Procedure #16-UV, Sigma Diagnostics, St. Louis, MO, USA). Insulin was determined using a radioimmunoassay (Coat-A-Count Insulin, Diagnostic Products, Los Angeles, CA, USA) previously validated for equine insulin (Reimers et al., 1982). The

intraassay CV of duplicate samples was <1% for glucose and 5% for insulin.

Plasma [6,6-^{2}H]glucose enrichment was determined by gas chromotography-mass spectrometric analysis (GC-MS) with electron impact for penta-acetate derivatives of glucose. Protein was precipitated from 0.200 mL of sample plasma by adding 2 mL acetone. The mixture was centrifuged at 3000 g for 10 min at 4˚C and the removed supernatant vacuum-dried. The dried remnant was dissolved in 0.5 mL of a 2:1 v/v acetate anhydride/pyridine and heated on a heating block at 60˚C for 10 min. After cooling to room temperature (5-10 min), 1.0 µL of the solution was injected on the GC-MS system (Hewlett-Packard 6890 GC with 5973N Mass Selective Detector, Hewlett-Packard, Palo Alto, CA). Percent isotope enrichment was determined from the ratio of peak areas for ions 100 (labeled glucose) and 98 (unlabeled glucose) using the selective ion-monitoring mode (Sunehag and Haymond, 2003).

Muscle biopsies

A muscle biopsy samples were taken prior to beginning the kinetics exercise test. After the kinetics test, horses rested for 30 minutes, then performed a stepwise exercise test where speed was increased 1 m/s every 2 min until the horse was unable to continue despite encouragement. A second biopsy was then taken. Muscle biopsy samples were collected percutaneously (6 cm depth) from the middle gluteal muscle by needle-biopsy technique (Lindholm and Piehl, 1974). Muscle samples were placed immediately in liquid nitrogen and stored at -80 C until analysis, when they were freeze dried, dissected free of connective tissue and powdered, then extracted with NaOH, neutralized with HCl and citric acid, and finally incubated with amiloglucosidase (Spriet et al., 1989). Glygogen content was determined spectrometrically as glucosil units per mg dry muscle.

Modeling

Tracer as fraction of dose per L (Tr) was calculated as: $Tr(t) = [E \cdot G(t)] / D$, where t is the time in min, E is the plasma isotopic enrichment (%), G(t) is the plasma glucose concentration (mg/L) at t min, and D is the tracer dose (mg). Curves of Tr(t) were fit using WINSAAM software for two- and three-term exponential decay functions of the

form: $Tr(t) = A_1 \cdot e^{-a1 \cdot t} + A_2 \cdot e^{-a1 \cdot t} + A_3 \cdot e^{-a1 \cdot t}$. Fits for the two and three term exponential functions were compared by Akaike's Information Criterion (AIC_c), autocorrelation of the residuals (Durbin-Watson Statistic), and resolution of the parameter estimates. Parameters were considered unresolved by a fractional standard deviation ≥ 0.50.

Observational parameters [eigenvalues (a_n) and eigenvectors (A_n)] of the exponential equations were used to determine bounds for model microparameters (compartment volumes, mL/kg BW; masses, mg; rate constants, min^{-1}; and transport flow rates, mg/min) (Chen et al., 1985; DiStefano, 1983; Landaw et al., 1984).

Observational parameters were also used to solve for non-compartmental parameters. The determination of clearance rate (mL/min per kg BW) was adapted from non-compartmental analysis used in humans and horses (Geor et al., 2000c; Jeukendrup et al., 1999). Mean residence time (MRT, min) is the average time a glucose unit spends in a space between entering the system and leaving it irreversibly (Carson et al., 1983; Cobelli et al., 1992; Plusquellec and Houin, 1990). Turnover time (min) is the time required to transport a mass of glucose equal to the pool size (Shipley and Clark, 1972). Turnover rate (mg/min) is the sum of glucose being transported to (and from) a space per unit of time (Shipley and Clark, 1972).

Statistics

Statistics were performed using Intercooled Stata Version 9 (StataCorp, College Station, TX, USA). Effects of diet and exercise on hematocrit, total protein, plasma glucose, insulin and model parameters or their minimums and maximums were determined using a two-way ANOVA with horses nested in diet and, where appropriate, time was entered as a repeated measure. Interaction effects at specific time points were evaluated by regression with interaction expansion and clustering by horse. Comparison of bodyweight (BW) for the resting and exercise test was performed by paired t-test. The effect of diet adaptation on BW over 4 months was determined by repeated measures ANOVA with horse nested in diet. For comparison of diet groups within trials, the Kruskal-Wallis test was used. Significance was assessed at $P < 0.05$ and a trend at $P < 0.10$.

Results

Results of the proximate analysis for the feeds and pasture are shown in Table 3.1. Prior to diet adaptation, diet groups did not differ by weight, BC, or age (P = 0.27, 0.54 and 0.62, respectively). Horses weighed 456 ± 13 kg, had BC scores of 5.3 ± 0.3 (range 4 to 7), and were 11 ± 1 yrs old (range 5 to 16). There was no effect of diet or month of adaptation on BW (P = 0.36 and 0.14, respectively), nor was there a difference in BW between the exercise and resting test (P = 0.14). The ambient temperature on the days of the test was not different between diet groups or trials (P = 0.78 and 0.70, respectively). Mean temperature was 7±1˚C.

Basal heart rate was not different between diet groups or trials (P = 0.47 and 0.11, respectively). Mean heartrate during exercise ranged from 90 to 130 bpm for individual horses. Heartrate during exercise did not different across feeds (P = 0.42) and tended to decline (P = 0.059) although heartrate at the beginning of exercise (113 ± 4 bpm) did not differ (P = 0.37) from heartrate at completion (108 ± 3 bpm) (Figure 3.1).

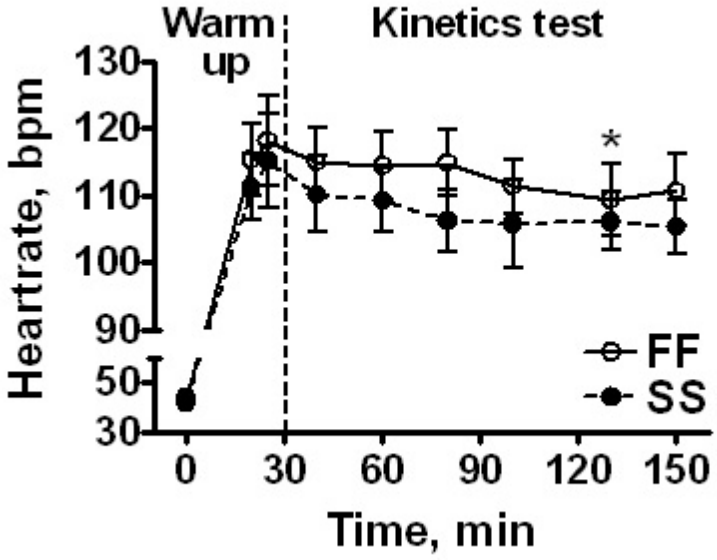


Figure 3.1. Heart rates of SS and FF-adapted horses during the exercise test. Resting baseline values were taken (0 min) followed by a 25 min warm-up after which horses ran at a constant speed. *Significantly different from 40 min across all 12 horses (P = 0.005).

Because predetermined lactate breakpoints were not different, there was no difference (P = 0.16) in the speed during the exercise test for FF horses (3.99 ± 0.09 m/s) compared to SS horses (3.73 ± 0.14 m/s).

Plasma glucose and insulin

Plasma glucose and insulin concentrations during the trials are shown in Figure 3.2. Glucose was approximately 8 mg/dL higher ($P = 0.022$) in SS horses compared to FF horses across both tests but was not different ($P = 0.32$) between rest and exercise. Insulin was not different ($P = 0.31$) between diet groups, but was lower ($P < 0.001$) throughout exercise than at rest.

The tracer glucose bolus at rest resulted in increased ($P \leq 0.013$) plasma glucose concentration compared to baseline from 4 to 35 min with the greatest increase (4 min post glucose bolus) being 15%. For the remainder of the trial, plasma glucose concentration were not different ($P \leq 0.12$) from baseline. During exercise there was a 12% increase ($P < 0.001$) from baseline after the glucose bolus, but basal glucose concentrations were reestablished by 10 min ($P \geq 0.070$). At 110 min, basal glucose values were lower ($P = 0.019$) than basal and continued to decline for the remainder of exercise reaching concentrations 13% lower than basal. At rest, plasma insulin value were increased ($P = 0.003$) by 6.8 mIU/L at 10 min post glucose bolus but had returned to basal values by 30 min. During exercise the glucose bolus had no apparent effect on insulin concentrations, however insulin concentrations were lower ($P = 0.009$) than basal after 70 min and remained lower at the completion of 150 min of exercise.

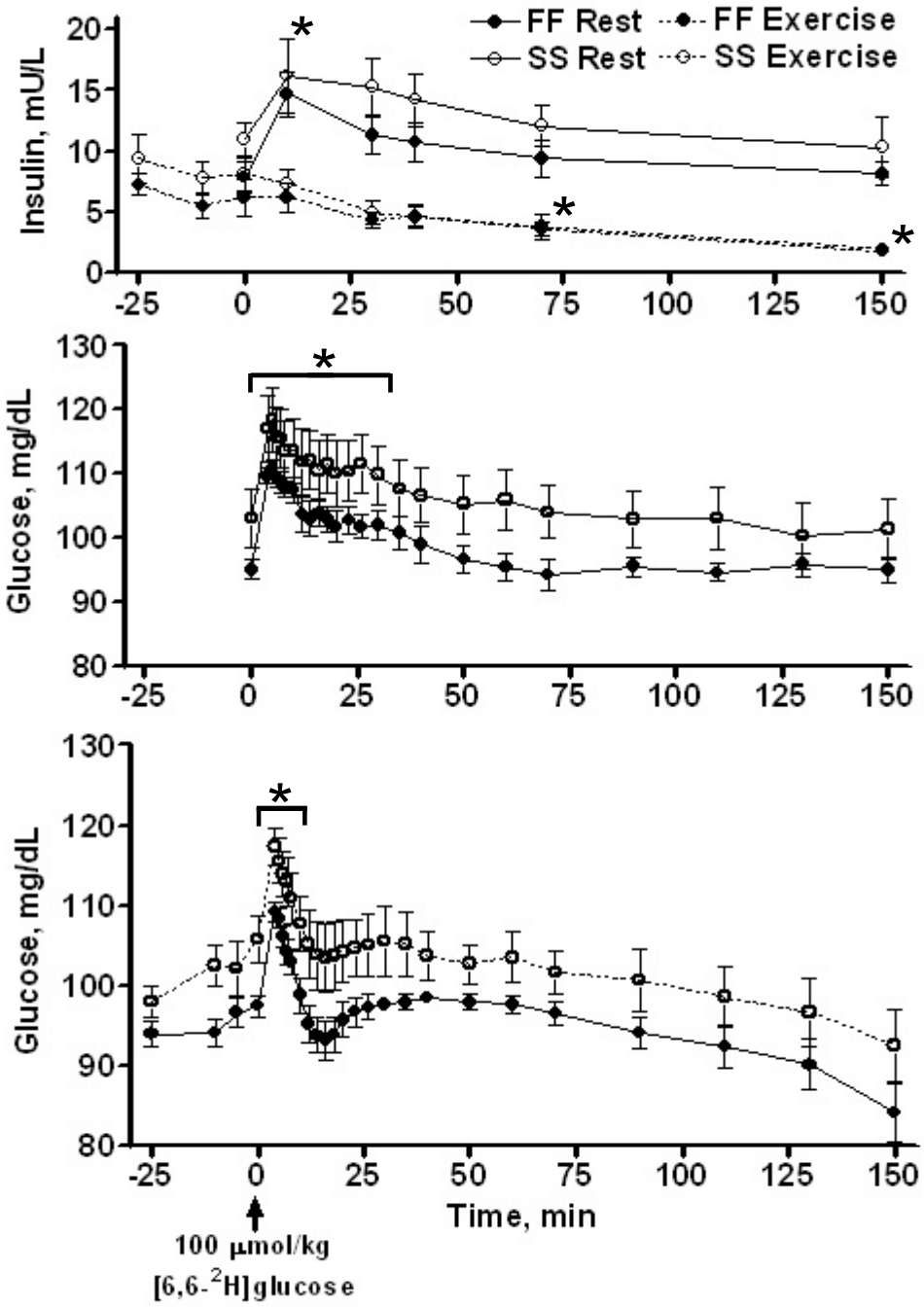


Figure 3.2. Plasma glucose and insulin concentrations at rest solid lines) and exercise (dotted lines) in trained Arabians adapted to diets rich in sugar and starch (SS, open circles) or fat and fiber (FF, closed circles). At 0 min, an intravenous bolus of 100 µmol/kg BW of [6,6-^{2}H]glucose was administered. For the exercise test, a 25 min warm-up occurred before the 0 min dose. *Significant difference ($P < 0.019$) from respective baseline (0 min value) across all horses.

Glucose tracer curves

Plasma enrichment curves are shown in Figure 3.3. An example of fits for one, two and three phase exponential equations during rest and exercise are shown for one horse in Figure 3.4.

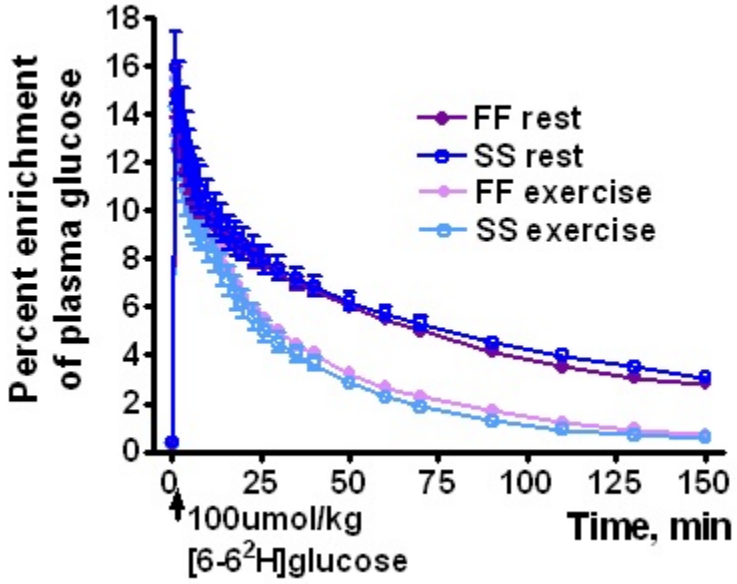


Figure 3.3. Plasma glucose tracer enrichment curves following a tracer glucose bolus. Curves represent horses adapted to feeds high in sugar and starch (SS, n=6) or fat and fiber (FF, n=6) at rest or during low intensity exercise.

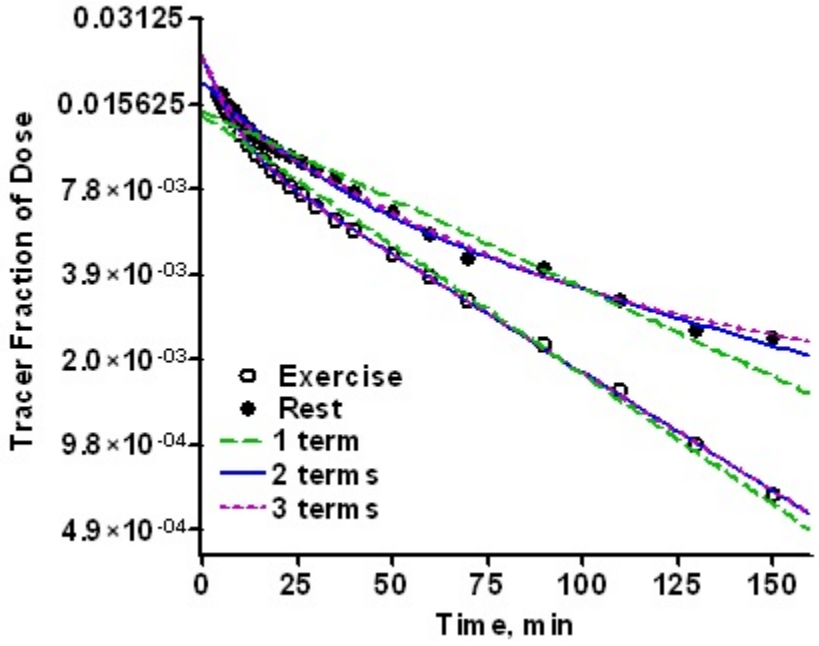


Figure 3.4. One, two and three term exponential decay functions fit to glucose tracer curves during rest and exercise for one trained Arabian gelding. Systematic deviations were apparent for the one term function (broken line) and improved by adding the second exponential term (solid line). Addition of a third exponential term (dotted line) did not condiserably improve the fit.

The one-phase exponential equation was discarded based on clear systematic deviation. At rest, the AIC_c recommended a three-phase curve for 8 (5SS, 3FF) of the 12 horses. Of these, only 6 (3SS, 3FF) demonstrated improved residuals, and subsequently, parameters could be satisfactorily resolved for only 3 horses (2SS, 1FF). None of the criteria supported a three-phase exponential curve during exercise. Accordingly, a conservative two-compartment model was selected for compartmental analysis (Figure 3.5C).

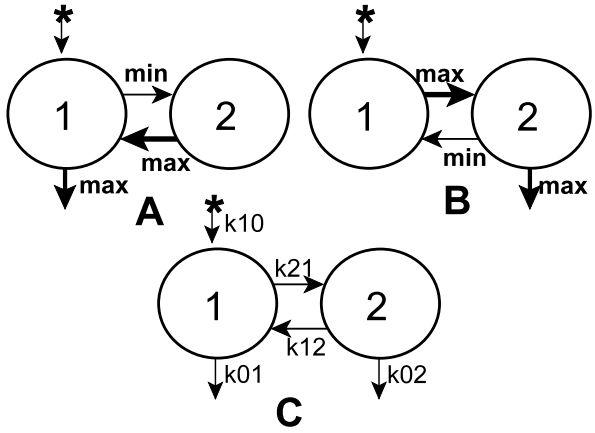


Figure 3.5. Two-compartment models of glucose kinetics. Models A and B are uniquely identifiable with parameters representing upper (max) and lower (min) bounds for the corresponding parameters (kij) in model C.

Model Analysis

Microparameter values or maximum and minimum bounds for the diet groups during exercise and rest are shown in Figure 3.6. Exercise increased ($P \leq 0.038$) the maximum bound of all rate constants (min^{-1}) between compartments by (approximately) 110%, all transport flows (mg/min) between compartments by 75%, and the total transport through the system by 170%. Total glucose transport through the system increased more during exercise in SS horses (from 1.6 ± 0.1 to 4.8 ± 0.3 mg/min per kg) than FF horses (from 1.7 ± 0.1 to 4.1 ± 0.3 mg/min per kg) ($P = 0.043$).

Exercise decreased the minimum bound for the volume (mL/kg) of the second compartment (V2) by 29% ($P = 0.005$) and tended to decrease the maximum bound of V2 by 15% ($P = 0.089$) and the volume of the primary compartment (V1) by 9% ($P = 0.069$). This change in V1 was attributable to an exercise induced drop in V1 of 16% in FF adapted horses ($P = 0.019$) compared to a non-significant 4% in SS adapted horses

(P = 0.99). An associated trend for an interaction (P = 0.087) between feed and exercise on the mass of compartment 1 (Q1) was also observed. This was attributable to an exercise-induced drop in Q1 of 15% in FF adapted horses (P = 0.037) with an non-significant change in Q1 for SS adapted horses (P = 0.67). However there was no difference in the volumes or masses of either compartment between the different feed groups within trials ($P \geq 0.15$).

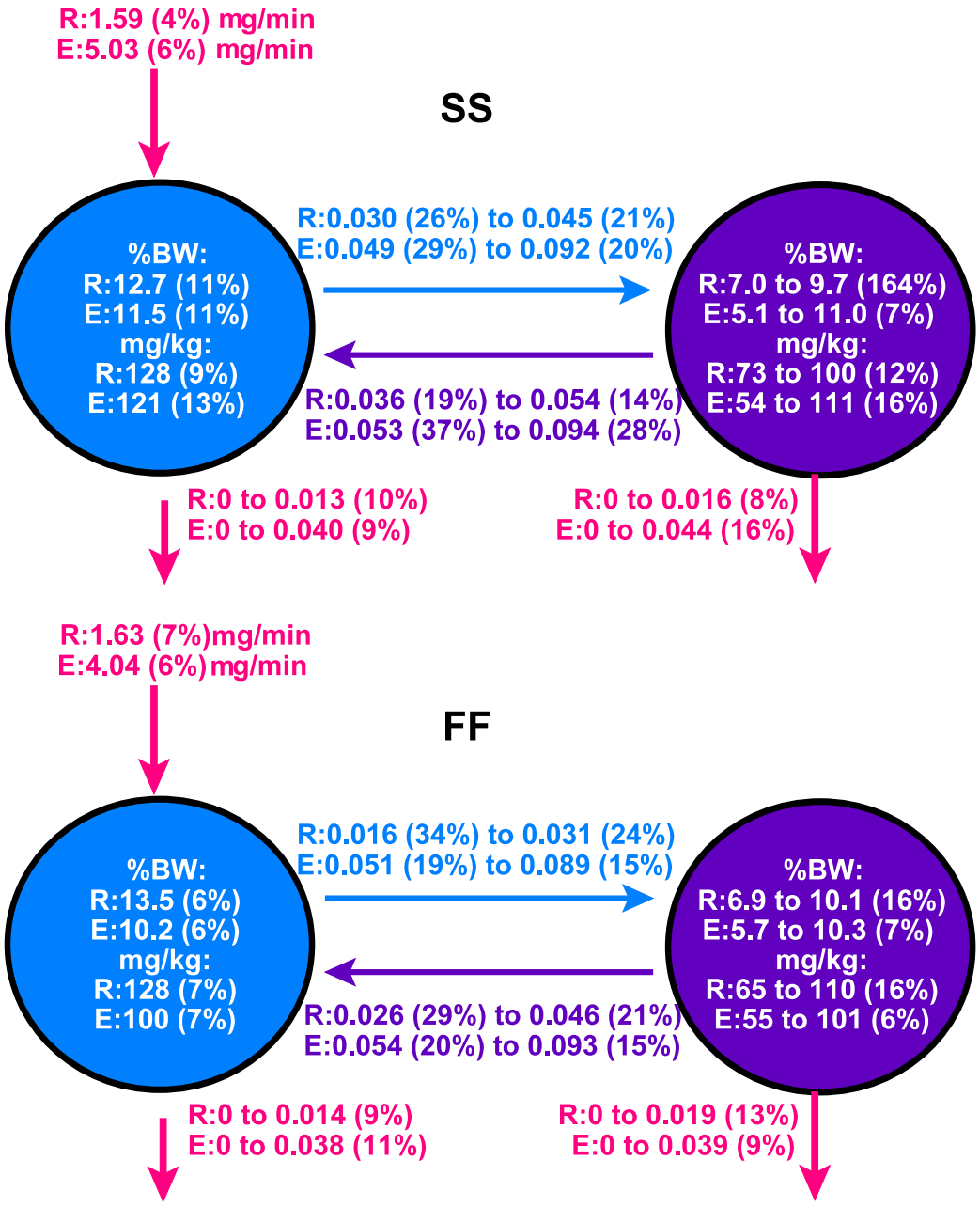


Figure 3.6. Two compartment model of the glucose space at rest (R) and exercise (E) for horses adapted to diets rich in sugar and starch (SS) or fat and fiber (FF). Numbers (min to max) represent rate constants (min^{-1}) unless stated. Data are reported as medians (coefficient of variation).

Results for observational parameters, clearance rate, MRT, and indices of turnover are reported in Table 3.2.

Table 3.2. Observational and non-compartmental parameters of glucose kinetics at rest or during exercise in trained Arabian geldings adapted to feeds rich in sugar and starch (SS) or fat and fiber (FF). Exercise significantly affected all parameters ($P \leq 0.002$) except for the minimum turnover rate for compartment 2 ($P = 0.067$). There were no diet effects or interaction on any parameter ($P \leq 0.12$).

		Rest		Exercise	
	Feed	**FF**	**SS**	**FF**	**SS**
Eigenvectors	$A_1 \times 10^2$	0.95 ± 0.08	1.17 ± 0.17	1.10 ± 0.05	0.95 ± 0.05
	$A_2 \times 10^2$	0.93 ± 0.05	1.08 ± 0.12	1.12 ± 0.05	1.12 ± 0.14
Eigenvalues	$a_1 \times 10^2$	-8.3 ± 1.7	-11.5 ± 2.6	-18.5 ± 1.3	-21.1 ± 4.0
	$a_2 \times 10^2$	-0.76 ± 0.05	-0.76 ± 0.08	-2.32 ± 0.18	-2.61 ± 0.23
Clearance Rate, mL/min per kg BW		1.7 ± 0.1	1.5 ± 0.1	4.2 ± 0.3	4.5 ± 0.2
Mean Residence $Time_{system}$ (MRT) , min		123 ± 9 to 149 ± 12	129 ± 14 to 150 ± 17	40 ± 3 to 50 ± 4	35 ± 2 to 45 ± 4
MRT_1, min		74 ± 7	73 ± 9	25 ± 2	23 ± 1
MRT_2, min		48 ± 5 to 75 ± 7	56 ± 7 to 77 ± 9	15 ± 1 to 25 ± 2	12 ± 1 to 22 ± 3
Turnover rate $constant_1$, (k11) $\times 10^2$, min^{-1}		-4.6 ± 1.0	-6.5 ± 1.6	-10.3 ± 0.6	-10.6 ± 1.3
Turnover rate $constant_2$, (k22) $\times 10^2$, min^{-1}		-4.5 ± 0.8	-5.8 ± 1.1	-10.5 ± 1.0	-13.1 ± 3.0
Turnover $rate_1$, mg/min per kg BW (= Turnover $rate_2$ maximum)		5.1 ± 0.8	6.3 ± 1.3	10.2 ± 0.5	11.6 ± 1.6
Turnover $rate_2$ minimum, mg/min per kg BW		3.5 ± 0.8	4.8 ± 1.2	6.1 ± 0.3	6.8 ± 1.5
Turnover $time_1$, min		26.4 ± 4.9	21.2 ± 5.0	9.9 ± 0.6	10.2 ± 1.2
Turnover $time_2$, min		26.4 ± 4.7	21.3 ± 4.5	9.8 ± 0.7	9.6 ± 1.7

Pre-exercise muscle glycogen was not different between diet groups (FF: 518 ± 21 versus SS: 515 ± 13 mmol glucosyl units/kg dry weight, P = 0.87). Muscle glycogen use across the kinetics plus incremental test also did not differ between diet groups (FF: 107 ± 29 versus SS: 129 ± 16 mmol glucosyl units/kg dry weight, P = 0.52). Despite the indirect nature of the data, muscle glycogen use across both tests was correlated to the maximal rate of glucose loss from the primary compartment determined by the kinetics test (Figure 3.7).

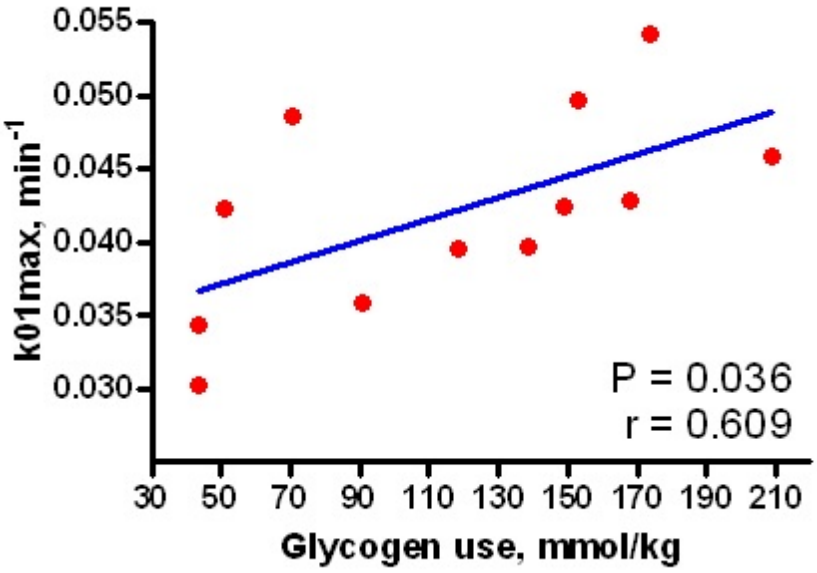


Figure 3.7. Regression of glucose irreversible loss rate and muscle glycogen use during exercise on the same day.

Discussion

This study demonstrated that single injection tracer kinetics can be applied at rest and during constant, low-intensity exercise to provide detailed compartmental models of the glucose system. Enhancement of glucose kinetics was demonstrated during exercise, with a greater exercise-induced increase in total glucose transport in horses adapted to a high-glycemic sugar and starch feed compared to horses adapted to a lower-glycemic feed incorporating fat and fiber to replace hydrolyzable carbohydrates.

Tracer kinetics studies rely on the assumption that the tracee remains at steady state, ensuring linearity so that parameters can be solved based on steady state relationships). Glucose steady-state during exercise has been considered difficult to achieve and single-injection tracer models have rarely been applied (Finegood et al., 1992). However, by providing a period of acclimation (approximately 30 min),

constant low or moderate intensity exercise results in relatively constant plasma glucose concentrations, as demonstrated in this study. The absolute changes in glucose both at rest and during exercise were small in absolute terms (< 15 mg/dL deviation from baseline) and at all times concentrations were within the normal range for horses. The small insulin response at rest (an increase of < 7 mIU/L) and no insulin response during exercise provide further confidence that these data represent a near steady state with minimal perturbations. Simultaneous modeling of tracer and tracee curves from this study (see Chapter 3, Supplement 2) resulted in less than 1% change in the estimates for EGP, consistent with simulations of non-steady states in rabbits (Atkins, 1980a).

Selection of a conservative two compartment model is consistent with most glucose kinetics models in animals (Anwer et al., 1976; Atkins, 1980b; Radziuk et al., 1978). A third glucose compartment may also be elucidated under certain conditions and assumptions, particularly when the sample period is several hours long (Cobelli et al., 1984; Gastaldelli et al., 1997; Jacquez, 1992). This three compartment model is considered to represent the plasma compartment, a rapidly exchanging interstitial compartment, and a slowly exchanging interstitial compartment. For this study, the third compartment could not be satisfactorily characterized in 150 min, with the variability in parameter estimates too large to provide useful information. The two-compartment model selected does not differentiate the rapidly exchanging compartment from the central compartment, and therefore is theorized to represent a primary compartment of plasma and rapidly exchanging interstitial fluid, and a secondary compartment of slowly exchanging interstitial fluid.

The volumes estimated for these compartments in this study (and others) support their physiological interpretation. In the present study, the primary compartment represented 12% of BW at rest and 10% of BW during exercise. Approximately 4% of BW is estimated for plasma volume, 4% for red blood cells, with the remainder representing the rapidly exchanging interstitial fluid. The secondary compartment in this study made up the remainder of the glucose space to estimate a total glucose distribution of 20-24% BW at rest and 16-20% BW during exercise. These values for

rest are similar to values reported across species for glucose space and extracellular fluid (Baker et al., 1959; Kronfeld, 1977; Steele et al., 1956). To our knowledge, this is the first study to estimate the volume of glucose spaces during exercise. The tendency for the volumes of compartment 1 and 2 to be lower during exercise translated into a 15-20% lower volume of the total glucose space during exercise. Similar magnitudes of loss for plasma volume are reported with exercise in the horse and other species independent of sweat loss (Fortney et al., 1981; Nyman et al., 2002; Sejersted et al., 1986). This loss of plasma and interstitial volume is likely attributable to fluid shifts redistributing vascular volume and moving fluid into active muscle (Harrison, 1985).

A two compartment representation of glucose kinetics has been used extensively in the past to describe single-injection tracer experiments. Originally, evaluation of single-injection curves involved the calculation of irreversible loss and total entry rate (in this case all glucose entering the sampled compartment). Because the loss of tracer is directly proportional to the loss of tracee and, under steady-state conditions the amount of tracee lost equals the amount of tracee arriving, irreversible glucose loss can be calculated from the proportion of tracer loss applied to the glucose tracee mass, and total entry rate calculated from the total loss of tracer corrected for the glucose tracee mass (White et al., 1969) (see Chapter 3, Supplement 1: Equation 5). The discrepancy observed between total entry rate and irreversible loss indicated that some glucose was exchanging with another compartment, thus a new term was applied, attributing this difference to ‘recycling’. These factors essentially describe a two compartment model with input and output from the sampled compartment (Figure 3.4A) and recycling from a second compartment which may represent the space on which insulin acts to increase glucose disposal (Ni et al., 1997; Vicini et al., 1997). The other identifiable model (Figure 3.5B) suggests that the sampled plasma-containing compartment exchanges with a peripheral compartment (e.g. interstitial fluid) from which glucose is cleared irreversibly into tissue (Atkins, 1980b).

The two compartment models described above are a priori identifiable, meaning that unique solutions can be determined for all rate constants describing exchange amongst compartments (Shipley and Clark, 1972). However as there is no firm

physiological basis for the assumption that glucose disposal does not occur from one compartment and since both compartments are considered to contain interstitial fluid from which glucose could be expected to be irreversibly cleared into tissue, we chose to evaluate a two compartment model with possible loss from both compartments (Figure 3.5C). This final model is not a priori identifiable, therefore minimal and maximal values for parameters are estimated. These ranges are determined by selectively setting the irreversible loss from each compartment to its minimal value (i.e. 0), thus the minimal and maximal values actually represent the parameter estimates identified by the models assuming loss from only one compartment (Figure 3.5A & B).

The rate of glucose entering the system in resting trained Arabian geldings (~1.5 mg/min per kg) was similar to that previously determined for trained, overnight fasted Thoroughbred, Arabian and Standardbred horses using glucose tracer primed-infusion (Geor et al., 2000a; Geor et al., 2000c; Pagan et al., 2002). As in the Arabians, exercise increased glucose entering and leaving the sampled pool in horses under glucose-tracer infusion, with the percent increase proportional to the exercise intensity (~250% (control diets) or ~150 % (fat-supplemented) at 35% VO_{2max} and ~400% at 50-55% VO_{2max}) (Geor et al., 2000a; Geor et al., 2000c; Jose-Cunilleras et al., 2002; Pagan et al., 2002). By comparison, the mean absolute speed run in the present study (3.9 ± 0.1 m/s) was close to that run at 35% VO_{2max} (4.4 ± 0.2 m/s) and glucose entering and leaving the glucose system in the present study increased by 200% (SS) and 150% (FF). Therefore kinetics studies in exercising horses demonstrate consistency despite differences in breed, training, modeling techniques, and the fasting versus grazing state.

The effect of exercise on additional parameters determined by compartmental modeling is not surprising as the augmented exchange of glucose facilitates the increase in glucose utilization. This augmentation may be attributable to catecholamines, which increase EGP directly and by suppressing insulin while increasing glucagon

(Gustavson et al., 2003; Sumida et al., 2003; Wasserman et al., 1989). The effects of epinephrine on glucose kinetics during exercise have been clearly demonstrated in the horse (Geor et al., 2000a; Geor et al., 2000b).

The volume of distribution for the sampled compartment of primed-infusion experiments has been assumed to be 162 mL/kg (or 65% of the extracellular space estimated as 25% of bodyweight) (Geor et al., 2000abc; Pagan et al., 2001). This value is based on mono-exponential fits to data in resting animals from 60 to 180 minutes, and assumed to remain constant despite observed changes in glucose concentrations (i.e. a non-steady state) (Steele et al., 1956). Primed-infusion studies in horses assumed this volume of 162 mL/kg for the sampled compartment in order to evaluate glucose kinetics during exercise under non-steady state conditions (Geor et al., 2000abc; Pagan et al., 2001; Jose-Cunilleras et al., 2002). Results from the present study, however, indicate that 162 mL/kg is an overestimation of the sampled compartment volume, attributable to the one-compartment assumption, the limited observation period, and a failure to account for fluid shifts which are expected to occur in the exercise state. Applying the volume revealed for the sampled compartment from the present single-injection study (104 mL/kg) to data from primed infusion studies indicates an overestimation of Ra and Rd of approximately 10% due to the volume assumption (Geor et al., 2000abc; Pagan et al., 2001; Jose-Cunilleras et al., 2002). Although noteworthy, this overestimation would not be expected to influence the physiological interpretation of data from primed-infusion studies as the exercise-induced increase observed in Ra and Rd is much greater than the potential overestimation and comparisons between exercising groups relative (Geor et al., 2000abc; Pagan et al., 2001). Likewise, the similar volumes during exercise between diet groups observed the present study suggests a similar overestimation of Ra and Rd across diet groups (assuming similar tracer enrichment values), therefore the difference observed between diet groups during the primed-infusion would not be affected (Pagan et al., 2001). These comparisons indicate the value of single-injection tests to provide a foundation for future kinetics studies.

Diet adaptation had no significant effect on any microparameters of glucose exchange at rest. The effects of diet on resting glucose kinetics has been evaluated in ponies (Anwer et al., 1976; Argenzio and Hintz, 1972; Ford and Evans, 1982) as well as a number of other species including pigs, cows, sheep, camels and fish (Chandrasena et al., 1979; Kronfeld et al., 1971; Machado et al., 1989; Pegorier et al., 1984; Steel and Leng, 1973). Rates of glucose transport have been shown to be proportional to energy intake (Ortigues-Marty et al., 2003; Steel and Leng, 1973) whereas feeds in the present study were supplemented to provide similar digestible energy. Grain concentrates have also been associated with increased glucose transport compared to high-roughage feeds, attributable to greater postprandial glucose loads and possible adaptations to increase the utilization of carbohydrate energy (Evans and Buchanan-Smith, 1975; Ortigues-Marty et al., 2003). Ponies adapted to a diet comprising oats and high soluble-carbohydrate content demonstrated increased glucose entering and leaving the sampled glucose compartment when compared to ponies adapted to alfalfa and beet-pulp (Argenzio and Hintz, 1972). The lack of an effect of diet adaptation on glucose kinetics at rest in this study may be attributable to an effect of exercise training which has been shown to alter both glucose and fat energy utilization (Brooks and Mercier, 1994; Donovan and Sumida, 1990; Manetta et al., 2002) and may have overwhelmed the influence of diet. Similarly no diet effect on resting glucose turnover was found in trained horses fed control or fat-supplemented feeds (Pagan et al., 2002). Interestingly, training alone was not shown to affect resting glucose turnover in horses adapted to the same diet (Geor et al., 2002).

During exercise an effect of diet adaptation on substrate utilization was observed. Glucose transport through the system increased ~2.5 mg/min per kg in FF horses with exercise, and ~3.5 mg/min per kg in SS horses. This difference probably represents greater utilization of plasma glucose for energy during exercise by SS horses as glucose leaving circulation is expected to be entering working tissue for metabolism

(Jeukendrup et al., 1999). Increased uptake of glucose in SS horses could be attributable to up-regulation of the glycolytic pathway, down-regulation of fat metabolism, or higher circulating glucose concentrations. The latter is supported by the similar clearance rates observed between diet groups during exercise. Increased circulating glucose and glucose leaving circulation has not been shown to spare energy stores and appears to improve exercise performance only in subjects at risk for developing hypoglycemia (Coggan and Coyle, 1991). In contrast, lower rates of glucose appearance and disappearance in the plasma during glucose infusion in horses adapted to a fat-supplemented diet suggest a decrease in carbohydrate utilization, likely compensated by increased fat utilization as has been observed in horses during primed-infusion studies (Pagan et al., 2001). Compensation for decreased glucose and glycogen utilization by increased utilization of lipid energy sources has been demonstrated in fat-adapted humans as well (Burke et al., 2000; Helge et al., 2001). The association between glucose loss rate and muscle glycogen utilization in this study suggest a similiar metabolic pattern.

Another interesting diet effect was the tendency for greater exercise-related decreases in the volume and mass of the primary compartment in FF horses compared to SS horses. The differences appeared to be associated with a greater resting volume in FF horses, and a lower glucose mass during exercise in FF horses. The explanations and implications for these differences are unclear.

This study has demonstrated that kinetic analysis of single-injection stable-isotope tracers can be applied both at rest and during constant exercise to develop detailed models of glucose kinetics in accessible and non-accessible compartments. The glucose space was quantified at rest and exercise, with a lower volume demonstrated during exercise and a possible influence of diet adaptation on glucose distribution. Parameter estimates for glucose transport increased during exercise, enhancing glucose availability to working tissues. Glucose transport through the system was greater in horses adapted to a feed high in hydrolyzable carbohydrates compared to horses adapted to a feed replacing hydrolyzable carbohydrates with fat and fiber. These results suggest that horses adapted to diets high in hydrolyzable carbohydrates rely more on circulating glucose as an energy source during exercise.

CHAPTER 3 Supplement 1:
Determination of kinetic parameters

The equations used to solve for minimal and maximal bounds of microparameters for the two compartment model are as follows:
The curves for tracer fraction of dose (y(t), L^{-1}) as shown in Figure 3.2 were fit by the following exponential equation in WINSAAM:

$$y(t) = A_1^{-a_1 \cdot t} + A_2^{-a_2 \cdot t} \quad (1)$$

The volume of the primary compartment (V1, mL per kg BW) was determined as the dilution of the tracer dose extrapolated to t = 0 (Landaw et al., 1984):

$$V1 = \frac{1000mL}{L} \cdot \left[\frac{1}{(A_1 + A_2)}\right] \cdot BW^{-1} \quad (2)$$

From the known steady-state glucose concentration (C1, mg/dL) the mass of glucose in the primary compartment (Q1, mg per kg BW) could be determined (Landaw et al.,1984):

$$Q1 = V1 \cdot C \cdot \frac{0.01dL}{mL} \quad (3)$$

The fraction of glucose leaving compartment i (kii, fractional turnover rate constant, min^{-1}), is defined as:

$$kii = -\sum_j kji \quad (4)$$

where kji is the fractional rate constant (min^{-1}) of glucose from compartment i arriving in compartment j. Therefore k21 is the fraction of compartment 1 transferred to compartment b per min, and k01 is the fraction of compartment 1 which is irreversibly lost each minute. Using the observational parameters of the tracer curve, k11 can be determined as an average of the eigenvalues weighted by the coefficient (Landaw et al., 1984):

$$k11 = \frac{(a_1 \cdot A_1 + a_2 \cdot A_2)}{A_1 + A_2} \tag{5}$$

Because the sum of the eigenvalues is equivalent to the sum of kii, k22 for the two-compartment model can be solved by difference:

$$k22 = a_1 + a_2 - k11 \tag{6}$$

Finally, k12k21, which is the product of k12 and k21 can be determined from k22 (Landaw et al., 1984):

$$k12k21 = \frac{A_1 + A_2}{\left[\frac{A_1}{(k22 - a_1)^2} + \frac{A_2}{(k22 - a_2)^2}\right]} \tag{7}$$

From the above uniquely identifiable microparameters, parameter bounds for the remaining model parameters of *a priori* unidentifiable models can be determined by sequentially setting the loss from each compartment to 0, thereby maximizing the possible loss from the other compartment as shown in Figure 3.4. Specifically, by assuming no loss from compartment 1 (i.e. k01 = 0), then all loss from compartment 1 must arrive in compartment 2, thus k21 is maximized and identifiable:

$$k21\max = -k11 \tag{8}$$

Similarly, the maximum for glucose exchange from compartment 2 to compartment 1 (k12) is determined by setting k02 to zero and using equation 4 for k22, then:

$$k12\max = -k22 \tag{9}$$

Since k12k21 is also known, the minimum values for k12 and k21 are solved for by matching them with their complementary maximums:

$$k21\min = \frac{k12k21}{k12\max} = \frac{k12k21}{-k22} \tag{10}$$

$$k12\min = \frac{k12k21}{k21\max} = \frac{k12k21}{-k11} \tag{11}$$

From these minimums and equation 4, maximal irreversible loss for each compartment can be determined when loss to the other compartment is minimal:

$$k01\max = -k21\min - k11 \tag{12}$$

$$k02\max = -k12\min - k22 \tag{13}$$

From the ki1 and Q1, flow rates (Ri1, mg/min) and turnover rates (Rii, mg/min) can be calculated:

$$Rij = Qj \cdot kij \tag{14}$$

By definition, in the steady-state, the sum of glucose mass arriving in any compartment equals the sum of the mass leaving irreversibly and to all of the n other compartments in the system:

$$\sum_{i=0}^{n} Rij = \sum_{i=0}^{n} Rji \tag{15}$$

As the only glucose mass entering compartment 2 is R21, parameter bounds for the mass of compartment 2 (Q2) can be determined:

$$Q1 \cdot k21 = R12 + R02 = Q2 \cdot (k12 + k02) = Q2 \cdot -k22 \quad (16)$$

Therefore:

$$\frac{Q1 \cdot k21\min}{-k22} \le Q2 \le \frac{Q1 \cdot k21\max}{-k22} \quad (17)$$

Also, because:

$$Vi = \frac{Qi}{C} \quad (18)$$

bounds for V2 and subsequently Vd (total volume of distribution) can be determined:

$$\frac{V1 \cdot k21\min}{-k22} \le V2 \le \frac{V1 \cdot k21\max}{-k22} \quad (19)$$

$$V1 + V2\min \le V2 \le V1 + V2\max \quad (20)$$

R12 is uniquely identified and boundaries for R21 determined using equations 14 and 16, while:

$$R12 = \frac{-Q1 \cdot k12k21}{k22} \quad (21)$$

$$-Q2\min \cdot k22 \le R21 \le -Q2\max \cdot k22 \quad (22)$$

Finally, the total irreversible loss of glucose (and therefore the EGP) is calculated from the difference between the mass of glucose arriving in compartment 1 from compartment 2 (R12) and the total mass leaving compartment 1 (R11):

$$\sum_{i=1}^{2} R01 = \frac{Q1 \cdot k12k21}{k22} - k11 \cdot Q1 = Q1 \cdot \left(\frac{k12k21}{k22} - k11 \right) \quad (23)$$

The equation for clearance rate (CR, mL/min per kg BW) was adapted from non-compartmental analysis used in humans and horses (Geor et al., 2000c; Jeukendrup et al., 1999):

$$CR = \frac{\sum_{i=1}^{2} R0i}{C} \tag{24}$$

Mean residence times (i.e. the average time a glucose unit spends in a compartment after entering the system and before leaving it irreversibly) were determined for the total system (MRT_{total}) and each compartment (MRT_i) (Carson et al., 1983; Cobelli et al., 1992; Plusquellec and Houin, 1990):

$$\frac{Q1 + Q2\min}{\sum_{i=1}^{2} R0i} \le MRT_{total} \le \frac{Q1 + Q2\max}{\sum_{i=1}^{2} R0i} \tag{25}$$

$$MRT_1 = \frac{-k22}{k11 \cdot k22 - k12k21} \tag{26}$$

$$MRT_2 = \frac{k21}{k11 \cdot k22 - k12k21} \tag{27}$$

Turnover time (TT) (i.e. the time required to move a mass of glucose equal to the pool size) for compartment i is defined as (Shipley and Clark, 1972):

$$TT_i = \frac{-1}{kii} \tag{28}$$

Turnover rate (TR) (i.e. the total rate of glucose input and output, mg/min) for compartment i is defined as (Shipley and Clark, 1972):

$$TR_i = -Qi \cdot kii \tag{29}$$

Maximum percent range for bounded parameters (p) can be calculated as (DiStefano et al., 1988):

$$\%\Delta p = 100\% \cdot \frac{p\max - p\min}{p\min} = 100 \cdot \left(\frac{p\max}{p\min} - 1 \right) \tag{30}$$

CHAPTER 3 Supplement 2:

Evalutation of steady-state assumption in non-steady state

The tracer glucose dose of 100 µmoL/kg resulted in slight but significant increase in circulating glucose and insulin concentrations in the test subjects which may have violated the assumption of a glucose steady state. To evaluate the potential influence of a non-steady state on the results of the compartmental analysis, tracer curves from the mean of FF or SS groups at rest and exercise were modeled simultaneously with their respective tracee glucose masses using WINSAAM software (Figure 3S2.1).

Figure 3S2.1. Example of WINSAAM model for simultaneously fitting mean tracer and tracee data of 6 SS horses at rest. Compartments 1 & 2 represent the tracer model with loss from the primary compartment. Compartments 11 & 12 represent the simultaneous tracee data for compartments 1 & 2. Compartments 3 & 4 represent the tracer model with loss from the secondary compartment (i.e. compartment 4). Compartments 13 & 14 represent the simultaneous tracee data for compartments 3 & 4. In tracee models the units for the primary compartment (11 or 13) are mg/dL while the units for the secondary compartment (12 or 14) are in mg.

```
 A SAAM31          SS Rest Combined
2      21
H PAR
c K12MAX
  P(12)    0.03        0.001         1
C K21MIN
  P(21)    0.03        0.001         1
c K12min
  P(34)    0.02        0.001         1
C K21max
  P(43)    0.04        0.001         1
C BW
  P(30)=450
C GB MG/DL
  P(25)    95          75           125
C TRACER FRACTION OF DOSE / L AT TIME=0
  P(1)     0.018       0.01          0.03
  IC(1)=p(1)
  IC(2)=0
```

```
c V1-L01 ML/KG
  P(8)=(1000/P(1))/P(30)
C C1(0)-L01 MG/DL OF V1
  P(11)   115        95         140
  IC(11)=P(11)
c Q2(0)MIN-L01
  P(22)=P(27)*P(21)/P(12)
  IC(12)=P(22)
C V2MIN ML/KG
  P(24)=100*P(22)/P(25)
C Q1-L01 BASAL
  P(27)=P(8)*P(25)/100
C K01MAX
  P(20)   0.01       0.001      1
C EGP-L01, HPO
  P(10)=P(20)*P(27)
C L02 MODEL TRACER FRACTION OF DOSE/L (0)
  P(3)    0.018      0.01       0.03
  IC(3)=p(3)
  IC(4)=0
c V1-L02 ML/KG
  P(6)=(1000/P(3))/P(30)
C C1(0)-L02 MG/DL OF V1
  P(13)   115        100        140
  IC(13)=P(13)
c Q2(0)MAX-L02
  P(44)=P(49)*P(43)/(P(34)+P(40))
  IC(14)=P(44)
C V2MAX ML/KG
  P(46)=100*P(44)/P(25)
C Q1-L02 BASAL
  P(49)=P(6)*P(25)/100
C K02MAX
  P(40)   0.01       0.001      1
C EGP-L02, HPO
  P(50)=P(40)*P(44)
H DAT
C TRACEE MG/DL OF V1
X UF(11)=(P(12)*F(12)*100/P(8))-
 P(21)*F(11)-P(20)*F(11)+P(20)*P(25)
X UF(12)=P(21)*F(11)*P(8)/100-P(12)*F(12)
C TRACER FRACTION OF DOSE / L OF V1
x UF(1)=P(12)*F(2)*100/P(8)-P(21)*F(1)-P(20)*F(1)
x UF(2)=P(21)*F(1)*p(8)/100-P(12)*F(2)
H DAT
C TRACEE MG/DL OF V1
X UF(13)=(P(40)*P(44)*100/p(6))+
 (P(34)*F(14)*100/P(6))-
 P(43)*F(13)
```

```
X UF(14)=(P(43)*F(13)*P(6)/100)-P(34)*F(14)-
  P(40)*F(14)
C TRACER FRACTION OF DOSE / L OF V1
x UF(3)=P(34)*F(4)*100/P(6)-P(43)*F(3)
x UF(4)=P(43)*F(3)*P(6)/100-P(34)*F(4)-P(40)*F(4)
113F(13)                    fsd=0.02
        0
        4          117.0783
        5          118.5258
        6          115.6875
        7          115.5133
        8          113.6008
        10          113.5425
        12          111.93
        14          112.1083
        16          110.6267
        18          111.4842
        20          110.0583
        23          110.44
        26          111.6208
        30          109.8342
        35          107.7
        40          106.5917
        50          105.2833
        60          105.91
        70          103.98
        90          102.9117
        110          103.0833
        130          100.3567
        150          101.3633
103F(3)                     fsd=0.02
        0
        4          0.01756851
        5          0.0169752616666667
        6          0.015667175
        7          0.0150329716666667
        8          0.0144136183333333
        10          0.0136620016666667
        12          0.0128180816666667
        14          0.012335619
        16          0.011698018
        18          0.0114518495
        20          0.010992465
        23          0.0106092965
        26          0.010273987
        30          0.00961581966666667
        35          0.008932594
        40          0.00841561083333334
        50          0.0074972875
```

```
        60          0.00690725966666667
        70          0.0062374505
        90          0.00520675133333333
        110          0.00451066866666667
        130          0.00383585066666667
        150          0.0033213055
114F(14)
        0
2        1                    150
104F(4)
        0
2        10                    15
111F(11)                        fsd=0.02
        0
        4          117.0783
        5          118.5258
        6          115.6875
        7          115.5133
        8          113.6008
        10          113.5425
        12          111.93
        14          112.1083
        16          110.6267
        18          111.4842
        20          110.0583
        23          110.44
        26          111.6208
        30          109.8342
        35          107.7
        40          106.5917
        50          105.2833
        60          105.91
        70          103.98
        90          102.9117
        110          103.0833
        130          100.3567
        150          101.3633
101F(1)                         fsd=0.02
        0
        4          0.01756851
        5          0.0169752616666667
        6          0.015667175
        7          0.0150329716666667
        8          0.0144136183333333
        10          0.0136620016666667
        12          0.0128180816666667
        14          0.012335619
        16          0.011698018
        18          0.0114518495
```

```
        20          0.010992465
        23          0.0106092965
        26          0.010273987
        30          0.00961581966666667
        35          0.008932594
        40          0.00841561083333334
        50          0.0074972875
        60          0.00690725966666667
        70          0.0062374505
        90          0.00520675133333333
        110         0.00451066866666667
        130         0.00383585066666667
        150         0.0033213055
112F(12)
        0
2       1                   150
102F(2)
        0
2       10                  15
```

This model assumed constant hepatic glucose production equivalent to the total glucose loss at basal steady state glucose concentration. The masses of the compartments changed, however, as reflected by the changes in glucose concentrations of the primary compartment (i.e. plasma glucose concentrations). This model also assumed linear rate constants and no additional clearance functions, therefore fits to the plasma glucose concentrations represent only the best-fit exponential decay function of the tracee data (Figure 3S2.2). Fractional standard deviations for all parameters of all fits were less than 0.5 and most less than 0.1. The resulting differences in parameters estimations are shown in Table 3S2.1.

Figure 3S2.2. Results from the combined tracer/tracee model for 6 SS horses at rest. Above: Model fit (red line) to tracer in the primary compartments (c1, mg tracer/mg dose per L, open circles). Calculated curves for tracer fraction in secondary compartments (q2, mg tracer/mg dose) when loss is from the primary compartment (L01, blue line) or from the secondary compartment (L02, green line). Below: Model fit (red line) to plasma glucose concentrations in the primary, sampled compartment (C1, mg/dL, open circles). Calculated curves for glucose mass in the secondary compartment (Q2, mg/kg BW) when loss is from the primary compartment (L01, blue line) or from the secondary compartment (L02, green line).

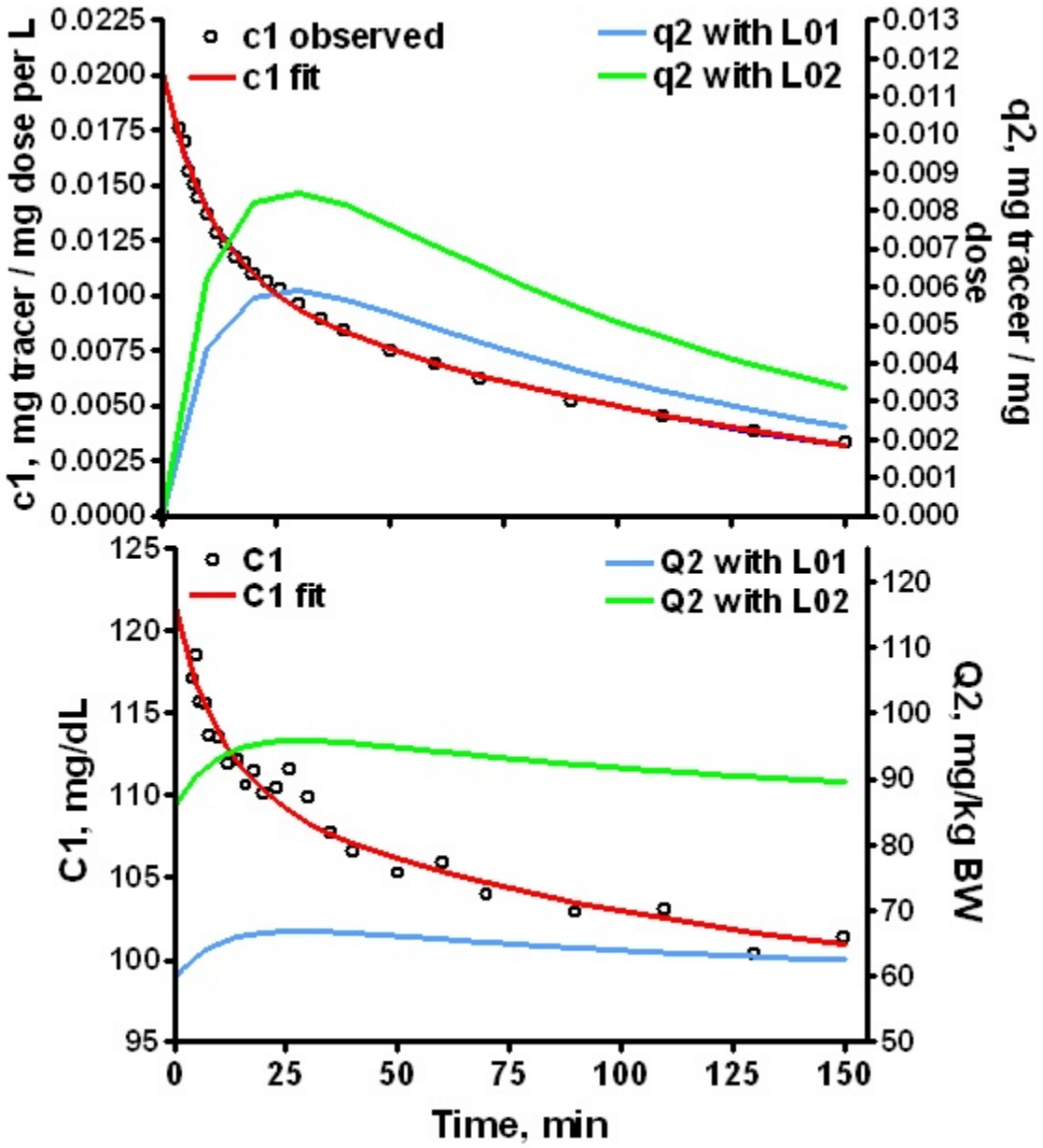


Table 3S2.1. Estimated transport from the glucose system from the model under steady-state assumptions or with simultaneous modeling of tracee data (combined model).

Diet/Trial	k12 min-max (min^{-1})	k21 min-max (min^{-1})	k01,k02 (min^{-1})	Total system transport (g/min)	V1 (mL/kg)	V2 min-max (mL/kg)
FF/Rest	0.022-0.040	0.018-0.030	0.013, 0.017	1.506	131	58-103
FF/Rest Combined	0.024-0.041	0.018-0.031	0.013, 0.017	1.509	130	59-101
SS/Rest	0.040-0.057	0.032-0.046	0.014, 0.017	1.484	110	62-88
SS/Rest Combined	0.040-0.057	0.032-0.046	0.014, 0.017	1.484	111	62-88
FF/Exercise	0.059-0.100	0.053-0.092	0.038-0.042	3.598	104	55-95
FF/Exercise Combined	0.063-0.104	0.058-0.098	0.039, 0.042	3.590	102	57-95
SS/Exercise	0.044-0.092	0.036-0.075	0.039, 0.048	4.498	119	46-96
SS/Exercise Combined	0.046-0.093	0.037-0.077	0.039, 0.048	4.495	118	47-96

Although the combined model provides only an estimate it attempts to correct for changes in plasma glucose concentrations, giving an idea of the potential influence of non-steady state, which in these examples was less than 1% for total glucose transport. Another study evaluating the effects of simulated steady-state violations showed a similar difference in estimated glucose transport also considerably smaller than the observed difference in plasma glucose concentrations (Atkins, 1980a).

CHAPTER 4:

Insights from combined kinetics and minimal model studies

The dynamic minimal model and the glucose tracer kinetic model complement each other by characterizing the unperturbed glucose system (kinetics) and then characterizing the response of that system to a perturbation, in the case of the minimal model hyperglycemia and subsequent hyperinsulinemia. Therefore certain parameters from the kinetic model should relate to those from the minimal model.

The minimal model assumes that endogenous glucose production (EGP_{MM}, mg/dL per min) at steady state is Sg·Gb, where Sg is the glucose effectiveness parameter (min^{-1}) and Gb is the basal glucose concentration (mg/dL). The kinetic test provides an estimate of EGP (mg/min) which can be compared to EGP_{MM} when divided by the volume (in dL) of the primary compartment into which it arrives to produce EGP_k (mg/dL). No difference (P = 0.35) was found by paired t-test between EGP_{MM} (2.60 ± 0.25 mg/dL per min) and EGP_K (2.84 ± 0.31 mg/dL per min) across both rest and exercise trials. The EGP_{MM} and EGP_k were correlated (r = 0.623; P = 0.001) and had a concordance coefficient of 0.656 (Lin, 1989) (Figure 4.1). These results indicate agreement between the kinetic and dynamic tests.

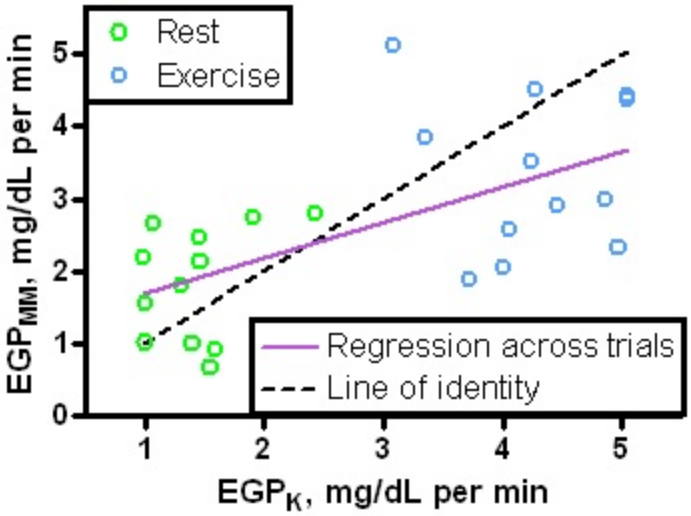


Figure 4.1. Association between estimates for endogenous glucose production from the minimal model (EGP_{MM}) and kinetics (EGP_K) studies. Values are for trained horses tested at rest (green) or during exercise (blue).

When exercise and resting tests are considered separately, there was a tendency (P = 0.095) for EGP_{MM} to be 22% higher than EGP_K at rest, while EGP_{MM} was 26% lower (P = 0.040) than EGP_K during exercise. When corrected for units, the regression between EGP_{MM} and EGP_K was almost identical to that reported for resting humans which observed overestimation of minimal model Sg (hence EGP_{MM}) when glucose loss rate was low (e.g. at rest) and underestimation when glucose loss rate was high (i.e. during exercise), attributed to confounding by incomplete mixing (Ni et al., 1997).

The tendency for the minimal model to overestimate Sg at rest, particularly in diabetic patients has prompted the addition of a second compartment to the minimal model to reduce the bias of mixing in a nonuniform space (Avogaro et al., 1989; Callegari et al., 2003; Ni et al., 1997). In order to ensure identificability, the two compartment minimal model (2CMM) requires additional assumptions or a priori knowledge (Avogaro et al., 1989; Callegari et al., 2003; Vicini et al., 1997).

In the present study, microparameters identified by the kinetics tracer test potentially represent the necessary a priori knowledge. By combining an identified two compartment model and the remote insulin compartment of the minimal model as affecting output from one of the two compartments, a 2CMM could be used to fit FSIGT data in these horses (Figure 4.2).

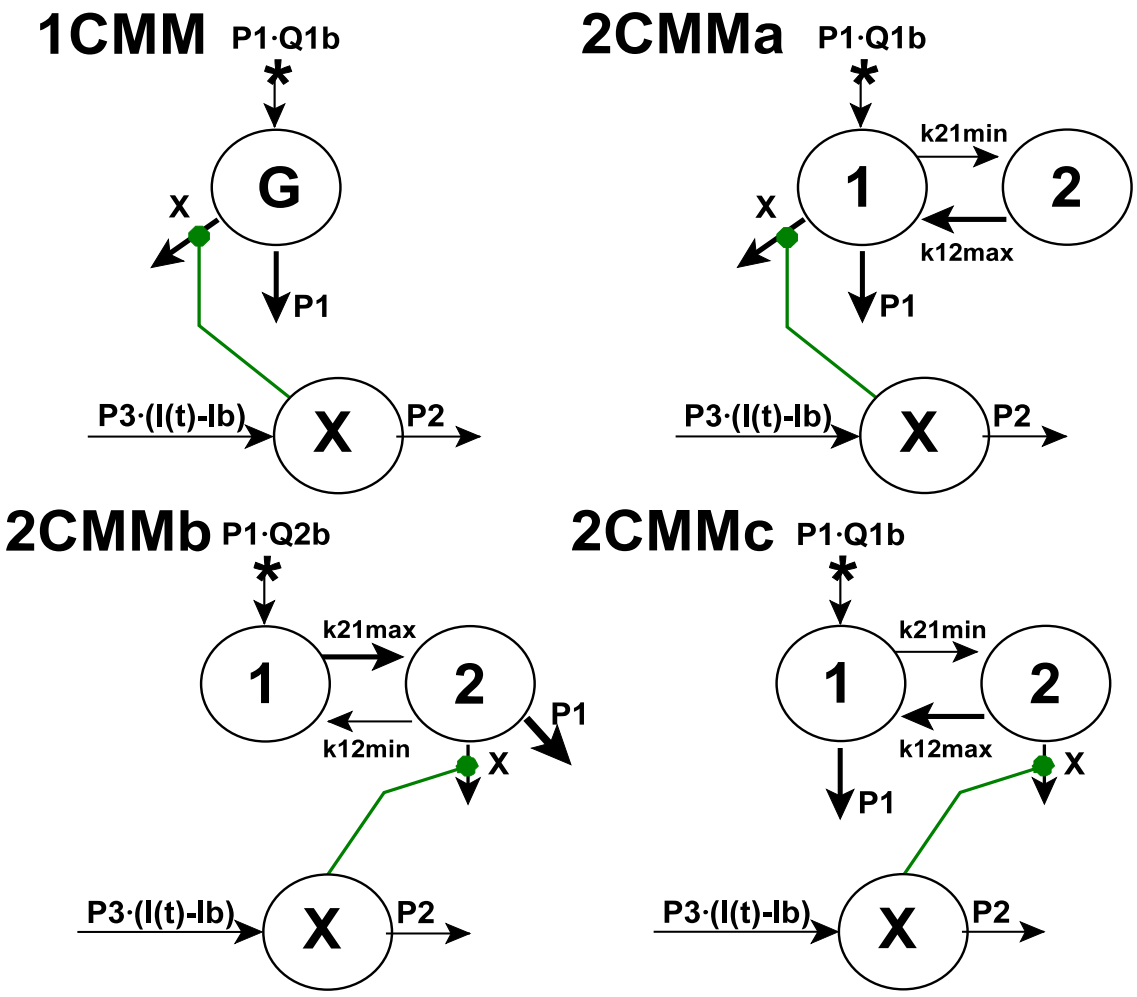


Figure 4.2. Models of glucose and insulin dynamics. Traditional one-compartment minimal model (1CMM) with single glucose distribution space (G), remote compartment of insulin action (X), glucose loss at basal insulin (p1, min^{-1}) and insulin-mediated glucose loss (p2/p3, min^{-1} per mIU/L insulin). Change in plasma insulin concentration is indicated by the difference between plasma insulin concentration at time = t [I(t)] and basal insulin (Ib). Two compartment minimal models are illustrated similarly (2CMMa-c).

The models from Figure 4.2 were fit to the FSIGT data using WINSAAM with the following assumptions. Basal glucose concentration in the sampled compartment (G or 1) was represented by the final (150 min) value (Callegari et al., 2003). Compartment volumes (V1, V2min for models 2a and 2c, and V2max for model 2b) were provided a priori by the kinetic analysis for each horse. Estimates for parameters k12 and k21 for individual horses were also provided a priori by the kinetic study as appropriate (Figure 3.4). Estimates for k12 and k21 within the 2CMM given only the known value of parameter k12k21 (the product of k12 and k21) were attempted and yielded satisfactory parameter estimates. However, parameter estimates for p1 and k12 were highly correlated, therefore k12 and k21 were assumed known. For the one compartment model, G(0) (i.e. the initial concentration of the sampled compartment) was estimated and this estimate used to calculate the volume of distribution of the one compartment model (Vd_{1C}, mL/kg BW):

$$Vd_{1C} = \frac{D}{G(0) - Gb}$$

where D is the glucose dose in mg/kg BW and Gb is the basal glucose concentration in mg/dL. The models are described by the following equations:

One compartment minimal model:

$$G'(t) = -G(t) \cdot [p1 + X(t)] + p1 \cdot Gb$$
$$X'(t) = -p2 \cdot X(t) + p7 \cdot p2 \cdot [I(t) - Ib]$$
$$X(0) = 0$$

where I(t) is insulin at time = t and Ib is basal insulin.

Two compartment model 2CMMa:

$$Q1(t) = -Q1(t)\cdot[p1 + X(t) + k21] + p1\cdot Q1b + Q2(t)\cdot k12$$
$$Q2(t) = Q1(t)\cdot k21 - Q2(t)\cdot k12$$
$$X'(t) = -p2\cdot X(t) + p7\cdot p2\cdot[I(t) - Ib]$$
$$X(0) = 0$$
$$Q1(0) = Q1b + D$$
$$Q2(0) = Q1b\cdot\frac{k21}{k12}$$

Two compartment model 2CMMb:

$$Q1(t) = -Q1(t)\cdot[k21] + p1\cdot Q2b + Q2(t)\cdot k12$$
$$Q2(t) = Q1(t)\cdot k21 - Q2(t)\cdot[k12 + X(t) + p1]$$
$$X'(t) = -p2\cdot X(t) + p7\cdot p2\cdot[I(t) - Ib]$$
$$X(0) = 0$$
$$Q1(0) = Q1b + D$$
$$Q2(0) = Q1b\cdot\frac{k21}{k12 + p1}$$

Two compartment model 2CMMc:

$$Q1(t) = -Q1(t)\cdot[k21 + p1] + p1\cdot Q2b + Q2(t)\cdot k12$$
$$Q2(t) = Q1(t)\cdot k21 - Q2(t)\cdot[k12 + X(t)]$$
$$X'(t) = -p2\cdot X(t) + p7\cdot p2\cdot[I(t) - Ib]$$
$$X(0) = 0$$
$$Q1(0) = Q1b + D$$
$$Q2(0) = Q1b\cdot\frac{k21}{k12}$$

where Q1 and Q2 are the mass (mg/kg BW) of their respective compartments and values, Q1b and Q2b are the basal (i.e. steady state) masses of their respective compartments, and Q1(t) is defined by multiplying the glucose concentration curve for the primary compartment by V1.

Data for all horses during exercise were fit to the 4 dynamic models. For one horse (#11) fits for models 2CMMb or 2CMMc were not possible. Difficulty fitting model 2CMMb and 2CMMc was encountered for one additional horse (#5). This difficulty may have been attributable to these horses having the most extreme hypoglycemia (~30 mg/dL) observed during the FSIGT. Difficulty fitting model 2CMMb (but not 2CMMc) was also encountered for 2 more horses (#1 & 2). The results for difficult fits produced high, probably spurious values for insulin sensitivity (Figure 4.4). For all models, horses, and parameters, fractional standard deviations of the parameter estimates were less than 0.5 and most were less than 0.1. For all models and horses, inter-parameter correlations were less than 0.95. The sum of squared residuals for each 2CMM was not different ($P \geq 0.089$) from that of the 1CMM, but was lowest in 8 of 12 horses for the 1CMM.

The volume of glucose distribution from the 1CMM (Vd_{1C}) was 157 ± 7 mL/kg BW. This volume was higher ($P < 0.001$) than the volume of the primary compartment (V1) estimated by the two compartment kinetic model (104 ± 4 mL/kg) and lower ($P = 0.003$) than the two compartment maximum volume of distribution (205 ± 12), but not different ($P = 0.54$) from the two compartment minimum volume of distribution (162 ± 8) (Figure 4.3). These results are consistent with the fact that Vd and Vdmin represent the total distribution space of models with loss from the sampled compartment only.

The parameters normally associated with glucose effectiveness (Sg, p1) and insulin sensitivity (SI, p7) have different absolute values depending on the volume (or mass) of the glucose space on which they act. Therefore in order to compare the results from the various models, Sg and SI were normalized to SgV (mL/min per kg BW) and SIV (mL/min per mIU/L insulin per kg BW) by multiplying the estimated parameters (p1 or p7) by the volume of the compartment they affect (Callegari et al., 2003) (Table 4.1).

Figure 4.3. Estimates of volume for the glucose distribution space during exercise where Vdmax (red) and Vdmin (blue) are the maximum and minimum values estimated for the total distribution space of the two compartment kinetic model, V1 (purple) is the volume of the primary compartment of the two compartment kinetic model, and Vd_{1C} (green) is the total volume of distribution estimated for the single compartment of the 1CMM.

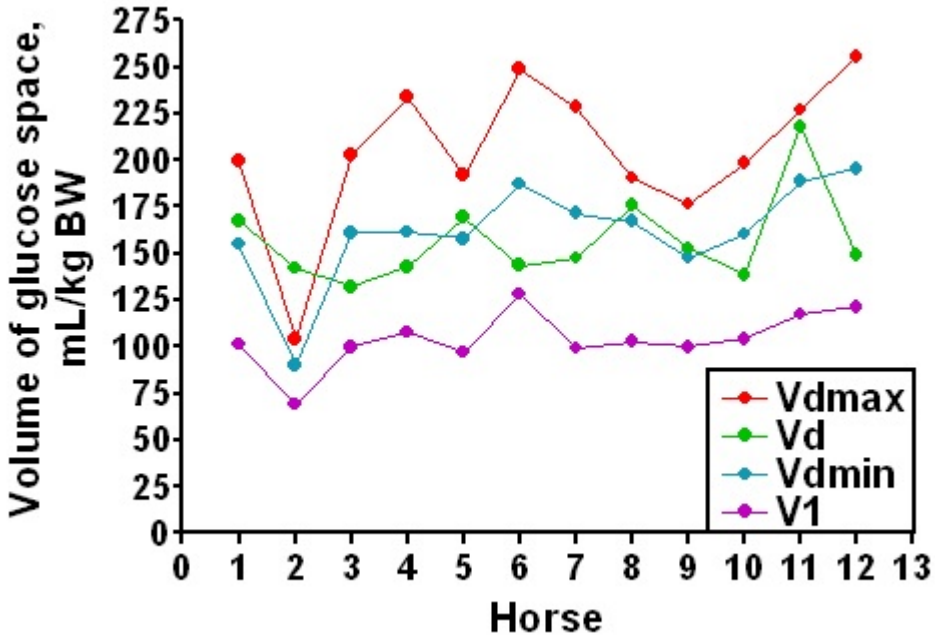
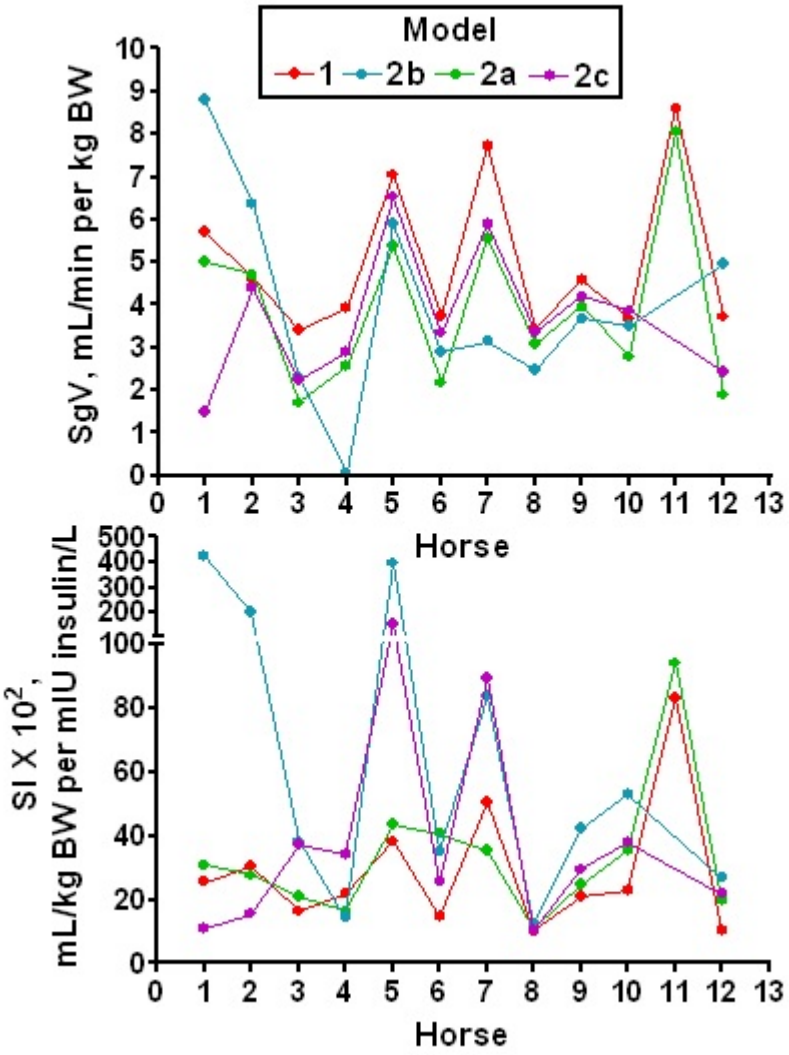


Table 4.1. Determination of glucose clearance parameters SIV and SgV for the 1CMM and 2CMMs.

		Compartmental Model		
Parameter	1CMM	2CMMa	2CMMb	2CMMc
Glucose clearance at basal insulin (SgV, mL/min per kg BW)	p1·Vd	p1·V1	p1·V2max	p1·V1
Insulin-mediated glucose clearance (SIV, mL/min per mIU/L insulin per kg BW)	p7·Vd	p7·V1	p7·V2max	p7·V2min

Results for SgV and SIV are shown in Figure 4.4. Paired t-tests revealed that SgV from the 1CMM was ~20% higher ($P \geq 0.023$) than SgV from models 2CMMa and 2CMMc. The same test demonstrated that SIV tended to be higher ($0.094 > P > 0.054$) for model 2CMMb than for other models.

Figure 4.4. Glucose effectiveness (SgV) and insulin sensitivity (SIV) during exercise for the 1CMM (red) and 2CMMs (2CMMa, green; 2CMMb, blue; and 2CMMc, purple) for 12 trained Arabian geldings.



Regressions comparing SgV for the 1CMM to each 2CMM are shown in Figure 4.5. The SgV of the 1CMM was correlated to SgV from 2CMMa and 2CMMc and the regression approached the line of identity. The regression for SgV of 2CMMb and SgV of 1CMM was not significant (P = 0.21). As Sg and SI were correlated for all models ($0.73 \geq r \geq 0.89$) results were similar for regressions comparing SIV of 1CMM to each 2CMM.

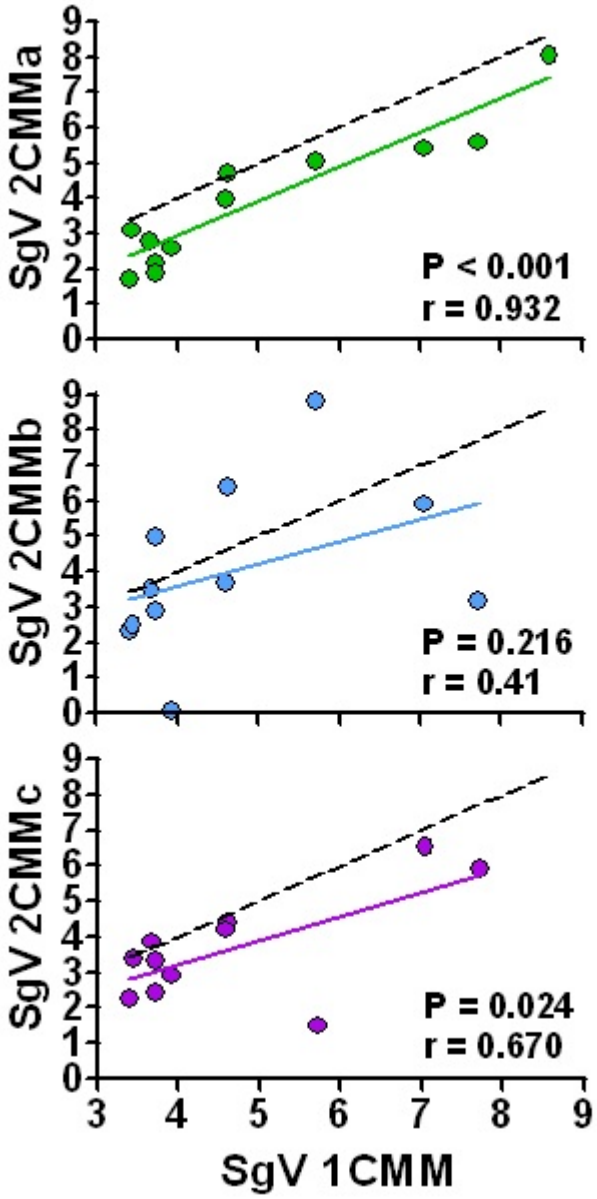
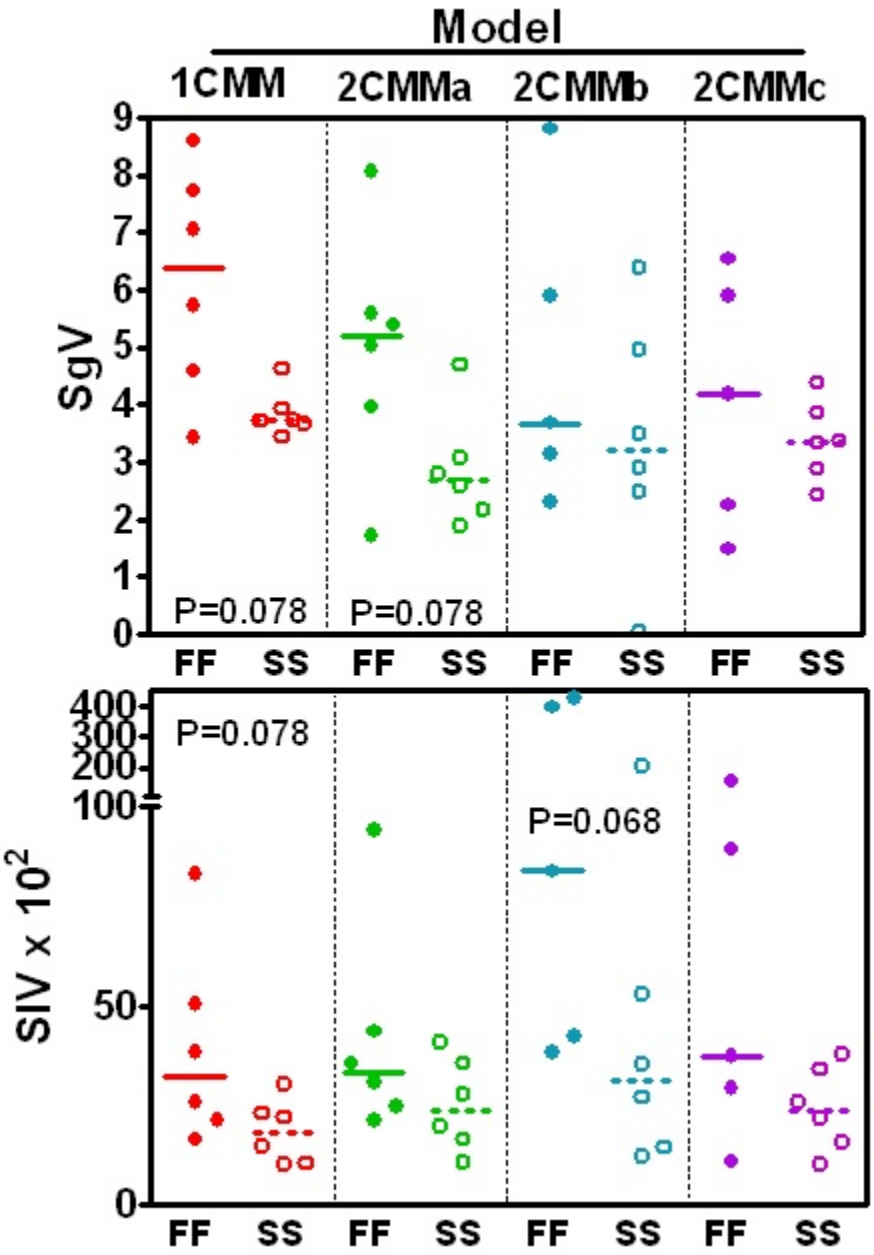


Figure 4.5. Regressions of glucose effectiveness (SgV) for two-compartment minimal models (2CMMs) versus the one-compartment minimal model (1CMM). Dotted lines represent the line of identity.

As described in previous chapters, the horses were adapted to feeds rich ineither fat and fiber (FF, n=6) or sugar and starch (SS, n=6). There was no significant feed effect on Vd1C of SgV of SIV for any model according to the Kruskal-Wallis test. However FF horses tended to have higher SgV from models 1CMM and 2CMMa (P = 0.078) and higher SIV from models 1CMM (P = 0.078) and 2CMMb (P = 0.068) (Figure 4.6).

Figure 4.6. Glucose effectiveness (SgV, mL/min per kg BW) and insulin sensitivity (SIV, mL/min per mIU/L insulin per kg BW) for the one-compartment minimal model (1CMM) and two-compartment minimal models (2CMMs). P-values indicate a tendency for difference within models between horses adapted to a feed high in fat in fiber (FF) and those adapted to sugar and starch (SS).



These models show that single-injection tracer glucose studies coupled with FSIGT tests can provide estimates of glucose effectiveness (Sg) and insulin sensitivity (SI) for a two compartment minimal model. Three identifiable 2CMMs are possible (Figure 4.2) of which 2CMMa and 2CMMc have been reported in human literature (Callegari et al., 2003; Ni et al., 1997). Another 2CMM has been proposed, but was not explored here due to the number of inherent assumptions which cannot be justified, as yet, for the horse (Vicini et al., 1997). This study assumed that k12 and k21 estimated from a separate steady-state kinetic study were applicable to the dynamic model under glucose and insulin-stimulated conditions. In humans, k12 and k21 estimated from a 2CMM and a prior kinetic tests were shown to be similar (Callegari et al., 2003).

The volumes of glucose space observed for exercising horses were lower (~30%) than those observed in resting humans and dogs, most likely attributable to fluid shifts into the working muscle tissue. The values for SgV in exercising horses are comparable to those reported for resting humans (1CMM and 2CMMa: (Callegari et al., 2003)) and slightly higher than for resting dogs (model 2CMMc: (Ader et al., 1997)). The values for SIV are higher than that of resting humans (1CMM and 2CMMa: (Callegari et al., 2003)). Higher values for Sg and SI are expected during exercise compared to rest (see Chapter 2).

The overestimation of SgV by the 1CMM compared to 2CMMa agrees with the results observed in resting humans (Callegari et al., 2003), although the magnitude of the overestimation was not as great as that in resting humans, possibly due to the higher glucose loss rate during exercise and therefore less confounding of the 1CMM by mixing. A 13% increase in SIV from the 1CMM to the 2CMMa was also reported in humans (Callegari). Although this difference was not significant in the present study, 9 of 12 horses had higher SIV for model 2CMMa (Figure. 4.3).

In selecting the preferred model for use, model 2CMMb seems least likely to be appropriate. This model was by far the most difficult to fit and produced inconsistent result in 3 of 11 horses. This model assumes all loss from the secondary compartment. When this compartment is small relative to the primary compartment and return to the primary compartment (k12) is large, the rate of irreversible loss from compartment 2 (p1

+ X(t)) must be very large to account for the observed net loss from the primary compartment. This could explain the extreme values for SIV observed in some horses. Models 2CMMa and 2CMMc responded similarly and provided possibly improved estimates of SgV. At the same time, the strong correlations between 1CMM and 2CMMa or 2CMMc parameters, and the lack of improvement in the sum of squares by the 2CMMs suggest that the 1CMM is a dependable tool for evaluating glucose and insulin dynamics in horses.

PART II - INSULIN RESISTANCE, THRIFTINESS AND LAMINITIS

CHAPTER 5:LITERATURE REVIEW

Thrifty Strategy

Glucose is an fundamental energy metabolite, fueling the central nervous system and fetal development. Many populations may not have access to glucose via their diet and must supply themselves through alternative means, primarily gluconeogenesis from volatile fatty acids (hindgut fermentors and ruminant herbivores) or proteins (carnivores) (Morris et al., 1983). Insulin resistance aids in the sparing of glucose by promoting gluconeogenesis and preventing glucose from entering peripheral muscle and adipose tissues which can utilize fat as an alternative energy source (Donkin and Armentano, 1995). Thus populations which have evolved on low-glucose diets such as ponies, ruminants, cats and even hunter-gatherer humans may possess a related metabolic predisposition to insulin resistance, known as the 'thrifty genotype' (Neel, 1962).

The adaptive thrifty genoptype can become detrimental when nutrition is altered (for example as result of new agricultural technology or species domestication/captivity) and glucose and energy become available in excess. In addition to digestive upsets, the influx of hydrolyzable carbohydrates interacts with underlying insulin resistance to contribute to metabolic disease states including dyslipidemia, type 2 diabetes and possibly laminitis (Colagiuri and Brand Miller, 2002; Zoran, 2002). At the same time, decreasing activity and increasing obesity in modern humans and domesticated animals further exacerbate insulin resistance. These perturbations confound the underlying dietary and metabolic interactions while making management strategies even more critical. The rising incidence of metabolic disease in our own species and the species we shepherd has spurred an increasing interest in balancing nutrition and performance goals with the limitations and advantages of an individual's physiological predisposition.

Pony breeds originate from areas of harsh climates with rocky, mountainous terrain and semi-barren moorlands. Decreased nutrient availability may have promoted selection of the insulin resistant genotype. A study comparing non-fat ponies to normal Standardbred horses showed that healthy ponies have increased insulin secretion with decreased glucose disposal following an oral glucose test and delayed glucose disposal following an insulin challenge (Jeffcott et al., 1986), all of which are indicators of insulin resistance. Ponies also demonstrated a 50% lower glucose disposal rate compared to horses during the euglycemic-hyperinsulinemic clamp (Rijnen and van der Kolk, 2003). The clamp has also showed lower glucose disposal rates in ponies compared to sheep and pigs (Kaske et al., 2001).

Camelids represents another population adapted to restricted nutrition in which insulin resistance may optimize energy use and survival. Ponies were shown to have higher glucose disposal rates compared to camels (Elmahdi et al., 1997). However this difference may be have due to the lower insulin response to the glucose challenge in camels compared to ponies, rather than insulin resistance. A more recent study found no difference in insulin-mediated glucose clearance rates were observed between ponies and camels (Kaske et al., 2001). Further, studies using the minimal model studies have demonstrated low insulin sensitivity in camelids (Araya et al., 2000) compared to healthy Thoroughbred geldings, but similar to obese geldings considered to be insulin resistant (Hoffman et al., 2003a).

Some populations of humans also seem predisposed to insulin resistance leading to type 2 diabetes mellitus. These generally include human populations which have persisted on hunter-gatherer diets where food, particularly carbohydrates, would not have be consistently available (Cordain et al., 2000). Thus, as for ponies and camels, insulin resistance may have been selected for during human evolution. The advent of the agricultural revolution 10,000 y ago may have relaxed selection pressures for insulin resistance in Western cultures but not in continuing hunter-gatherer societies such as the Pima Indians, Pacific Islanders, Mexicans, Africans, and Australian Aborigines (Brand-Miller and Colagirui, 1999; Zimmet and O'Dea, 1993). This would explain the high rates of glucose intolerance and type 2 diabetes (Cohen et al., 1988)

and the prevalence of genotypes associated with insulin resistance (Bellary, 2002) observed in only recently Westernized subpopulations.

Animals demonstrating the same disposition for insulin resistance can provide models for metabolic dysfunction (Barnett et al., 1994; Ziv et al., 1996). Large animal species such as ponies provide a unique to study the onset and long-term implications of insulin resistance due to these animals' longer life-span (Araya et al., 2000).

Recently a new role has been proposed for insulin resistance in species' survival. Insulin resistance could promote the storage of energy as intramyocellular triglyceride (IMTG), by decreasing muscle glucose uptake and suppressing lipolysis (Shulman, 2000). This IMTG would improve physical stamina, allowing for more food gathering as well as an increased capacity for heat production through muscle contraction (shivering), both of which would increase the chance of survival for populations where food is limited and conditions are harsh (Stannard and Johnson, 2004). This theory has implications for the role of exercise in human evolution and the maintenance of metabolic health.

In conclusion, insulin resistance, although associated with a number of metabolic dysfunctions in animals and humans, may be a positive adaptation when nutrition is limited. Modern diets provide readily available carbohydrates which, coupled with decreased physical activity, interact negatively with insulin resistance, contributing to obesity and metabolic disease. Awareness of the 'thrifty genotype' in certain populations, human and animal, can allow for improved diet and lifestyle management. This may decrease the risk of disease states associated with insulin resistance, such as the epidemic of obesity in Western cultures and some domesticated animals and possibly pasture-associated laminitis.

Insulin resistance in Equids – Possible role in laminitis

Treiber, K.H., Kronfeld, D.S. and Geor, R.J. (2006) Insulin resistance in equids: possible role in laminitis. J Nutr 136, 2094S-2098S.

Abstract

Insulin is a major regulatory hormone in glucose and fat metabolism, vascular function, inflammation, tissue remodeling and the somatotropic axis of growth. Insulin resistance alters insulin signaling by decreasing insulin action in certain resistant pathways while increasing insulin signaling in other unaffected pathways via compensatory hyperinsulinemia. In humans, altered insulin signaling is implicated in reduced glucose availability to insulin sensitive cells, vasoconstriction and endothelial damage, and inflammatory response. Although no direct evidence exists for insulin's role in these mechanisms in the laminitic horse, changes in the glucose availability, vasculature, and inflammation have all been demonstrated in hoof separation. Insulin resistance was first implicated in the pathogenesis of laminitis in the 1980's using tolerance tests. Recent work has provide the first specific evidence of insulin resistance as a major predisposing condition for laminitis. Specific quantitative characterization of insulin resistance is essential towards identifying 1) ponies in need of special management to avoid laminitis, and 2) potential management strategies to avoid laminitis by increasing insulin sensitivity, including reducing obesity, increasing exercise, and moderating dietary carbohydrates, particularly starch.

Introduction

Insulin resistance was first implicated in the pathogenesis of laminitis in the 1980's using oral glucose and intravenous insulin tolerance tests (Coffman and Colles, 1983; Jeffcott et al., 1986). These tests indicated relative glucose intolerance and resistance to exogenous insulin in ponies which had previously experienced laminitis, compared to ponies with no history of laminitis. Field and Jeffcott proposed a mechanism of insulin resistance-mediated vasoconstriction in laminitis (Field and Jeffcott, 1989). Since then, our understanding of insulin resistance in laminitis has

improved, largely due to technological advances in measurement. Advancements include the use of specific and quantitative techniques to characterize the dynamic glucose and insulin system and more convenient proxies that statistically predict specific quantitative parameters (Kronfeld et al., 2005a; Treiber et al., 2005d). Using such techniques can better define the role of insulin signaling and insulin resistance in the development of equine laminitis. Here we provide the background of insulin resistance and its assessment, present existing data connecting insulin resistance to equine laminitis, and discuss possible mechanisms. Our goal is to promote a better understanding of the role of insulin resistance in the development of equine laminitis, ultimately to improve management to avoid the disease.

Definition of insulin resistance

Insulin resistance is a general term for the inability of a normal concentration of insulin to produce a normal response from target tissues (Kahn, 1978). Insulin resistance is a characteristic of type 2 diabetes and if different from a reduction in insulin action due to reduced circulating insulin as occurs in type 1 diabetes. Insulin signaling refers to the stimulation of a response by insulin.

Insulin resistance could pertain to a breakdown in insulin-signaling mediators prior to the insulin receptor which reduce circulating insulin or down-regulate insulin-receptors. More commonly observed, however, are alterations in the post-binding signal transduction associated with decreased insulin receptor-autophosphorylation and decreased tyrosine kinase activity resulting in decreased insulin-receptor substrate-1 (IRS-1) phosphorylation and reduced activation of phosphatidylinositol 3-kinase (PI 3-Kinase) (Lamothe et al., 1998; Smith et al., 1997; Zierath et al., 1997). In addition, disruption of intracellular glucose metabolism regulated by enzymes such as hexokinase and glycogen synthase could reduce insulin-mediated glucose uptake and storage (Lamothe et al., 1998; Saltiel and Kahn, 2001; Smith et al., 1997; Zierath et al., 1997). To distinguish receptor-level and intracellular inhibition of insulin action we have adopted two terms: *insulin sensitivity*, which describes reduced insulin-mediated glucose transport into the cell, and *insulin ineffectiveness*, which describes a failure of insulin-facilitated intracellular glucose metabolism (Kronfeld et al., 2005b).

Assessment of insulin sensitivity

Various types of evidence have been used to document insulin insensitivity in horses. Analogy to human metabolic syndrome provides a tempting cornucopia of possible disease associations, mechanisms and interventions, but care should be taken to avoid the danger of unsupported assumptions. Similarly association with risk factors such as obesity or equine Cushing's disease that suggest insulin resistance is an insufficient basis for establishing genuine insulin resistance.

Non-specific methods: Common methods for determining insulin sensitivity are unstandardized and indefinite. These include basal value of glycemia and insulinemia which represent the end-point of considerable regulation, and tolerance tests which provide curves for which trends can be inferred, but no specific characteristics at the level of the tissue derived. Non-specific tests therefore only suggest, often ambiguously, alterations in the complex interactions between glucose, insulin and tissue.

Specific methods: Specific quantitative methods of determining insulin sensitivity characterize distinct properties of the dynamic glucose and insulin system. These methods, like the tolerance tests, involve observing the response of the system to controlled perturbations. Unlike tolerance tests, however, specific methods quantify the physiological response for statistical comparison and standardization.

Clamp methods: Clamp methods include the euglycemic-hyperinsulinemic clamp, the hyperglycemic clamp, and the insulin suppression test (Ferrannini and Mari, 1998). These techniques infuse glucose and insulin, fixing one at a steady state to observe concomitant infusion requirements to maintain a steady-state of the other. For example, the euglycemic-hyperinsulinemic clamp fixes insulin at a constant hyperinsulinemic level, then measures the rate of glucose infusion required to maintain steady-state glycemia. The glucose infusion rate is assumed to represent the simultaneous rate of glucose disposal from the plasma and is relative to the clamped level of hyperinsulinemia. The euglycemic-hyperinsulinemic clamp has been used extensively to study insulin sensitivity in numerous species, including the horse (Annandale et al., 2004) and has been considered historically as a 'gold-standard'. However the contribution of clamp studies in ponies and horses has been overstated

(Rijnen and van der Kolk, 2003; van der Kolk et al., 2005) and questions remain regarding the non-physiological nature of the clamp.

Minimal Model of glucose and insulin dynamics: The Minimal Model is a unique quantitative method which currently provides the most detailed assessment of glucose and insulin dynamics. The minimal model is a physiological compartmental representation of the glucose and insulin regulatory system (Bergman, 1989). Application involves an iv glucose tolerance test superimposed after a delay by an iv insulin dose. The pattern of glucose disposal is then modeled according to the compartmental design.

The minimal model allows for several determinations (Bergman, 1989). 1). The change in the rate of glucose disposal in response to a known exogenous insulin dose illustrates the insulin sensitivity index (SI) of the tissue. 2) The difference between the rates of insulin-stimulated glucose disposal and total glucose disposal reveals the insulin-independent component of glucose disposal (Sg). 3) The endogenous insulin response following the glucose dose (AIRg) quantifies pancreatic β-cell response. 4) The appropriateness of this response (DI) can be determined by comparing insulin secretion to insulin sensitivity.

Measurements of insulin sensitivity determined by the minimal model have been shown to be correlated to results from the euglycemic-clamp (Pratt et al., 2005). However, the minimal model is now being widely accepted based on its merits as a more physiological test of the glucose-insulin regulatory system. Unlike the clamp, the minimal model observes insulin activity at physiological levels. Due to its physiological and dynamic nature, the minimal model has proven robust and flexible, allowing application to a number of physiological states. Finally, the minimal model is the only method currently differentiating insulin-dependent and insulin-independent glucose disposal.

Basal proxies for specific methods: An attempt has been made to standardize simple proxies derived from basal glucose and insulin concentrations to test for insulin sensitivity and insulin response. Proxies derived in humans include HOMA, QUICKI and ratios of basal glucose and insulin (Gungor et al., 2004; Katz et al., 2000; Matthews et

al., 1985). These proxies provide standardized, quantitative estimates of insulin sensitivity and response which have been correlated to specific tests for insulin sensitivity or response. These proxies are also still based on circulating insulin and glucose values which are easily perturbed and describe the outcome (not the process) of glucose and insulin dynamics. Nevertheless basal proxies provide an example of the chronic state of the subject and so far are the most practical test for insulin resistance and insulin response for large numbers of tests.

Proxies for the horse: We have recently determined and statistically standardized basal proxies, RISQI and MIRG, for insulin sensitivity and insulin response in the horse (Treiber et al., 2005d). Unlike other proxies, RISQI and MIRG were designed to estimate specific quantitative parameters, respectively SI and AIRg of the Minimal Model, with determined variability. These proxies were also subjected to statistical tests of equivalence and predictive power.

These proxies require a single basal blood sample collected between 8 and 10 am from relaxed horses which have been grazing overnight on pasture or hay. The equations to calculate the proxies from basal plasma insulin (mIU/L) and glucose (mg/dL) are as follows:

Reciprocal of insulin square-root index (for determining insulin sensitivity, SI)

$RISQI = 1/\ insulin = insulin^{-0.5}$

Modified insulin ratio to glucose (for determining insulin response, AIRg)

$MIRG = [800\quad 0.30 \bullet (insulin\quad 50)^2]/(glucose\quad 30)$

RISQI and MIRG were evaluated, selected, and standardized by correlation, Bland-Altman plot and concordance. We also considered the sensitivity, specificity and total predictive power of each proxy based on appropriate differentiation of horses in lowest quintile of SI or AIRg. The linear relationship between each proxy and its minimal model parameter was well-defined and conserved within sub-populations. There was some individual variation attributable to horse and/or sampling, an important

consideration when using single sample surrogates for individual cases.

The need for proxies exists when constraints (in particularly large sampling size) preclude the use of time and labor-intensive tests like the Minimal Model or the clamp method. Proxies would be beneficial for repeated monitoring of individual cases or, as we have shown, for studies on large populations.

Observations on insulin resistance and laminitis

Tolerance tests: The ability for exogenous insulin to induce hypoglycemia has been shown to be reduced in ponies with previous incidence of laminitis compared to normal ponies (Coffman and Colles, 1983; Jeffcott et al., 1986). In addition, glucose intolerance in previously laminitic fat ponies was observed following oral glucose loading (1g/kg bodyweight) (Jeffcott et al., 1986). Peak glucose values were higher in these ponies compared to normal ponies and glucose failed to return to baseline. A similar pattern but to a lesser degree was observed in fat ponies which had no history of laminitis compared to normal ponies. Concomitant insulin responses were also higher in ponies with previous laminitis. This result further suggests insulin resistance as well as compensatory insulin response in the predisposition to laminitis.

Basal proxies: The proxies RISQI and MIRG have been used to evaluate insulin sensitivity and insulin response in 160 ponies (Hess et al., 2005; Treiber et al., 2005e). Consistent with non-specific observations, ponies predisposed to laminitis had lower insulin sensitivity (RISQI) and higher insulin response (MIRG), indicating a compensatory exaggeration of pancreatic β-cell insulin secretion (Treiber et al., 2005d). In addition, cutoffs for RISQI or MIRG could be defined to differentiate ponies predisposed to laminitis from ponies not predisposed both with an accuracy (specificity, sensitivity and total predictive power each) of at least 70%.

Minimal Model: The population survey using basal proxies was followed by an application of the minimal model in 7 previously laminitic and 7 control ponies matched for obesity (Treiber et al., 2005c). Results from the Minimal Model validated the basal proxies in ponies and corroborated conclusions of compensated insulin resistance in previously laminitic. Further, previously laminitic ponies were shown to be at greater risk

for failed glycemic control. With specific quantitative characterization this test consummates the association between altered insulin signaling and the risk of developing laminitis.

Mechanisms of insulin resistance and laminitis

Insulin resistance has been associated with a predisposition to laminitis in ponies (Coffman and Colles, 1983; Jeffcott et al., 1986; Treiber et al., 2005c; Treiber et al., 2005e). It is important to recognize the juxtaposition of insulin resistance and compensatory hyperinsulinemia, such that insulin-resistant pathways of insulin signaling will be suppressed, while other pathways will be over-stimulated. In humans, altered insulin signaling is implicated in reduced glucose availability to insulin sensitive cells, vasoconstriction and endothelial damage, and inflammatory response (DeFronzo and Ferrannini, 1991; Fonseca et al., 2004). These processes have been demonstrated to promote hoof separation. Perhaps similar insulin signaling mechanisms to those in humans exist in the pony and in combination with 'trigger factors' associated with digestion of starch/fructan contribute to hoof failure.

Suggested trigger factors in the horse include exotoxins, endotoxins, and amines (Bailey et al., 2004a). Exotoxins (proteases) are released by disturbed gut microflora and activate collagenases which break down hoof connective tissue. Matrix metalloproteinases (MMPs) -2 and -9 are particularly implicated in this pathogenesis of laminitis (Mungall et al., 2001; Pollitt, 2004). In human cells, insulin has been shown to suppress MMP-9 while glucose is MMP-9 stimulatory (Aljada et al., 2004; Dandona et al., 2003). Therefore insulin resistance and associated glucose intolerance could contribute to MMP activity and dissolution of the lamina.

Laminitis in horses has long been considered to involve a vascular component (Hinckley et al., 1996; Hood et al., 1978; Robinson et al., 1976), perhaps similar to the vascular changes observed in diabetes mellitus and cardiovascular disease in humans. Ischemia could reduce nutrient flow to sensitive tissues while the following reperfusion would instigate inflammation and possibly overload the weakened tissue with laminitis exacerbating factors such as activated MMPs.

Endotoxins (lipopolysaccharide) are released from the upset gut and have been shown to have detrimental effects on the vasculature and blood supply to the hoof (Elliott et al., 2003; Zerpa et al., 2005). Lipopolysaccharide has been shown to increase MMP-9 (Xue et al., 2005) and the inflammatory cytokine TNF-α (Fitzgerald, 2004), which induces insulin resistance (Hotamisligil, 1999; Rajala and Scherer, 2003; Ramos et al., 2003). In healthy humans, insulin may counter these inflammatory signals through stimulation of anti-inflammatory interleukin-6 (IL-6) release from adipose tissue in humans (Krogh-Madsen et al., 2004a; Krogh-Madsen et al., 2004b; O'Riordain et al., 1995). Insulin resistance could reduce this protective effect. Thus acute insulin resistance may be superimposed on chronic insulin resistance and other laminitis trigger factors.

Amines are also released by bacteria under acidic condition in the cecum (Bailey et al., 2004a). They have been shown to cause constriction in horse vascular tissue (Elliott et al., 2003; Zerpa et al., 2005) and reduce digital blood flow in standing horses (Bailey et al., 2004b). Insulin resistance may attenuate the sensitivity of tissue to amines, increasing their vascular effects (DeFronzo and Ferrannini, 1991; Fonseca et al., 2004).

Insulin itself is a vasoregulatory hormone, invoking vasodilation through pathways similar to those of insulin-mediated glucose metabolism in human cell cultures (Zeng and Quon, 1996b). Thus insulin insensitivity would occur simultaneous to a reduction in insulin's vasodilatory effect. Similarly, insulin's ability to counteract endothelin-1 associated vasoconstriction might be compromised in insulin resistance (Kolka et al., 2005) while compensatory hyperinsulinemia might stimulate increased endothelin-1 production (Juan et al., 2004a; Lteif and Mather, 2004). Altered insulin signaling could also impact factors including growth factors, neurohormones, and oxidative stress which are also associated with endothelial damage (El Midaoui et al., 2002; Fonseca et al., 2004).

Even when blood supply reaches laminar tissue, insulin insensitivity (exacerbated by inflammatory factors) could compromise glucose transport into insulin-dependent cells such as the laminar keratinocytes (Mobasheri et al., 2004). Glucose deprivation

has been shown in vitro to result in separation of equine hoof-to-bone connective tissue (Pass et al., 1998).

Countermeasures to insulin resistance

No countermeasures for pasture laminitis have been tested by randomized control trial. However, likely mechanisms have been suggested by patho-physiological and nutritional experiments, partially justifying intervention which is known to improve insulin sensitivity.

Diets which cause large postprandial fluctuations in plasma glucose and insulin are associated with decreased insulin sensitivity in the horse (Treiber et al., 2005a). Numerous factors influence digestion and absorption of glucose from starch and sugar, therefore contents of starch and sugar are not the sole indicator of a feed's glycemic response (Englyst et al., 2003). Fat and fiber feeds have been shown to reduce glycemic responses and promote normal glucose metabolism and gut function (Kronfeld et al., 2004; Stull and Rodiek, 1988). Certain polyunsaturated fats, such as omega-3 or omega-6 may modulate inflammatory signals (Hall et al., 2004) which could improve insulin sensitivity (Fitzgerald, 2004) in the horse, and omega-3 has been claimed to prevent starch-induced laminitis (Neeley and Herthel, 1997).

Exercise has been shown to improve insulin sensitivity in healthy horses for as long as 24 hours after a single bout (Powell et al., 2002; Treiber et al., 2005b). In addition to glucose metabolism, exercise stimulates fat metabolism and is influenced by diet (Duren et al., 1999; Jose-Cunilleras et al., 2002; Pagan et al., 2002).

Obesity is associated with diet and exercise, but obesity alone has consequences for insulin sensitivity and vascular function. Obesity in humans resembles an inflammatory state, with elevated circulating acute phase proteins and inflammatory cytokines associated with insulin resistance (Ramos et al., 2003; Xu et al., 2003). These factors elevate relative to the degree of obesity in humans (Ramos et al., 2003; Xu et al., 2003).

Weight management, proper diet and exercise are a synergistic recommendation for improving insulin sensitivity to avoid pasture laminitis. Recently, levothyroxine has

been shown to induce weight loss and improve insulin sensitivity, suggesting a possible supplement to reduce the risk of laminitis (Frank et al., 2005; Sommardahl et al., 2005). Because laminitis is associated with pain, biomechanical stress and increased risk of hypermetabolism, management of laminitic horses requires clinical judgements in regard to timing of exercise and weight reduction (Jeffcott and Field, 1985).

CHAPTER 6:

Use of proxies and reference quintiles obtained from minimal model analysis for determination of insulin sensitivity and pancreatic beta cell responsiveness in horses

Treiber, K.H., Kronfeld, D.S., Hess, T.M., Boston, R.C. and Harris, P.A. (2005) Use of proxies and reference quintiles obtained from minimal model analysis for determination of insulin sensitivity and pancreatic beta-cell responsiveness in horses. Am J Vet Res 66, 2114-2121.

Summary

Objective-To develop proxies calculated from basal plasma glucose and insulin concentrations that predict insulin sensitivity (SI; $L \cdot min^{-1} \cdot mU^{-1}$) and beta cell responsiveness (ie, acute insulin response to glucose [AIRg]; $mU/L \cdot min^{-1}$) and to determine reference quintiles for these and minimal model variables.

Animals-1 laminitic pony and 46 healthy horses

Procedure-Basal plasma glucose (mg/dL) and insulin (mU/L) concentrations were determined from blood samples obtained between 8:00 am and 9:00 am. Minimal model results for 46 horses were compared by equivalence testing with proxies for screening of SI and pancreatic beta cell responsiveness in humans and with 2 new proxies for screening in horses (ie, reciprocal of the square root of insulin [RISQI] and modified insulin-to-glucose ratio [MIRG]).

Results-Best predictors of SI and AIRg were RISQI (r = 0.77) and MIRG (r = 0.75) as follows: SI = 7.93(RISQI) -1.03 and AIRg = 70.1(MIRG) -13.8, where RISQI = plasma insulin concentration$^{-0.5}$ and MIRG = [800 – 0.30(plasma insulin concentration – 50)^2]/(plasma glucose concentration – 30). Total predictive powers were 78% and 80% for RISQI and MIRG, respectively. Reference ranges and quintiles for a population of healthy horses were calculated nonparametrically.

Conclusions and Clinical Relevance-Proxies for screening of SI and pancreatic beta cell responsiveness in horses of our study compared favorably with proxies used effectively for humans. Combined use of RISQI and MIRG will enable differentiation between compensated and uncompensated insulin resistance. The sample size of our study allowed for determination of sound reference range values and quintiles for healthy horses.

Introduction

Changes in insulin sensitivity (SI) are associated with certain diseases, including some forms of exertional rhabdomyolysis, osteochondrosis, hyperadrenocorticism and related syndromes, hyperlipidemia, and laminitis (Annandale et al., 2004; De La Corte et al., 1999; Garcia and Beech, 1986; Jeffcott et al., 1986; Pagan et al., 2001; Ralston, 1996). Insulin sensitivity is also an important component of energy regulation during exercise, pregnancy and lactation, aging, and obesity (Fowden et al., 1984a; Hoffman et al., 2003a; Powell et al., 2002; Treiber et al., 2005a). Specific quantitative methods for determining insulin resistance are limited by their technical complexity and expense (Ferrannini and Mari, 1998; Kronfeld et al., 2005a) and have been applied only to studies of obesity, reproduction, exercise, and polysaccharide storage myopathy in the horse (Annandale et al., 2004; Hoffman et al., 2003a; Powell et al., 2002; Treiber et al., 2005a) (Fitzgerald et al., 2002). Other common nonspecific indications of insulin resistance, such as fasting hyperinsulinemia and glucose intolerance, are ambiguous and unstandardized (Kronfeld et al., 2005a).

Simple, single-sample predictors of SI based on basal plasma concentrations of insulin and glucose have been used as surrogates and proxies in human studies (Fukushima et al., 1999; Gungor et al., 2004; Katz et al., 2000; Laaksonen et al., 2002; Legro et al., 1998; Parra et al., 1994; Uwaifo et al., 2002; Vuguin et al., 2001). Plasma samples for determination of basal glucose and insulin concentrations may provide good representations of the glucose-insulin system and predictors of disease because they describe the chronic unperturbed state of the subject. Reference quintiles for parameters of insulin resistance further allow for comparisons of individual data with

data collected from a large population. The combined use of proxies and reference quintiles facilitate the diagnosis and characterization of clinical cases.

The purpose of the study reported here was to apply the minimal model of glucose and insulin dynamics to 4 groups of healthy horses. Proxies calculated from plasma samples for determination of basal plasma concentrations of glucose and insulin were compared with parameters of the minimal model in terms of concordance and predictive power, and reference quintiles were defined for both types of indices. Data from 1 hyperlipidemic laminitic pony, the first such clinical case in which SI has been quantified by the minimal model, and from 1 horse for which the minimal model was unable to estimate SI, have been used to illustrate the value of simple tests and reference ranges. Some of the data have been published (Hoffman et al., 2003a; Treiber et al., 2005a).

Materials and Methods

Animals-The 46 healthy horses used in our study included 10 mature Thoroughbred geldings (tested in August 2002), 12 pregnant Thoroughbred mares (tested in April 2003), 12 Arabian geldings (tested in October and November 2003), and 12 Thoroughbred weanlings (tested in November 2002). Geldings were a mean ± SD of 12 ± 3 years old (range, 9 to 18 years old) with body condition scores of 5 to 8 (on a scale of 1 to 9) (Hoffman et al., 2003a). Pregnant mares were at 299 ± 3 days of gestation and were 11 ± 4 years old (range, 6 to 17 years old) with body condition scores of 4.5 to 5. Arabians were 11 ± 1 years old (range, 5 to 16 years old) with body condition scores of 4 to 7. Weanlings were 199 ± 5 days old with body condition scores of 5 or 6 (Treiber et al., 2005a). All horses were maintained on mixed grass-legume pasture and supplemented twice daily with feed high in sugar and starch, resembling a typical commercial sweet feed, or feed high in fat and fiber (Hoffman et al., 2003a). Supplements have been described previously (Hoffman et al., 2003a; Treiber et al., 2005a) and provided approximately a third or a half of daily energy requirements (NRC, 1989). Data was also obtained from 1 hyperlipidemic laminitic pony[a] and 1 obese insulin resistant Thoroughbred gelding.

Procedures-Basal plasma concentrations of glucose (mg/dL) and insulin (mU/L) were determined from the mean value of 2 or 3 baseline samples for each horse taken prior to conducting a frequent sample collection IV glucose tolerance test (FSIGT) for the minimal model. Horses were kept in stalls overnight and had free access to grass hay and water but no concentrate. Blood samples for determination of basal plasma concentrations of glucose and insulin were taken between 8:00am and 9:00am for all groups. Plasma glucose concentrations were determined by use of an enzymatic assay. Plasma insulin concentrations were determined by use of a radioimmunoassay previously validated for equine insulin (Freestone et al., 1991). The intra-assay coefficients of variation (CV) of duplicate samples was < 1% for glucose and 5% for insulin.

Results of the FSIGT for each horse were analyzed according to the minimal model of glucose and insulin dynamics (Bergman et al., 1981). The model is used to calculate values for SI ($L \cdot min^{-1} \cdot mU^{-1}$), glucose effectiveness (Sg; min^{-1}), acute insulin response to glucose (AIRg; $mU/L \cdot min^{-1}$), and disposition index (DI; dimensionless). The model effectively characterized the 46 horses and the hyperlipidemic laminitic pony but found no solution for the obese Thoroughbred gelding. Acronyms for proxies and equations used for their calculation are provided (Appendix 1).

Statistical analysis-Correlation coefficients and linear regressions were used to compare proxies and to determine the best-fitting equations to predict SI, Sg, AIRg, and DI from proxies. Concordance with SI was calculated for each proxy for SI according to Lin (Lin, 1989). Equivalence was tested with lines of identity and Bland-Altman plots (Bland and Altman, 1999, 1986).

Data sets for healthy horses were tested for normalcy by use of the Shapiro-Wilkes statistic then used to calculate reference ranges (Hahn and Meeker, 1991), and were divided into quintiles (Altman and Bland, 1994). Data in the lowest quintile of SI and AIRg were compared with corresponding values predicted by the reciprocal inverse square of basal insulin (RISQI) concentration and the modified insulin-to-glucose ratio (MIRG), respectively. Results were categorized as true positives (TP), false positives

(FP), true negatives (TN), and false negatives (FN). Sensitivity was calculated as TP/(TP + FN); specificity was calculated as TN/(FP + TN); and, total predictive value was calculated as (TP + TN)/(TP + FP + TN + FN) (Gibson, 1990).

Results

The new proxies for screening of SI and pancreatic beta cell responsiveness in horses were compared with standard proxies for humans by use of correlation coefficients, Bland-Altman limits of agreement, and concordance coefficients with their respective minimal model parameters (Table 6.1). Regression of SI on RISQI (insulin concentration$^{-0.5}$) gave the best-fitting predictive equation for SI as follows ($r = 0.774$, $n = 46$, $P < 0.001$):

$$SI = (7.93 \pm 0.99)(RISQI) + (-1.03 \pm 0.41)$$

Similarly, the regression of AIRg on MIRG ([800 – 0.30(insulin concentration – 50)^2]/[glucose concentration – 30]) gave the best-fitting predictive equation for insulin secretory response as follows ($r = 0.754$, $n = 46$, $P < 0.001$):

$$AIRg = (70.1 \pm 9.2)(MIRG) + (-13.8 \pm 41.1),$$

No satisfactory regression was found for DI or Sg.

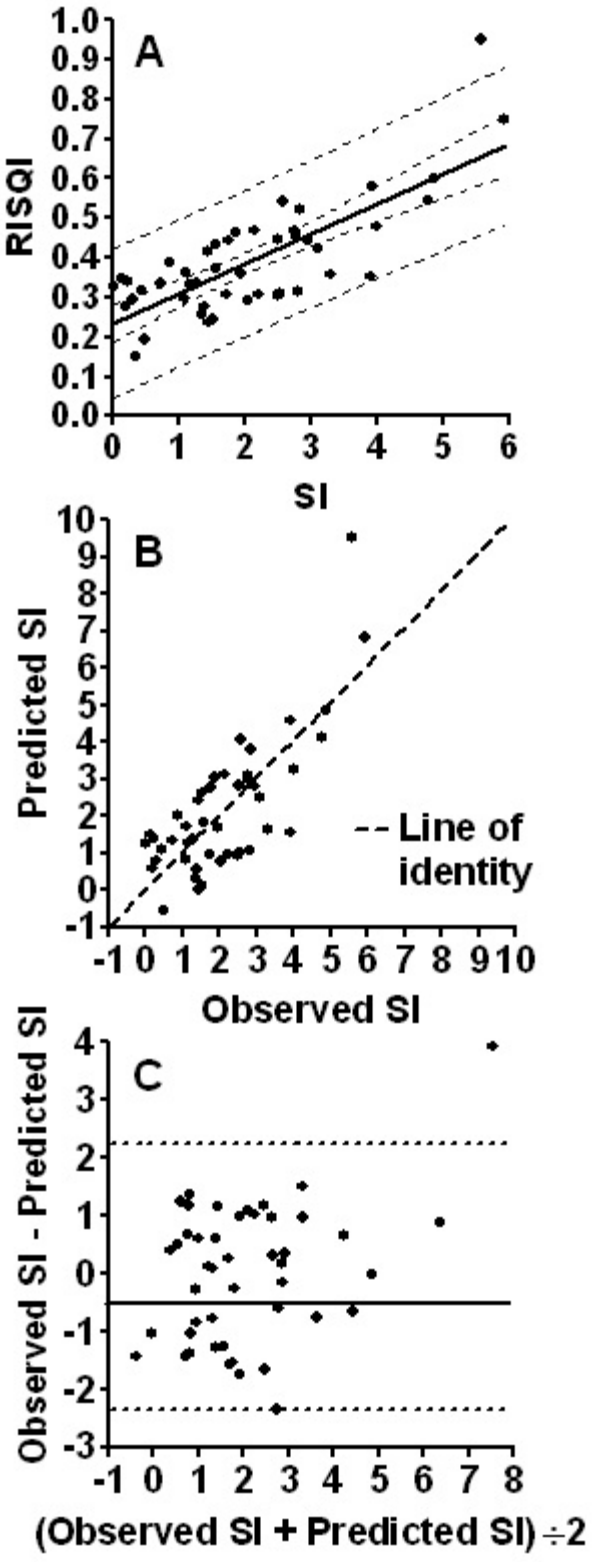


Figure 6.1. Example of regression and equivalence analysis for the reciprocal of the inverse square of basal insulin (RISQI) concentration. Units for insulin sensitivity (SI) are $L \cdot min^{-1} \cdot mU^{-1} \cdot 10^{-4}$. Units for RISQI are $(mU/L)^{-0.5}$. (A) Regression with SI, 95% confidence interval (heavy dashed line) and 95% prediction interval (light dashed line). (B) Plot of predictions for SI based on the calibration of RISQI values, including line of identity (heavy dashed line). (C) Bland-Altman plot comparing predicted and observed SI with mean difference (solid horizontal line) and 95% limits of agreement (dashed horizontal lines). All calculations are based on 46 healthy horses.

Table 6.1. Analyses of proxies compared with minimal model parameters of insulin resistance and beta cell function.

Comparisons*	Correlation coefficient	Bland-Altman 95% limits of agreement†	Concordance coefficient‡
Compared with SI			
Basal insulin concentration	-0.521	± 4.71	0.427
HOMA	-0.502	± 4.96	0.402
QUICKI	0.738	± 2.63	0.705
Basal glucose-to-insulin ratio	0.758	± 2.47	0.730
RISQI	0.774	± 2.36	0.749
Compared with AIRg			
Insulin-to-glucose ratio	0.725	± 323	0.689
HOMA-BC%	0.736	± 334	0.703
MIRG	0.754	± 306	0.725

*All relationships were significant ($P < 0.001$).
†Calculated as 2·SD in observed (Bland and Altman, 1986).
‡Evaluation of the degree to which coordinate pairs (observed, predicted) fall on the line of identity (Lin, 1989).
SI = Insulin sensitivity index. HOMA = Homeostasis model assessment. QUICKI = Quantitative insulin sensitivity check index. RISQI = Reciprocal of the square root of insulin. AIRg = Acute insulin response to glucose. HOMA-BC% = MIRG = Modified insulin-to-glucose ratio.

Reference ranges and reference quintiles for parameters of the minimal model and their proxies were calculated nonparametrically (Table 6.2). An example of graphic analysis for equivalence by use of RISQI is shown (Figure 6.1). The slope and intercept of the overall regression of SI for each proxy for SI was conserved in the 4 groups. No significant (P = 0.28) difference in regression slopes was found between groups adapted to diets rich in sugar and starch or in fat and fiber.

The predictive power of proxies calculated from basal plasma concentrations of glucose and insulin was also analyzed in terms of assessing the lowest quintile of SI from the population (Figure 6.2). All proxies of SI had similar specificity (approx 85%), sensitivity (approx 45%), and total predictive power (approx 78%). The same was true for all proxies of AIRg, which had specificities of approximately 88%, sensitivities of approximately 50%, and total predictive power of approximately 80%.

Data for the laminitic pony were as follows: SI = 0.089 $L \cdot min^{-1} \cdot mU^{-1}$, Sg ´ 10^2 = 1.37 min^{-1}, AIRg = 35 $mU/L \cdot min^{-1}$, DI ´ 10^4 = 3, basal insulin concentration = 101.3

mU/L, basal glucose concentration = 170.7 mg/dL, RISQI = 0.099 $(mU/L)^{-0.5}$, and MIRG = 0.075 $mU_{ins}^2/(10 \bullet L \bullet mg_{glu})$. Reference quintiles were expressed graphically and used to characterize deviations of the laminitic pony (Figure 6.3). The SI of the laminitic pony was in the lowest quintile of SI and RISQI, and despite high basal insulin secretion, this pony had a MIRG in the lowest quintile. A basal plasma insulin concentration of 48.38 mU/L, basal plasma glucose concentration of 112.34 mg/dL, RISQI of 0.144 $(mU/L)^{-0.5}$, and MIRG of 9.71 $mU_{insulin}^2/(10 \bullet L \bullet mg_{glucose})$ was found for the obese gelding that had no estimate of minimal model parameters.

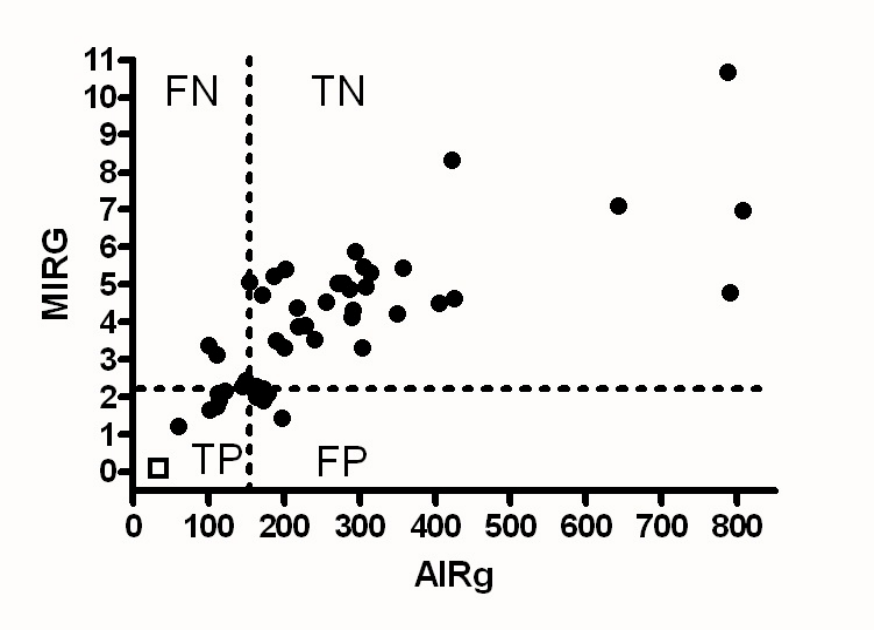


Figure 6.2 Plot of modified insulin-to-glucose ratio (MIRG) versus beta cell response (ie, acute insulin response to glucose [AIRg]) for 46 healthy horses (closed circles) and 1 hyperlipidemic laminitic pony (open square). Cutoff value for poor insulin responders is determined by the lowest quintile of AIRg (vertical dashed line). Cutoff value for poor insulin responders is determined by the lowest quintile of MIRG (horizontal dashed line). Points in each quadrant represent horses with results in the following respective categories: true positive (TP; n=5), false positive (FP; 4), false negative (FN; 4), or true negative (TN; 33). These numbers of horses are used to calculate sensitivity, specificity, and total predictive value of MIRG.

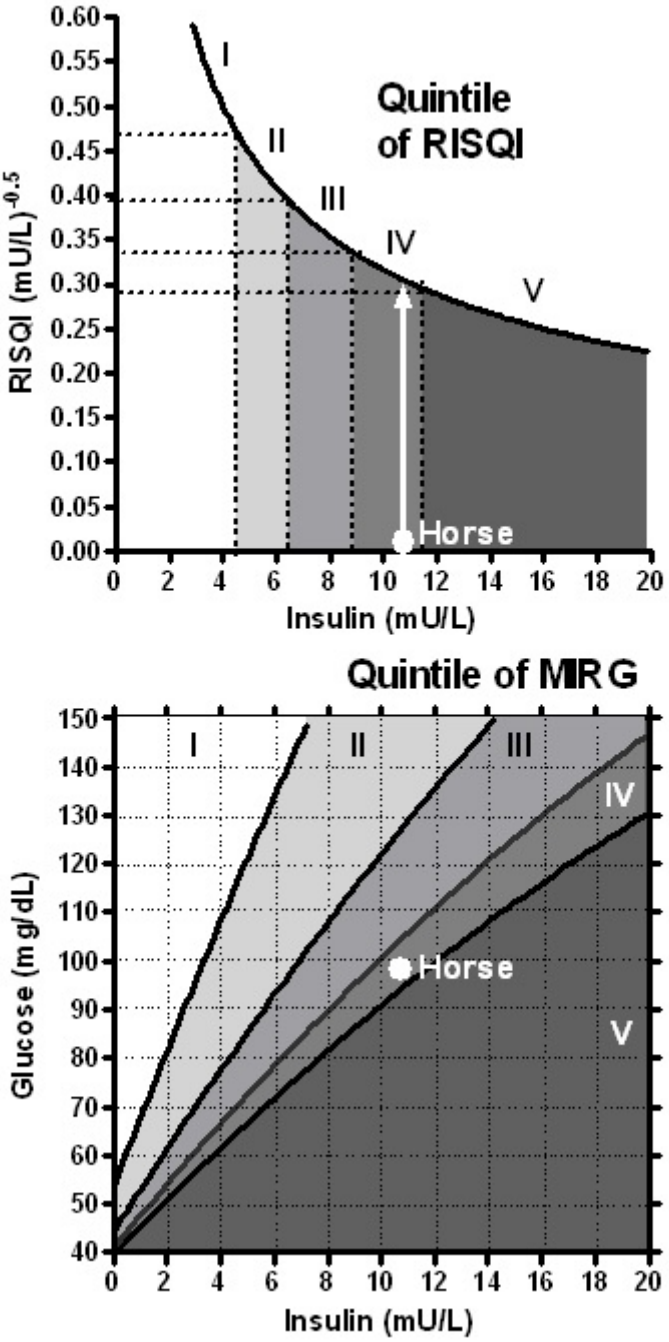


Figure 6.3 Plots of basal plasma concentrations of glucose and insulin to determine reference quintiles for a healthy horse. Top panel) Quintile of SI are determined by plotting basal plasma insulin concentration versus RISQI. Basal plasma insulin concentration of the horse was 10.7 mU/L. Notice that the horse has a SI within the fourth quintile (60th to 80th percentile). Bottom panel) Quintiles of MIRG are determined by plotting basal plasma insulin concentration versus basal plasma glucose concentrations. Basal plasma glucose concentration of the horse was 98.8 mg/dL. Notice that the horse has a MIRG within the fourth quintile.

Table 6.2. Summary statistics and reference ranges for minimal model variables and indices of insulin sensitivity (SI).

Variable	Mean	Median	95% reference interval*	Quintiles 1	2	3	4	5
SI x 10^4 [min^{-1}•$(mU/L)^{-1}$]	2.09	1.82	0.16 -5.88	0.14 - 0.78	0.79 - 1.50	1.51 - 2.27	2.28 - 3.04	3.05 - 5.94
Sg x 10^2 (min^{-1})	0.10	0.95	0.12 - 2.95	0.09 - 0.72	0.73 - 0.88	0.89 - 1.28	1.29 - 1.92	1.92 - 2.96
AIRg (mU/L•min)	270	218	67 - 805	60 - 148	149 - 190	191 - 273	274 - 337	338 - 808
DI x 10^4	479	381	39.3 -1675	30 - 207	208 - 316	317 - 427	428 - 817	818 - 1752
Basal insulin (mU/L)	9	7.9	1.22 - 40.40	1.10 - 4.53	4.54 - 6.48	6.49 - 8.93	8.94 - 11.46	11.47 - 43.29
Basal glucose (mg/dL)	101.7	97.8	73.9 - 124.7	73.2 - 90.4	90.5 - 94.8	94.9 - 101.2	101.3 - 119.5	119.6 - 124.9
RISQI $(mU/L)^{-0.5}$	0.392	0.356	0.159 - 0.917	0.152 - 0.295	0.296 - 0.335	0.336 - 0.393	0.394 - 0.470	0.471 - 0.953
MIRG ($mU_{insulin}^2$/[10•L•$mg_{glucose}$])	4.05	4.18	1.24 - 10.26	1.20 - 2.12	2.13 - 3.48	3.49 - 4.54	4.55 - 5.27	5.27 - 10.67

*Confidence intervals determined nonparametrically as the 2.5th percentile to 97.5th percentile (Phillips et al., 1994).
DI = Disposition index. Sg = Glucose effectiveness. (*See* Table 6.1 for remainder of key.)

Table 6.3. Calculation of proxies

Proxies adapted for basal plasma glucose (mg/dL) and insulin (mU/L) concentrations
HOMA[26] = (glucose - insulin)/22.5
QUICKI[16] = [log (glucose • insulin)]$^{-1}$
Glucose-to-insulin ratio = glucose/insulin.
HOMA-BC%[26] = (20 • insulin)/(glucose - 63)
Insulin-to-glucose ratio = insulin/glucose
RISQI = 1/Öinsulin = insulin $^{-0.5}$
MIRG = [800 - 0.30•(insulin - 50)^2]/(glucose - 30)

HOMA = Homeostasis model assessment. QUICKI = Quantitative insulin sensitivity check index. HOMA-BC%= Percent HOMA-beta-cell function. RISQI = Reciprocal of the square root of insulin. MIRG = Modified insulin-to-glucose ratio

Discussion

Results of our study indicate that reference quintiles and proxies for screening insulin sensitivity and pancreatic beta cell responsiveness in healthy horses can be obtained from calculated parameters of the minimal model. Correlations of the new proxies for screening of SI and pancreatic beta cell responsiveness in horses and minimal model parameters of SI and AIRg compare favorably with the best proxies for humans that have been used successfully in public health (Fukushima et al., 1999; Gungor et al., 2004; Katz et al., 2000; Laaksonen et al., 2002; Legro et al., 1998; Parra et al., 1994; Uwaifo et al., 2002; Vuguin et al., 2001). Combined use of RISQI and MIRG, which represent SI and insulin response, will enable assessment of compensatory insulin secretion in apparently healthy horses and insulin signaling failure in hyperglycemic horses.

Proxies are less accurate than the specific quantitative parameters they predict. However, proxies identify well-studied properties of insulin resistance and regulation with statistically determined power, and are therefore superior to nonspecific indications, such as basal hyperinsulinemia or glucose intolerance, or analogy to diseases in other species (Kronfeld et al., 2005a). In comparison to specific quantitative tests, proxies

calculated from basal plasma concentrations of glucose and insulin greatly increase the efficiency of resource use for more extensive generation of information. Proxies calculated in this manner have been useful in human research on large populations and in clinical situations requiring convenient and cost-effective evaluations of insulin resistance (Fukushima et al., 1999; Gungor et al., 2004; Katz et al., 2000; Laaksonen et al., 2002; Legro et al., 1998; Parra et al., 1994; Uwaifo et al., 2002; Vuguin et al., 2001), and should be equally useful in equine studies.

Insulin sensitivity in the minimal model (SI, $L \cdot min^{-1} \cdot mU^{-1}$) represents insulin-mediated glucose disposal or the rate of glucose clearance from plasma ($L \cdot min^{-1}$) per unit of plasma insulin (mU) (Bergman, 1997). Proxies for SI are based on plasma insulin and plasma glucose concentrations. The correlation coefficient (r = 0.774) of RISQI and SI may be compared with corresponding proxies used in studies of public health (r = 0.603, homeostasis model assessment [HOMA] (Fukushima et al., 1999); r = 0.73, glucose-to-insulin ratio (Legro et al., 1998); r = 0.50, fasting insulin (Legro et al., 1998); r = 0.89, HOMA and the quantitative insulin sensitivity check index [QUICKI] (Conwell et al., 2004); r = 0.91, glucose-to-insulin ratio (Conwell et al., 2004); r = 0.533, HOMA (Tripathy et al., 2004); and, r = 0.52, QUICKI (Katz et al., 2000).

Fasting hyperinsulinemia has been used previously as an indicator of insulin resistance in horses and humans (Craig et al., 2002; Fowden et al., 1984a; Jeffcott et al., 1986). This association is attributed to increased secretion of insulin by pancreatic beta cells to compensate for decreased SI. In humans, fasting plasma insulin concentrations may decline as the beta cells fail. This decompensation defines the advent of type 2 diabetes mellitus. Decompensation also occurs in the diabetic-like condition of late pregnancy that is associated with pregnancy loss in women and perhaps mares (Craig et al., 2002; Fowden et al., 1984a; Kronfeld, 2002).

Fasting hyperglycemia is an unreliable index for insulin resistance because plasma glucose concentration is maintained in a limited range by strong glucose homeostasis involving several regulators, including insulin. Our results indicate that a high basal plasma glucose concentration is not a common risk factor in healthy horses. Elevated basal plasma glucose concentration is more likely to be a sign of current

disease, such as hyperlipidemia, which is common in ponies (Jeffcott et al., 1986), or possibly type 2 diabetes mellitus, which is infrequently observed in horses (Baker and Ritchie, 1974; Muylle et al., 1986; Tasker et al., 1966).

From similar results in the equivalence tests, the proxies for SI can be categorized into 2 groups, those with basal insulin concentrations in the numerator (HOMA and basal insulin concentration) and those with insulin concentrations in the denominator (QUICKI, the basal glucose-to-insulin ratio, and RISQI). Proxies obtained by calculations with insulin concentration in the numerator had lower correlation and concordance, and higher Bland-Altman limits of agreement than proxies obtained by calculations with insulin concentration in the denominator. This is largely the result of several horses with low SI and high basal insulin concentrations. In humans, high insulin secretion is generally preempted by beta cell failure, where insulin secretion reaches a limit; at this point, hyperglycemia and type 2 diabetes mellitus ensues (Bland and Altman, 1986). The premise behind HOMA was to take advantage of this reciprocal relationship between basal insulin and glucose concentrations to avoid the ambiguity of low insulin concentrations that could indicate high SI or failed insulin secretion (Matthews et al., 1985). In humans, the HOMA index has shown good correlation to the euglycemic-hyperinsulinemic clamp technique and, to a lesser extent, the hyperglycemic clamp technique, although its precision was poor (Bergman, 1997). The HOMA index has also been shown to correlate with SI determined from the minimal model (Fukushima et al., 1999). In the horse, however, beta cells appear particularly resistant and high basal insulin concentrations are commonly observed in insulin resistant horses, as was the case for several horses in our study, whereas type 2 diabetes mellitus rarely occurs (Baker and Ritchie, 1974; Muylle et al., 1986; Tasker et al., 1966). As a result, proxies obtained from calculations with insulin concentration in the denominator (QUICKI, basal glucose-to-insulin ratio, and RISQI) would be expected to more accurately estimate SI with a positive correlation and high insulin values normalized to the remaining data.

The QUICKI was developed in humans as a simple method to assess SI as determined by the hyperinsulinemic-euglycemic clamp technique and is based on the

same reciprocal relationship between insulin and glucose as the HOMA (Katz et al., 2000). The QUICKI has been shown to improve precision in SI estimation compared with the HOMA index in adult human subjects (Katz et al., 2000; Laaksonen et al., 2002) but this improvement is inconsistent in children and adolescents (Gungor et al., 2004; Uwaifo et al., 2002). In humans (including nonobese, obese, and diabetic subjects) QUICKI was shown to correlate better than HOMA with SI as determined by the minimal model and the glucose clamp technique (Katz et al., 2000). Results from the present study also show improved precision with the use of QUICKI, compared with HOMA.

The basal glucose-to-insulin ratio is derived from a differing concept on the relationship between glucose and insulin, where the insulin-secreting response of the beta cells is exaggerated despite normal glucose concentrations. Therefore a low glucose-to-insulin ratio indicates low SI. The glucose-to-insulin ratio was originally used as an indicator of polycystic ovary syndrome in women (Craig et al., 2002; Parra et al., 1994). The ratio has since been shown to correlate with SI in women with polycystic ovary syndrome (Legro et al., 1998) and girls with premature adrenarche (Vuguin et al., 2001). It has been shown to increase precision in estimates of SI, compared with HOMA or QUICKI (Craig et al., 2002; Gungor et al., 2004), which was also the case in our study.

The QUICKI and basal glucose-to-insulin ratio include basal glucose in their assessment. In our study, basal glucose concentration had no relationship to SI and was not shown to substantially improve estimations of SI, compared with the reciprocal of basal insulin concentration alone. This finding reflects the large variation in basal insulin concentration (CV = 88%) relative to basal glucose concentration (CV = 15%). Results of human studies have similar variation in basal glucose concentrations for healthy subjects but increased variability in diabetic subjects (Bergman et al., 2003; Matthews et al., 1985). As in humans, larger variation in basal glucose concentration may be expected in sick horses, in which case the glucose-to-insulin ratio and QUICKI may provide additional information, compared with RISQI. However we recommend comparing 2 proxies, RISQI and MIRG that separate SI and insulin secretion and avoid

ambiguous determinations of the dynamic glucose and insulin system. Also, the simpler test with the use of basal insulin concentration only is preferable as glucose sample collection and analysis add an extra source of error.

The insulin response to a glucose challenge is measured for the first 10 minutes of the minimal model and defines the acute response (phase 1) of insulin to glucose (AIRg) (Bergman, 1997). This measurement of insulin secretion has been considered an estimate of beta cell activity (Garca-Estevez et al., 2004; McManus et al., 2001). High values of AIRg have been associated with low SI and described as a compensation for insulin inefficiency. Low values of AIRg in conjunction with hyperglycemia indicate a state of decompensation, where beta cells are failing to provide adequate insulin to maintain glucose homeostasis. All the proxies for AIRg are based on the insulin-to-glucose ratio, which estimates the amount of insulin secretion stimulated per unit of circulating glucose.

The correlation coefficient (r = 0.754) of MIRG and AIRg may be compared with corresponding proxies used in studies of public health (r = 0.60, percent HOMA-b-cell function [HOMA-BC%]; r = -0.66, glucose-to-insulin ratio) (Conwell et al., 2004). The HOMA estimate of beta cell function was first proposed by Matthews et al (Matthews et al., 1985) and was correlated with beta cell functions estimated by use of the hyperglycemic clamp technique, IV glucose tolerance test, and continuous glucose infusion model assessment. The HOMA-BC% was shown to correlate slightly (r = 0.58) with AIRg in human children and adolescents (Uwaifo et al., 2002). The HOMA-BC% was also shown to correlate well to first-phase insulin response in groups of people with varying glucose tolerance and fasting hyperglycemia, but no consistent relationship was observed across the groups (Tripathy et al., 2004).

The insulin-to-glucose ratio closely resembles the HOMA-BC% and is an adaptation of the insulinogenic index, which is the ratio of insulin-to-glucose 30 minutes after an oral glucose tolerance test (Phillips et al., 1994; Wareham et al., 1995). Single sample proxies derived from oral tolerance tests are highly speculative as ingestion, digestion, and absorption can vary dramatically between subjects. Nevertheless, the insulinogenic index has been correlated to first phase insulin response to IV

administration of glucose (Phillips et al., 1994; Uwaifo et al., 2002). A fasting version of the insulinogenic index has also been shown to correlate to first and second phase insulin response during the hyperglycemic clamp technique, but only second phase insulin response in women with polycystic ovary syndrome (Gungor et al., 2004). Results of human studies indicate that surrogate estimates of beta cell function are adequate for groups with similar health status, but not across groups, so proxies for beta cell function are evaluated carefully for characterizing disease states (Gungor et al., 2004; Tripathy et al., 2004). In our study, MIRG successfully predicted AIRg for the healthy population and the hyperlipidemic laminitic pony.

Together RISQI and MIRG identify apparently healthy individuals that are compensating for low SI with increased beta cell activity. The combination also allows for assessment of the ability of an individual to tolerate increases in plasma glucose that might be encountered following meals of concentrated feeds, when grazing rich pasture, or during veterinary treatment. In addition, these proxies provide a means to determine whether changes in glucose tolerance are occurring between plasma insulin and its target action at the level of the tissue or whether the changes result from altered beta cell responsiveness. This can then identify affected horses where insulin administration is indicated or when various management strategies will be effective.

Reference ranges are usually based on 95% confidence intervals of the sample distribution from approximately 50 healthy animals, so that appropriate determinations of normalcy can be achieved.[d] Reference ranges for insulin resistance have been proposed on the basis of data from 4 ponies and 5 horses (Rijnen and van der Kolk, 2003), despite the inadequacy of these small sample sizes. The meager number of observations in studies of horses with the glucose clamp technique (Rijnen and van der Kolk, 2003) vitiates the power of such experiments and reinforces the need for proxies (Kronfeld et al., 2005a; Kronfeld et al., 2005b).

The comparison of SI and response in individuals to a larger population of horses is facilitated by the use of reference quintiles, especially when expressed graphically. Reference quintiles can be used to characterize individual deviations and to monitor the progress of an affected horse. For the laminitic pony[a] of our study, SI was in the lowest

quintile of SI and RISQI, indicating insulin resistance, which has been implicated in the pathogenesis of hyperlipidemia and laminitis (Jeffcott et al., 1986). Despite a high basal insulin secretion, this pony had a MIRG in the lowest quintile, indicating a reduced capacity for beta cell secretion. This decompensation was made evident by hyperglycemia (basal glucose concentration in the highest quintile), and indicates that this pony could benefit from exogenous insulin administration.

The obese Thoroughbred gelding provides another example of the use of the proxies and reference quintiles. When adapted to the sugar and starch diet, data from the FSIGT provided no solution to the minimal model for this horse (Hoffman et al., 2003a). The RISQI of the gelding was the lowest observed in healthy horses, however, qualifying it for the lowest quintile of SI. This result is consistent with the association of obesity and insulin resistance (Hoffman et al., 2003a). Despite a low SI, the high MIRG of the gelding demonstrates effective compensation and glucose homeostasis was maintained. Diet and exercise management might benefit this horse. Indeed, adaptation of the gelding to a diet with a low glycemic index was increased the RISQI by 11% and MIRG by 10%, suggesting improved SI and beta cell function.

Reference quintiles and proxies for SI should be especially valuable in research on physiologic conditions (such as obesity, pregnancy, and exercise) and diseases (such as exertional rhabdomyolysis, osteochondrosis, hyperlipidemia, laminitis, and pituitary adenoma) that may be characterized by alterations in glucose and insulin regulation (Annandale et al., 2004; De La Corte et al., 1999; Fowden et al., 1984b; Garcia and Beech, 1986; Hoffman et al., 2003a; Jeffcott et al., 1986; Pagan et al., 2001; Powell et al., 2002; Ralston, 1996; Treiber et al., 2005a).

For groups of horses, and perhaps for serial samples obtained while monitoring an affected horse, proxies calculated from basal plasma concentrations of glucose and insulin appear to be reliable predictors of corresponding sets of SI and AIRg data. This assertion is supported by strong correlations, tight confidence intervals, and conservation of regression lines between groups in our study.

The observed correlation coefficient of 0.744 for the association between RISQI and SI corresponds to a coefficient of determination of 0.55, or 55% of the variation in

SI being accounted for by the variation in RISQI. Similarly, 57% of the variation in AIRg can be accounted for by variation in MIRG. In contrast, < 10% of the variation in Sg and < 20% of the variation in DI could be accounted for by variation in proxies obtained from basal insulin and glucose concentrations.

We found no human study of proxies for SI that performed Bland-Altman analysis to test equivalence. Simple correlation can be misleading as correlations do not account for measurement bias and are influenced by the range of the data (Bland and Altman, 1986). The Bland-Altman plot provides a quantitative analysis of the agreement between predicted and observed means while taking into consideration the variability in each. Calibration ensured no bias in the mean prediction for SI or AIRg by its respective proxies, compared with observed SI or AIRg. The prediction interval of the regression lines and the 95% limits of agreement of the Bland-Altman plots, however, revealed a degree of approximation for all proxies. For RISQI, 95% of estimates from the proxy should fall within 2.5 SI units of the actual SI. This imprecision indicates that proxies calculated from basal plasma concentrations of glucose and insulin should be used with caution when estimating individual values of SI.

The concordance correlation coefficient is another means to assess equivalence between 2 measurement techniques (Lin, 1989). Concordance describes the agreement between paired measurements and their relation to the line of identity. Concordance values of > 0.7 indicate that the difference between the proxy estimates and their quantitative measurement was < 30% of the deviation expected for unrelated pairs of measurements.

The predictive power of proxies calculated from basal plasma glucose and insulin concentration was also analyzed in terms of assessing the lowest quintile of SI from the population. Predictive power analysis has been used for previous comparisons of indices of insulin resistance (Legro et al., 1998; Parra et al., 1994; Uwaifo et al., 2002). Use of these indices allowed appropriate selection of > 85% of horses within the top 4 quintiles of SI, but were only sensitive enough to identify approximately 45% of individuals within the lowest quintile of SI. Sensitivity should improve with a larger sample size of healthy horses. Also, it should be tested further by comparison of data

from clinically ill horses to the reference quintiles of healthy horses.

Results of our study indicate that the use of proxies obtained from single basal plasma concentrations of glucose and especially insulin facilitates the assessment of SI and insulin responsiveness as well as the determination of compensatory insulin secretion and decompensation leading to hyperglycemia in horses. These aids should be useful in situations requiring multiple or repeated assessments that are unsuitable for complicated and expensive specific quantitative methods, namely the euglycemic-hyperinsulinemic clamp technique and the minimal model, and are inadequately described by nonspecific indications, such as fasting hyperinsulinemia. The use of proxies enables multiple determinations that are needed in population studies and in the initial characterization and subsequent monitoring of clinical cases. Proxies may be used with reference to quintiles, and this use is facilitated by graphs in which basal insulin concentration is entered to locate the quintile for SI, and insulin and glucose concentrations are entered to locate the quintile for beta cell response.

a. Case No. 02-44-68, Marion DuPont Scott Equine Medical Center, Leesburg, VA.
b. Beckman Instruments, Glucose Procedure No. 16-UV, Sigma Diagnostics, St. Louis, MO
c. Coat-A-Count Insulin, Diagnostic Products, Los Angeles, CA
d. Clinical Pathology Laboratory, Cornell College of Veterinary Medicine Website. Reference Intervals. Available at: www.diaglab.vet.cornell.edu/clinpath/reference Accessed April 6, 2005

CHAPTER 7:

Insulin resistance and compensation in laminitis-predisposed ponies characterized by the Minimal Model

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Abstract

Glucose metabolism in Welsh and Dartmoor ponies and ponies predisposed to pasture laminitis was characterized using the Minimal Model of glucose and insulin dynamics. Laminitis-predisposed ponies were selected based on farm records of previous disease incidence and non-parallel hoof-lines. All ponies were maintained on pasture and in early March each underwent a frequent sampling iv glucose tolerance test which involved a bolus of 300 mg/kg glucose followed 20 min later by an iv bolus of 20 mIU/kg insulin. Sampling began just prior to the glucose dose and continued for 4 h. Glucose and insulin curves were modeled to determine insulin sensitivity, glucose effectiveness, acute insulin response to glucose and disposition index. Laminitis-predisposed ponies were less insulin sensitive and demonstrated compensatory insulin hypersecretion compared to ponies not at risk. Management of at-risk ponies to avoid insulin resistance and triggers for insulin secretion could reduce the likelihood of developing clinical laminitis.

Keywords: Minimal model, laminitis, insulin resistance, pony, glucose

German Title:

Karakterisierung von Insulin Resitenz und ihre Kompensation in Hufrehe anfällige Ponies durch dem Minimalen Modell

German Summary:

Glukose Stoffwechsel in Welsh und Dartmoor Ponies die zu Gras verursachten Hufrehe anfälling sind wurde karakterisiert durch dem Minimalen Model der Glukose und Insulin Dinamik. Dreizehn nicht tragende Wesh und Dartmoor Pony Stuten und einem Pony Wallach wurden auf einer Weide gehalten bis das Experiment in der ersten Woche im März durchgeführt wurde. Sieben Stuten waren Hufrehe anfällig (PL), dies wurde durch der ehemaligen Krankheits Geschichte und das Auftreten von unparalelle Huflinien. Die anderen 7 Ponies hatten keine Anfälle der Krankheit (NL). Die Gruppen wurden gepaart für Rasse und Körperkondition mit Bewertung von 1 zu 9. Alle Ponies wurden als fett beurteilt (BC 7.1 ± 0.2, von 6-8). An allen Ponies wurde der Intravenösem Glukose Toleranz Test mit frequenter Blut kollektion (FSIGT) zwischen 8:00 und 10:00 am durgeführt. Der FSIGT startete mit einer basalen Blutsammlung (0), die von einer intravenösen 300 mg/kg Körpergewicht Dosis von Glukose gefolgt wurde. Die Glukose wurde durch einem Katheter schnell injektiert. Blutentzapfung wurde um 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, und 19 Minuten durchgeführt. Zwanzing Minuten nach der basalen Blutentnahme wurden 20 mIU Insulin/kg Körpergewicht injektiert. Blutentnahme ging weiter um 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 100, 120, 150, 180, 210, und 240 Minuten nach der Glukose Injektion. Die Minimale Modell Analyse wurde auf die herauskommende Glukose und Insulin-kurven durchgeführt zur Determinierung der Insulin Sensibilität (SI), der Glukosen Effektivität (Sg), der akuten Insulin Antwort zu Glukose (AIRg), und des Dispositions Indexes (DI). Normalität wurde durch dem Shapiro-Wilkes Test getested, Gruppen wurden bei dem two sample T-test verglichen, und der Kruskal-Wallis wurde anderswo benutzt. Die SI ($L \cdot min^{-1} \cdot mU^{-}1$) war kleiner (P = 0.007) in PL Ponies (0.09 ± 0.03) verglichen zu NL Ponies (0.39 ± 0.07). Die Sg war nicht verschieden zwichen PL und NL Ponies. Die AIRg ($mU/L \cdot min^{-1}$) war grösser (P = 0.045) in PL Ponies (885 ± 187) verglichen zu NL Ponies (172.7 ± 48.5). Keine Rassen Unterschiede wurden gefunden für Sg, SI, oder DI (P > 0.23), aber AIRg hatte die Tendenz grösser in Welsh Ponies (858 ± 197) im Vergleich zu Dartmoors (436 ± 67; P = 0.085) zu sein. Eine gute Korrelation (P < 0.01, r > 0.68) wurde zwischen den

Minimalen Modell Parametern und den Basalen Proxies RISQI (SI) und MIRG (AIRg) (abgeleited von der basalen Blutentnahme). Dieses ist das erste Experiment, in welches Insulin Resitenz durch einer spezifischen quantitativen Methode karakterisiert wurde. Insulin Resistenz kann als eine adaptative Stategie in Pfereherden vorkommen, insbesndere in Pony Rassen, die sich in harschen Zustände endwickelt haben. Deswegen haben die Ponies in diesem Studium den niedrigsten SI Quintile von gesunden Pferden, was ein gewissen Grad von Insulin Resistenz den Ponies zuschreibt. Eine gesteigerte Insulin Sekretion (AIRg) in PL Ponies kompensiert teilweise für einer geringeren Antwort von dem Gewebe zu Insilin. Zusammen können Insulin Resitenz und Kompensation zur Hufrehe beitragen indem sie das Signalisieren welches zum Glukose Angebot zur vaskularen Funktion und Hufrehe erregende Faktore wie Citokinen und Wachstum Faktore beinflussen. Ausserdem kann die Einnahme von Stärke (zum Beispiel in Frühlinggrass) mit Insulin Resitenz zusammen den Risk für Hufrehe vergrössern. Die Karakterization von Insulin Resistenz und Proxies zur Identifikation von Ponies, die einem Risk zur Hufrehe haben, könnte helfen gute Massnahmen zur Prevention der Krankheit zu halten.

Stichwörter: Minimal Modell, Hufrehe, Insulin Resistenz, Pony, Glukose

Introduction

Laminitis involves separation of laminae of the inner wall of the hoof from the pedal bone resulting in acute and sometimes irreversible hoof damage (Hood, 1999). In vitro studies have shown associations between failed glucose metabolism and hoof-wall separation (French and Pollitt, 2004; Pass et al., 1998). Few studies have considered glucose and insulin dynamics in laminitis-predisposed ponies (Field and Jeffcott, 1989), and no studies have applied specific quantitative methods to characterize insulin resistance in these ponies.

Recently, specific and statistically derived proxies for insulin sensitivity (SI) and acute insulin response to glucose (AIRg) as assessed by the Minimal Model of glucose and insulin dynamics (Treiber et al., 2005d) indicated compensated insulin resistance in

ponies with a previous history of laminitis compared to those never affected by the disease (Treiber et al., 2005e). To test the validity of these proxies and further characterize glucose metabolism in laminitis-disposed ponies we have applied the Minimal Model (Hoffman et al., 2003a; Treiber et al., 2005a) to 14 ponies having either a history of recurrent laminitis on spring pasture (PL, n = 7) or no history of laminitis (NL, n = 7).

Materials and Methods

Thirteen barren Welsh and Dartmoor mares and one Welsh gelding were maintained on pasture until the morning of the study which was performed in the first week of March. Ponies were selected based on their individual history of laminitis as well as the occurrence of laminitis in members of their pedigree. Groups were matched for breed and body condition on a scale of 1-9 (Henneke et al., 1983). All ponies were considered overweight (BC 7.1 ± 0.2, range 6-8) and weighed 312 ± 6 kg. Three NL and 3 PL ponies were Dartmoor and 4 NL and 4 PL ponies were Welsh. The NL ponies were younger (median 4 y, range 4 – 6 y) than PL ponies (median 8 y, range 6 – 21 y).

All ponies underwent a frequently sampled intravenous glucose tolerance test (FSIGT) beginning between 8:00 and 10:00 am. The FSIGT began with a basal sample followed by a glucose dose of 300 mg/kg BW glucose (Dextrose Solution 50%, Phoenix Pharmaceutical, Inc. St. Joseph, MO) administered rapidly (within 2 min) through a venous catheter which defined 0 min of the test. Sampling occurred at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 min. At 20 min, 20 mIU insulin/kg BW (Humulin R, Eli Lilly and Company, Indianapolis, IN) was rapidly administered (within 30 sec) through the catheter. Sampling continued at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 100, 120, 150, 180, 210 and 240 min post-glucose dose.

Blood samples were immediately transferred to heparinized sample tubes (Vacutainer, Fisher Health Care, Chicago, IL) and placed in ice water until centrifuged at 3000 g for 10 min. Plasma was removed within 30 min of collection and frozen at -4º C until analysis. Plasma glucose was analyzed by enzymatic assay (Beckman Instruments, Glucose Procedure #16-UV, Sigma Diagnostics, St. Louis, MO). Insulin

was determined using an 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.

The minimal model was applied to the glucose and insulin curves using MinMod Millenium (Boston et al., 2003) to calculate SI ($L \bullet min^{-1} \bullet mU^{-1}$), AIRg ($mU/L \bullet min^{-1}$), glucose effectiveness (Sg, min^{-1}), and disposition index (DI, dimensionless ratio) (Bergman, 1997). In addition, basal proxies for SI, the reciprocal of the insulin square root index (RISQI) and AIRg, the modified insulin ratio with glucose (MIRG), which were developed for use in horses were evaluated for validity in these pony breeds (Treiber et al., 2005d).

The Shapiro-Wilkes test for normality was applied to the minimal model data. When the assumption of normality was valid, groups were compared by two-sample t-test. When data was not normally distributed, the Kruskal-Wallis test was used. Correlations were determined by simple linear regression with outliers determined by Studentized (jackknifed) residuals. Statistics were performed using Stata software (StataCorp, 2003).

Results

Glucose and insulin curves are shown in Figure 7.1.

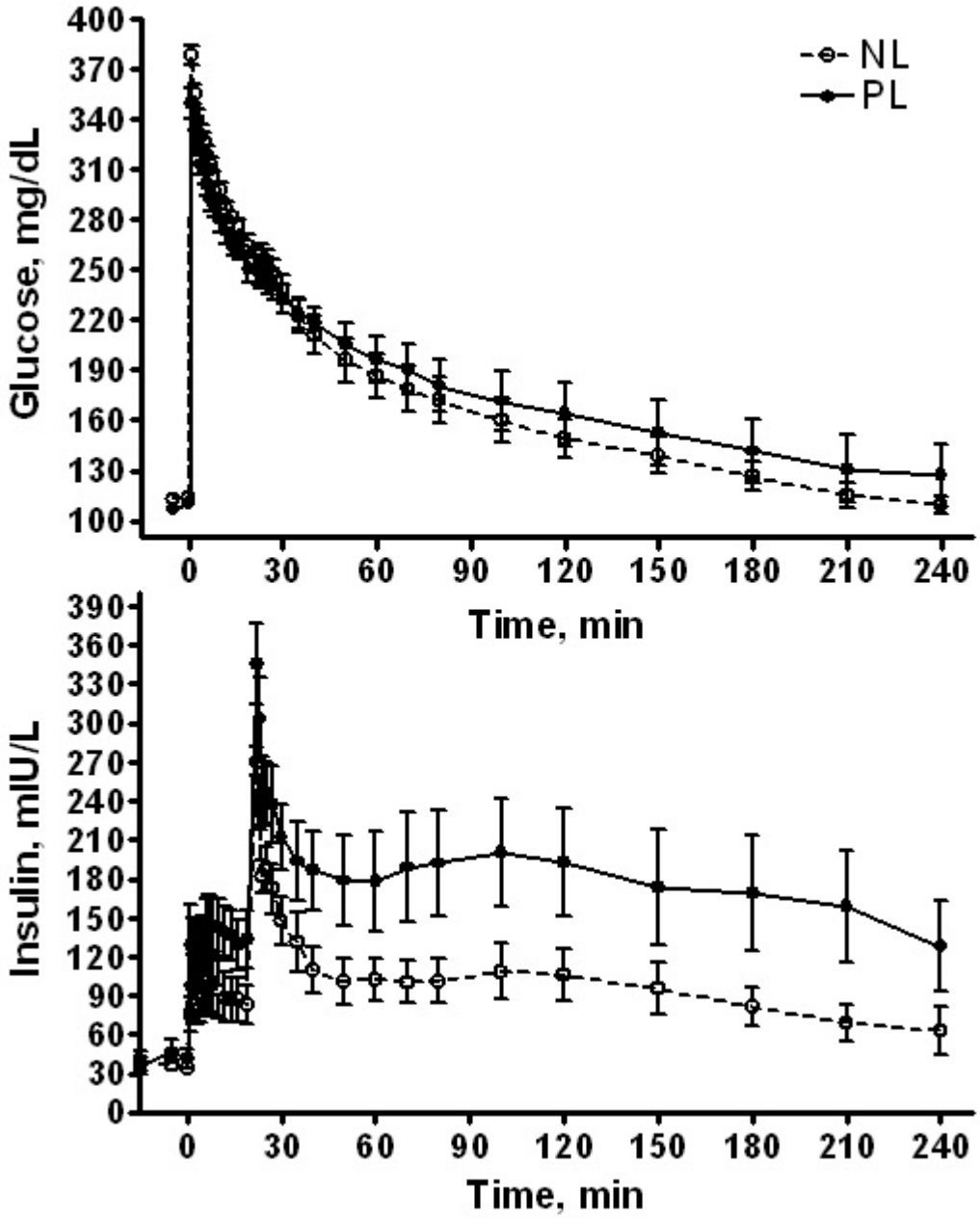

Figure. 7.1: Glucose (a) and insulin (b) curves during the frequent sampling glucose tolerance test for ponies predisposed to laminitis (PL, closed circles) and ponies not predisposed (NL, open circles). Data reported as means ± standard error.

Results for the minimal model analysis are shown in Table 7.1.

Table 7.1: Minimal model parameters for Welsh and Dartmoor ponies predisposed to pasture laminitis (PL) or not predisposed to laminitis (NL).

Minimal Model Parameter	NL	PL	*P-value*
Sg x 10^2, min^{-1}	1.3 ± 0.2	1.3 ± 0.3	0.95
SI, $L \bullet min^{-1} \bullet mU^{-1}$	0.39 ± 0.07	0.09 ± 0.03	0.007
AIRg, $mU/L \bullet min^{-1}$	405 ± 65	885 ± 187	0.045
DI	173 ± 49	93 ± 42	0.037

In PL ponies, SI was 77% lower (P = 0.007), AIRG 220% higher (P = 0.045), and DI 50% lower (P = 0.037) compared to values for NL ponies. One pony in the PL group was determined to be an outlier by Grubb's test for SI (Z = -8.50, P < 0.001). One pony in the NL group was determined by Grubb's test to be an outlier for AIRg (Z = 5.61, P < 0.001). Both of these ponies were excluded from analysis of DI, which is the product of SI and AIRg. For PL ponies, SI and DI were lower and AIRg higher compared to the NL group.

Age was not correlated to any parameters of the minimal model (r < 0.35; *P* > 0.24). The BC was also not correlated to any parameters of the minimal model (r < 0.33; *P* > 0.29). There was no breed difference for Sg, SI or DI (P > 0.23), but AIRg tended to be higher in Welsh ponies (858 ± 197) compared to Dartmoors (436 ± 67; P = 0.085). The one gelding was not exceptional for any variable.

Regressions of RISQI on SI and MIRG on AIRg showed good correlation (P = 0.005, r = 0.724 and P = 0.010, r = 0.681, respectively) (Figure 7.2). One horse was an outlier (P < 0.007) in both basal insulin and glucose and not included in either regression analysis. Another horse was an outlier for SI (P = 0.010) and removed from the regression of RISQI on SI, although this point had little affect on the goodness-of-fit.

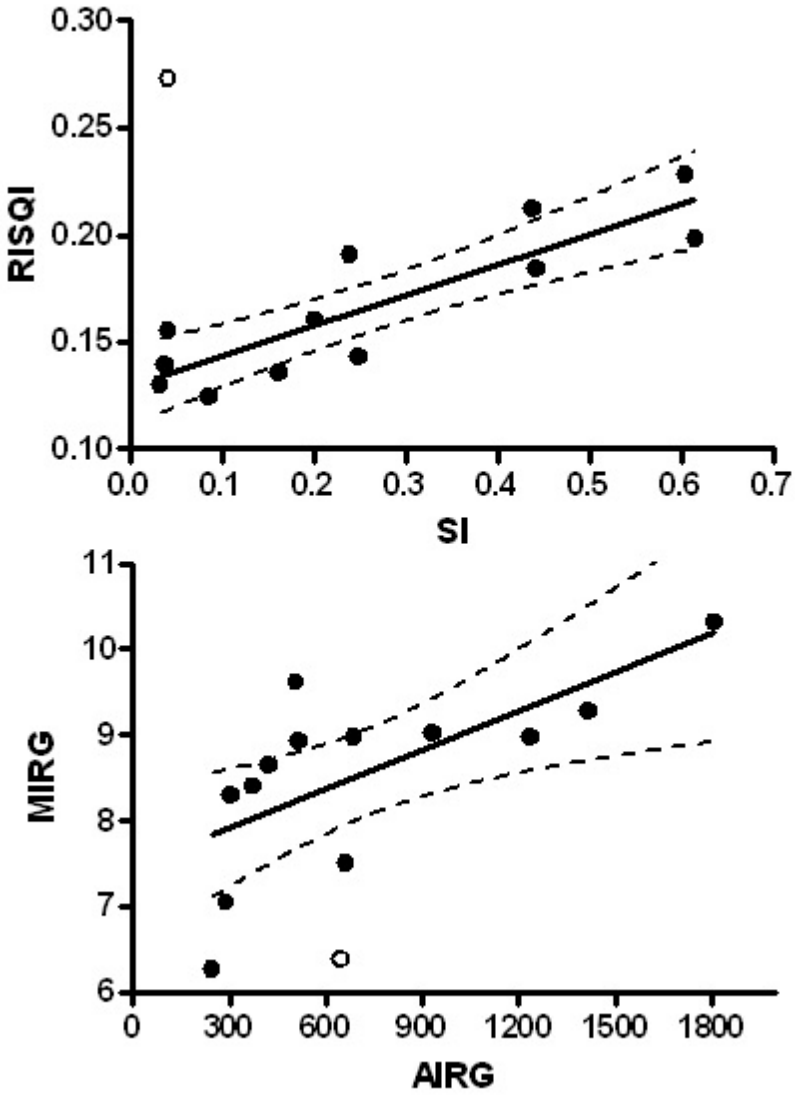

Figure 7.2. Regressions of RISQI on SI and MIRG on AIRg. The 95% CI is shown by the broken lines. One horse was an outlier for basal values (open circles) and was not included in the regression analysis.

Discussion

These results indicate compensated insulin resistance in ponies predisposed to laminitis, where insulin secretion is increased to compensate for decreased tissue responsiveness to insulin. Also specific proxies for Minimal Model parameters were shown to be good predictors of insulin sensitivity and insulin response.

The present study is the first to characterize insulin resistance in laminitis-predisposed ponies using a specific quantitative method. All ponies had insulin sensitivity in the lowest quintile of healthy horses (Treiber et al., 2005d), indicating a general degree of insulin resistance in these pony breeds. Ponies in the PL group,

however, had only 1/5th the insulin sensitivity of NL ponies and this insulin resistance was not associated with age or degree of obesity.

Insulin resistance may decrease glucose availability to insulin-sensitive cells, such as laminar basal cells or laminar keratinocytes responsible for laminar tissue turnover (French and Pollitt, 2004; Mobasheri et al., 2004). Increased insulin resistance may also alter insulin-mediated effects on blood flow and pressure (Coffman and Colles, 1983; Field and Jeffcott, 1989; Lind et al., 2000).

Ponies in the NL group had an insulin secretory response similar to that previously reported in obese Thoroughbreds (Hoffman et al., 2003a) while the secretory response was more than twofold higher in PL ponies. Increased insulin secretion compensates partially for decreased tissue response to insulin and has been observed in Thoroughbreds, calves and humans (Hoffman et al., 2003a; Stanley et al., 2002; Welch et al., 1990). However, insulin is also an important hormone in regulating fat metabolism, growth, and circulation. Compensatory hyperinsulinemia may therefore be another factor contributing to metabolic disorders such as hyperlipidemia, osteochondrosis and laminitis (Field and Jeffcott, 1989; Jeffcott and Field, 1985; Ralston, 1996). The tendency for Welsh ponies to have higher insulin secretion may place this population most at risk and indicates a possible difference in the expression of ‘thriftiness’ in particular pony pedigrees (Splan et al., 2005).

In some cases, compensation by increased insulin secretion may not be sufficient to overcome profound insulin resistance and hyperglycemia may result. None of the ponies in this study were hyperglycemic at baseline, however their tolerance of the glucose dose varied. The disposition index, DI, is the product of SI and AIRg and quantifies the effectiveness of an individual’s insulin response relative to the insulin sensitivity of their tissue (Bergman, 1997). As such, the DI is considered to be an indicator of the risk of developing hyperglycemia. Despite their greater insulin response, PL ponies had lower DI than NL ponies indicating an additional risk of potential disease. The DI in PL ponies was comparable to that observed in obese Thoroughbreds suggesting that the DI is a weaker discriminator of the laminitis-disposition than insulin sensitivity and compensatory hyperinsulinemia.

The strong correlations in ponies between one-sample basal proxies and SI parameters validates the use of these proxies in a new population of equines. At the same time, one horse was an outlier in both basal measurements and regressions either due to basal sample handling or temporary metabolic perturbation. This example demonstrates the limitation of a single sample in evaluating individual horses.

Insulin resistance in ponies is an energy-conserving ‘thrifty’ condition which could be the expression of a ‘thrifty genotype’ suggested by an identified mode of inheritance (Neel, 1962). Animals with the thrifty disposition would have a reduced tolerance for high dietary carbohydrates, and exposure could result in diseases associated with metabolic dysfunction such as hyperlipidemia and especially laminitis (Jeffcott and Field, 1985). Certain populations and family-lines expressing this thrifty genotype therefore may have an increased risk of developing laminitis. Management of these individuals to avoid insulin resistance, for example reducing obesity and avoiding of carbohydrate-rich grains or pasture, could reduce the risk of disease.

Conclusions

The present study characterizes insulin resistance in two pony breeds, with extreme insulin resistance and compensatory hyperinsulinemia associated with a predisposition for laminitis. Insulin resistance may present an adaptive strategy in populations of equines, particularly pony breeds, which evolved in nutritionally sparse environments. This predisposition may increase the risk of laminitis when insulin resistance and insulin secretion are exacerbated by other factors such as obesity and high-glycemic diets. The present study with the Minimal Model also validates the use of basal proxies for insulin sensitivity and insulin secretion in ponies.

CHAPTER 8:

Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies

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Abstract

Objective-To evaluate genetic and metabolic predispositions and nutritional risk factors for development of pasture-associated laminitis in ponies.

Design-Observational cohort study.

Animals-160 ponies.

Procedure-A previous diagnosis of laminitis differentiated 54 ponies (PL group) from 106 nonlaminitic ponies (NL group). Pedigree analysis was used to determine a mode of inheritance for the PL disposition. In early March, ponies were weighed and scored for body condition, and basal venous blood samples were obtained. Plasma was analyzed for glucose, insulin, triglycerides, nonesterified fatty acids, and cortisol. Basal proxies for insulin sensitivity (reciprocal of the square root of insulin [RISQI]) and insulin secretory response (modified insulin-to-glucose ratio [MIRG]) were calculated. Observations were repeated in May, when some ponies exhibited signs of clinical laminitis.

Results-Previous diagnosis of laminitis was consistent with the expected inheritance of a dominant major gene or genes with reduced penetrance. A prelaminitic metabolic profile was defined based on body condition, plasma triglyceride concentration, RISQI, and MIRG. Meeting 3 of these criteria differentiated PL- from NL-group ponies with a total predictive power of 78%. Determination of prelaminitic metabolic syndrome in

March predicted 11 of 13 cases of clinical laminitis observed in May when pasture starch concentration was high.

Conclusions and Clinical Relevance-Prelaminitic metabolic syndrome in apparently healthy ponies is comparable to metabolic syndromes in humans and is the first such set of risk factors to be supported by data in equids. Prelaminitic metabolic syndrome identifies ponies requiring special management, such as avoiding high starch intake that exacerbates insulin resistance.

Proposed summary:

Equine pasture-associated laminitis has been compared to metabolic syndromes in humans but no data has been collected to support this conclusion. The study reported here compared nonlaminitic ponies to ponies previously diagnosed with laminitis to develop a set of specific metabolic and physical criteria to characterize ponies predisposed to laminitis. In addition, pedigree analysis suggested a genetic link to the laminitis-disposition with pasture and nutrition playing an important role in the appearance of laminitis.

Abbreviations

BC	Body condition
RISQI	Reciprocal of the square root of insulin
MIRG	Modified insulin-to-glucose ratio

Introduction

Survival of species evolving in nutritionally sparse environments, including hunter-gatherer humans and rugged pony breeds, was probably facilitated by thrifty genes and insulin resistance(Neel, 1962). Since the agricultural revolution, grains and improved pastures have supplied abundant soluble carbohydrates to humans and horses. High carbohydrate diets exacerbate insulin resistance(Brand-Miller and Colagirui, 1999; Treiber et al., 2005a), transforming the evolutionary advantage of insulin resistance to a predisposition for certain diseases, notably type 2 diabetes

mellitus (WHO, 1999) and coronary heart disease (Adult Treatment Panel III, 2002) in humans and—so far somewhat speculatively—laminitis in equids (Jeffcott and Field, 1985; Johnson, 2002).

Pasture-associated laminitis accounts for 54% of cases of equine laminitis for which the initial cause is identifiable. Other initial causes include grain overload (8%) and miscellaneous feeding problems, diarrhea and colic, and complications of injury, obesity and pregnancy (each < 5%) (USDA, 2000).

Most research associated with laminitis in horses has used experimental models yielding results that pertain mainly to pathogenic events in the acute stage, when separation of the hoof wall from the pedal bone is beginning (Bailey et al., 2004a; Moore, 2005). Once separation occurs, there is little opportunity for effective intervention (Hood, 1999).

Preceding the acute stage of laminitis is a prodromal or developmental stage of many hours during which trigger factors possibly including exotoxins, endotoxins, amines, or inflammatory cytokines are circulating and presumably causing latent vasoactive, structural, and metabolic abnormalities (Bailey et al., 2004a). By this stage, effective intervention may not be possible. Intervention to avoid the release of triggers may prove more efficacious. In the opinion of the authors, however, the most likely opportunities for intervention reside in identifying predisposing conditions in ponies and avoiding environmental, mainly nutritional, risk factors to pre-empt the disease.

In humans, 2 metabolic syndromes have been defined that predict an increased risk of disease. One pertains to type 2 diabetes mellitus, with a diagnostic definition requiring insulin resistance or glucose intolerance, and any 2 of high triglycerides or HDL-cholesterol concentrations, hypertension, or microalbuminemia (WHO, 1999). The other pertains to coronary heart disease, with a diagnostic definition including any 3 of high glucose, triglycerides, or HDL-cholesterol concentrations, hypertension, or abdominal obesity (Adult Treatment Panel III, 2002). Extensive scientific evidence has been collected to identify these risk factors and define quantitative cut off values to characterize each predisposition. Because these metabolic syndromes are predictive, they are meaningful only in the diagnosis of apparently healthy individuals (Kahn et al.,

2005).

An equine metabolic syndrome has been proposed as a form of mild to moderate laminitis characterized by consistent fasting hyperinsulinemia and hyperglycemia and obesity in most but not all cases (Johnson, 2002). The existence of this syndrome has not been substantiated by published data that differentiate horses characterized with equine metabolic syndrome from other horses with mild to moderate laminitis or from healthy horses (Kronfeld et al., 2005a). In contrast, the study reported here derives a prelaminitic metabolic syndrome from substantial observations on apparently healthy ponies.

The original World Health Organization definition of a prediabetic metabolic syndrome stipulated the use of the euglycemic-hyperinsulinemic clamp to assess insulin resistance (WHO, 1999). Specific quantitative methods for assessing insulin resistance, such as the clamp and the minimal model are more precise than 1-sample proxy tests; however, they are technically complex and expensive. Proxies or surrogates have been developed for screening purposes and have been advocated in studies of metabolic syndrome in (Balkau and Charles, 1999; Ford and Giles, 2003).

In humans, the concept of a metabolic syndrome as a set of risk factors was advanced originally as syndrome X (Reaven, 1988), the expression of a genetic predisposition, which was exacerbated by a high carbohydrate diet. Results of pedigree analysis of families of 22 affected children have since suggested that metabolic syndrome in humans is inherited as an autosomal dominant trait (Kerem et al., 2001).

The purpose of the study reported here was to evaluate genetic and metabolic predispositions and nutritional risk factors for development of pasture-associated laminitis in ponies.

Materials and Methods

Ponies-A herd of 160 pure- and cross-bred Welsh and Dartmoor ponies was maintained on approximately 130 acres of mixed grass and legume pasture at geographic coordinates 39 N 78 W in northern Virginia. Ponies were kept in separate herds (15 to 60 individuals each) of stallions, colts and fillies, pregnant brood mares,

barren brood mares, or other. Pasture rotation occurred approximately monthly and varied between herds. Of the ponies, 102 (64%) were females, 24 (15%) were sexually intact males, and 34 (22%) were neutered males.

Previous episodes of laminitis were confirmed by a veterinarian after observation of classic diverging rings on the hoof wall with spaces between rings wider at the heel than at the toe. All ponies with rings were confirmed to have had laminitis by farm records of previous diagnosis of laminitis by a veterinarian. During the first week of March 2004, those ponies were allocated to a previous laminitis (PL, n = 54) group. The remaining ponies represented a control nonlaminitic (NL, n = 106) group.

In the last week of May 2004, 137 (87 NL and 50 PL) ponies were observed again, 4 ponies from the PL group and 19 ponies from the NL group sampled in March were not accessible for resampling. Thirteen ponies from the PL group had classic initial clinical signs of laminitis (reluctance to move, bounding digital pulses, and increased temperature of the hoof surface) in May and were reallocated to a third group (clinical laminitis [CL]).

Procedures and sample collection-On each observation day (March 4 to 10 and May 17 to 25, 2004), approximately 30 ponies were gathered from the pasture at 7:00 AM. Ponies were weighed on an electronic scale and body condition was assessed on a scale from 1 to 9 (Henneke et al., 1983). Blood samples were collected via jugular venipuncture between 8:00 and 10:00 AM.

Blood samples were immediately transferred to evacuated blood collection tubes[a] containing heparin and placed in ice water for < 30 minutes until centrifuged at 3,000 X *g* for 10 minutes. Plasma was stored at -20°C. Concentrations of glucose, triglycerides, and nonesterified fatty acids were assayed enzymatically by use of commercial kits.[b,c,d] Plasma insulin concentration was determined by a radioimmunoassay validated for equine insulin (Reimers et al., 1982).[e] Plasma cortisol concentration was determined by a radioimmunoassay validated for equine cortisol (Alexander and Irvine, 1998).[f] The intra-assay coefficient of variation of duplicate samples was < 1% for glucose, 5% for insulin, 5% for cortisol, 3% for nonesterified fatty acids, and 5% for triglyceride

concentrations.

Proxies for insulin sensitivity (RISQI) and pancreatic β-cell response (MIRG) as assessed by the minimal model of glucose-insulin dynamics were calculated from basal plasma concentrations of glucose (mg/dL) and insulin (mU/L) (Treiber et al., 2005d) as follows: RISQI = 1/[square root (basal insulin concentration)] = basal insulin concentration$^{-0.5}$ and MIRG = [800 - 0.3·(basal insulin concentration - 50)^2] / (basal glucose concentration -30).

Pastures were sampled by separating each 5 to 30 acre field into quadrants according to geographic orientation, relative elevation, and slope. Within each quadrant, forage samples were randomly collected every 10 m by clipping forage plants at a height of no less than 2.5 cm from the ground. A composite sample from each pasture was preserved immediately in liquid nitrogen and stored at –80ºC. Four samples from March and 4 from May were submitted for proximate analysis of simple sugars and starch by a Dairy Herd Improvement Association laboratory.[g]

Pedigrees for ponies in PL and NL groups were traced to common ancestors (5 to 10 generations), and analyzed by use of computer software[h] to investigate whether relationships among ponies, combined with the observation of PL, was consistent with simple models of inheritance.[i]

Statistical analysis-Data are reported as means ± SE unless otherwise stated and significance was defined by $P < 0.05$. Statistical analysis was performed using computer software.[j] Outliers were identified by a Grubbs test. Data for March were compared by 2-sample *t* tests. Comparisons among NL, PL, and CL groups in May were performed by use of ANOVA with Bonferroni's multiple comparisons. Comparisons of variables between May and March samples were performed by use of ANOVA. Body condition was compared across genders by ANOVA and across age by linear regression and Pearson's correlation.

Individual criteria were chosen on the basis of their ability to differentiate PL- from NL- group ponies. Predictive power of each criterion was determined by the appropriate categorization of PL- and NL-group ponies. Categories included true

positives (TP), false positives (FP), true negatives (TN), and false negatives (FN). Sensitivity was calculated as TP/(TP + FN); specificity was calculated as TN/(FP + TN); and total predictive power (TPP) was calculated as (TP + TN)/(TP + FP + TN + FN) (Gibson, 1990). Threshold values for each criterion in the prelaminitic metabolic syndrome were obtained by cut-point analysis according to the following formula: $M = ws + (1-w)\cdot p$, where w is the percentage of the population with laminitis, s is the sensitivity of the cut off value, and p is the specificity of the cut off value (McLaughlin et al., 2003). The cut off value was defined by a local maximum of M in which sensitivity and specificity were > 60%. Diagnostic values of criteria were compared by use of receiver operating characteristic curves (McLaughlin et al., 2003).[j] The statistical power was > 0.80 for each criterion and for prelaminitic metabolic syndrome.

Results

At the time of sampling, all ponies were in apparently good health and no lameness was observed except for the 13 ponies in the CL group in May that had uncomplicated laminitis assessed at day 1 or 2 of clinical signs. Ages of ponies ranged from 1 to 27 years. Mean age of ponies in the NL group was 5.7 ± 0.6 years (range, 1 to 27 years), mean age of ponies in the PL group was 12.6 ± 1.2 years (range, 5 to 32 years), and mean age of ponies in the CL group was 14.4 ± 2.1 years (range, 5 to 26 years).

Thirty-one mares sampled in March were pregnant (7 in the NL and 24 in the PL groups). Twenty-three of those resampled in May had foaled (4 in the NL and 19 in the PL groups) and 1 pony in the PL group was still pregnant in May. Pregnancy did not affect measured variables except to increase circulating triglyceride concentrations in mares in the PL group ($P < 0.001$) but not in mares in the NL group ($P = 0.78$). Lactation increased RISQI ($P < 0.001$) and decreased MIRG ($P = 0.002$) and triglyceride concentrations ($P < 0.001$) in ponies in PL and NL groups.

Laminitis was expressed in 34% of all ponies, and the prevalence of laminitis was 8-fold lower ($P < 0.001$) in mature stallions (1/18 [6%]) than in females (53/102 [52%]). Laminitis was not observed in any of the 34 geldings but those geldings may not have

been representative because they were young (age range, 2 to 7 years) and thus may not have had an opportunity to express the PL phenotype. Observed prevalence of laminitis was consistent with expected prevalence derived from the action of a major gene or genes expressed dominantly, but with reduced penetrance attributable to sex-mediated factors, age of onset, and further epigenetic factors. Nearly all ponies in the PL group were progeny of females in the PL group when status was known on both ponies. All female offspring of the 1 stallion in the PL group were also in the PL group.

In March, ponies in the PL group had significantly ($P < 0.001$) higher body condition scores (6.4 ± 0.1) than ponies in the NL group (5.8 ± 0.1). Generally, ponies in the PL group had 'cresty necks', i.e. large adipose tissue pads of varying sizes along the upper lines of the cervical region and *ligamentum nuchae*, as well as localized adipose tissue accumulation on the shoulder, upper portions of ribs, and at the tail-head. The body condition score was weakly correlated with age ($r \leq 0.16$; $P = 0.049$) but not different between sexes ($P = 0.12$). Hirsuitism was observed in only 4 pony mares (3 in the PL group and 1 in the NL group), approximately 17 to 30 years old, and was not associated with laminitis (odds ratio, 2.5; $P = 0.61$).

Overall, there was a significant ($P < 0.001$) increase in body condition scores from 6.1 ± 0.1 in March to 6.3 ± 0.1 in May. This increase was attributable to a significant ($P < 0.001$) increase of 0.3 in body condition score in ponies in the NL group, whereas an increase of 0.1 in body condition score in ponies in the PL group was not significant ($P = 0.24$). However, in May, ponies in the PL group maintained significantly ($P = 0.001$) higher absolute body condition scores than ponies in the NL group.

Hormones and metabolites-In March, the plasma concentration of insulin was significantly ($P < 0.001$) higher in ponies in the PL group (21.6 ± 2.2 mU/L) than in ponies in the NL group (10.7 ± 0.8 mU/L; Figure 8.1). The plasma concentration of triglycerides was significantly ($P < 0.001$) higher in ponies in the PL group (97.2 ± 10.7 mg/dL) than in ponies in the NL group (52.3 ± 3.6 mg/dL). The plasma cortisol concentration was significantly ($P < 0.001$) lower in ponies in the PL group (5.3 ± 0.3 µg/dL) than in ponies in the NL group (6.8 ± 0.2 µg/L). The plasma concentrations of

glucose (PL, 95.7 ± 1.3 mg/dL and NL, 93.5 ± 0.8 mg/dL) and nonesterified fatty acids (PL, 0.53 ± 0.04 mEq/L and NL, 0.58 ± 0.02 mEq/L;) were not significantly different (P = 0.13 and P = 0.11, respectively) between the PL and NL groups.

In May, plasma insulin concentrations were significantly (P < 0.001) higher in ponies in the CL group (103.7 ± 24.0 mU/L), compared with ponies in the NL (12.0 ±1.1 mU/L) or PL (21.5 ± 3.2 mU/L) groups (Figure 8.1). The plasma concentration of insulin was also significantly (P = 0.030) higher in ponies in the PL group than in the NL group. The plasma triglyceride concentration was significantly higher in ponies in the CL group (62.6 ± 5.3 mg/dL), compared with ponies in the PL (42.3 ± 4.2 mg/dL; P = 0.011) or NL (40.1 ± 2.1 mg/dL; P = 0.002) groups; however, it was not significantly (P > 0.59) different between the NL and PL groups. Plasma concentrations of cortisol, nonesterified fatty acids, and glucose did not differ (P > 0.05) among any groups. However, glucose values for 2 hyperglycemic ponies (plasma glucose concentrations, 146 and 191 mg/dL) in the CL group were statistical outliers.

From March to May, plasma triglyceride concentrations decreased significantly (P < 0.001) by 62% in ponies in the PL group and 27% in ponies in the NL group to similar values (PL, 42.3 ± 4.2 mg/dL and NL, 39.9 ± 2.1 mg/dL). Plasma concentrations of nonesterified fatty acids decreased significantly (P < 0.001) by 80% in ponies in the NL and PL groups. Conversely, plasma cortisol concentrations increased significantly (P < 0.001) by 59% in NL- and PL-group ponies to similar values (PL, 9.8 ± 0.6 µg/dL and NL, 10.4 ± 0.4 µmg/dL)).

In March, RISQI was significantly (P < 0.001) lower in ponies in the PL group (0.25 ± 0.01 $mU/L^{-0.5}$) than ponies in the NL group (0.37 ± 0.01 $mU/L^{-0.5}$). The MIRG was significantly (P < 0.001) higher in ponies in the PL group (7.29 ± 0.33 $mU_{insulin}^{2}/[10 \cdot L \cdot mg_{glucose}]$) than in ponies in the NL group (4.97 ± 0.23 $mU_{insulin}^{2}/[10 \cdot L \cdot mg_{glucose}]$; Figure 8.1).

In May, RISQI was significantly (P < 0.001) lower in ponies in the CL group (0.12 ± 0.01 $mU/L^{-0.5}$), compared with ponies in the NL (0.42 ± 0.03 $mU/L^{-0.5}$) or PL (0.33 ± 0.04 $[mU/L]^{-0.5}$) group. The RISQI was also significantly (P = 0.040) lower in ponies in the PL group, compared with the NL group. The MIRG was significantly (P < 0.003)

higher in ponies in the CL group (11.3 ± 0.8 $mU_{insulin}^2/[10 \cdot L \cdot mg_{glucose}]$), compared with ponies in the NL (5.2 ± 0.3 $mU_{insulin}^2/[10 \cdot L \cdot mg_{glucose}]$) or PL (7.3 ± 0.7 $mU_{insulin}^2/[10 \cdot L \cdot mg_{glucose}]$) groups. The MIRG was also significantly (P = 0.007) higher in ponies in the PL group, compared with ponies in the NL group. For all ponies, from March to May, RISQI increased significantly (P = 0.017) by 22%.

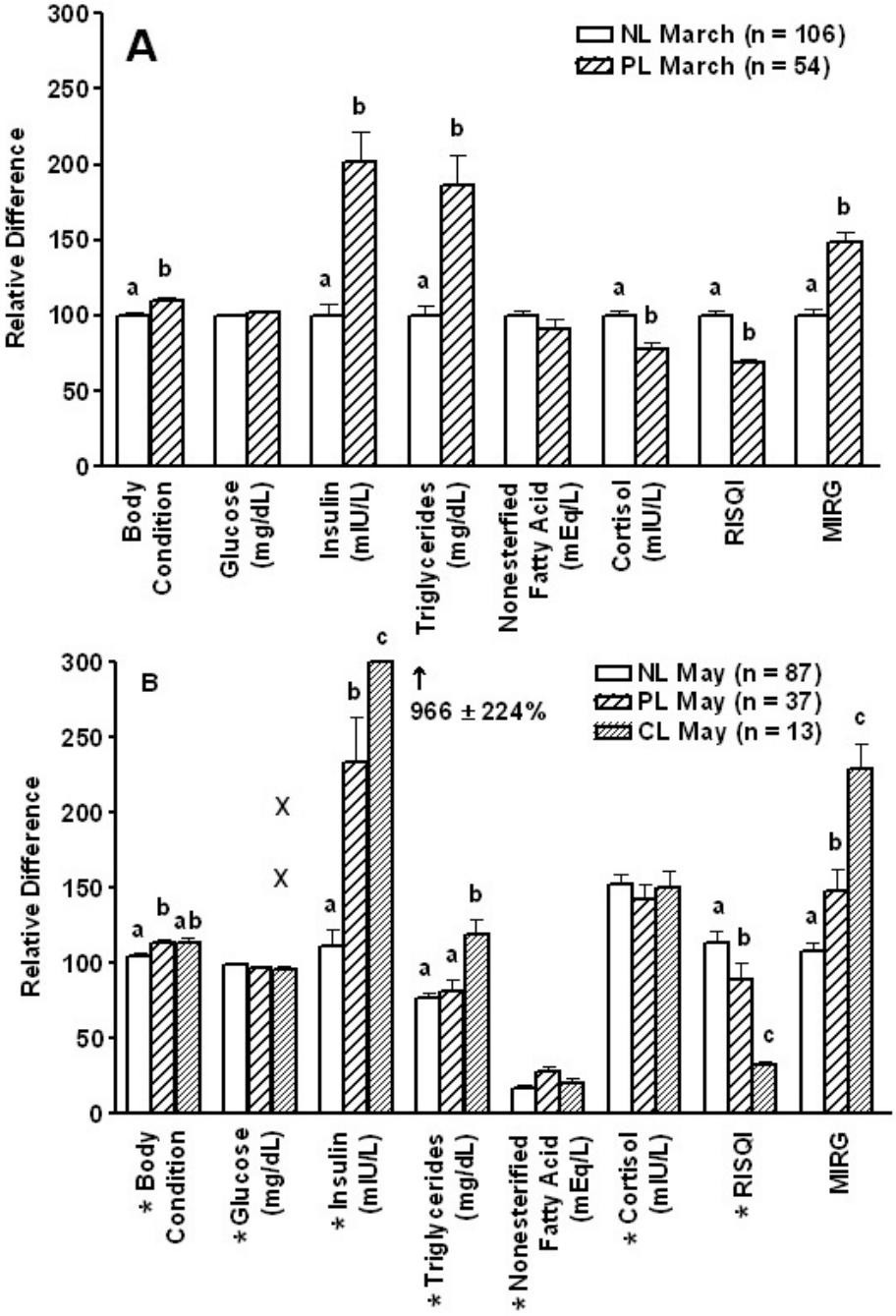
On the basis of metabolic differences detected between ponies in the PL and NL groups, we statistically derived criteria for prelaminitic metabolic syndrome in apparently healthy ponies that have 3 or more of the following characteristics: insulin resistance (RISQI < 0.32 $[mU/L]^{-0.5}$), compensatory β-cell secretory response (MIRG > 5.6 $mU_{insulin}^2/[10 \cdot L \cdot mg_{glucose}]$), hypertriglyceridemia (triglyceride concentration > 57.0 mg/dL), or obesity (body condition score > 6.0 with localized fat deposits on neck and tailhead).

Individual criteria had predictive powers of > 70% with sensitivity and specificity each > 64%. The area under each criterion's receiver operating characteristic curve was between 0.72 and 0.77 with no significant (P = 0.67) difference between criteria. Three or more criteria were chosen as the cut off to identify ponies with prelaminitic metabolic syndrome because this provided the highest total predictive power (78%) and balanced sensitivity (74%) and specificity (79%) to provide the overall best accuracy in differentiating ponies at increased risk from those not at risk (Figure 8.2).

Of ponies samples in May, 11 clinical cases of pasture-associated laminitis developed in 55 ponies with and 2 of 82 ponies without prelaminitic metabolic syndrome. An odds ratio was calculated as follows: odds ratio = (11/44) / (2/80) = 10.0 (90% confidence interval, 2.4 to 34.1, P < 0.001).

In March, pastures consisted primarily of tall fescue (*Festuca arundinacea*), orchard grass (*Dactylis glomerata*), Kentucky bluegrass (*Poa pratensis*), and white clover (*Trifolium repens*) with few weeds. Pastures were generally grazed down to approximately 5 cm, and availability of the seemingly dormant forage was minimal. In contrast, spring growth was evident in May when large forage plants, especially clover, were flourishing and general height had increased to approximately 12 cm. Clover was sparse in March, but abundant in May. Pasture starch content was significantly (P = 0.039) higher in May (7.8 ± 0.6% of dry matter) than March (4.2 ± 1.0%).

Figure 8.1 Metabolic profiles of nonlaminitic ponies (NL group), ponies with a previous history of pasture-associated laminitis (PL group), and ponies with clinical laminitis (CL group, May only) in March (A) and May (B). Means for PL- and CL-group ponies are given relative to corresponding means in NL-group ponies in March, which were assigned a value of 100%. In May, 4 ponies from the PL group and 19 ponies from theNL group sampled in March were not accessible for resampling. For each parameter, different superscript letters indicate significant ($P < 0.05$) differences among groups.*Significantly ($P < 0.05$) different from parameter in March NL- and PL-group ponies. X = Hyperglycemic ponies in the CL group that were outliers.



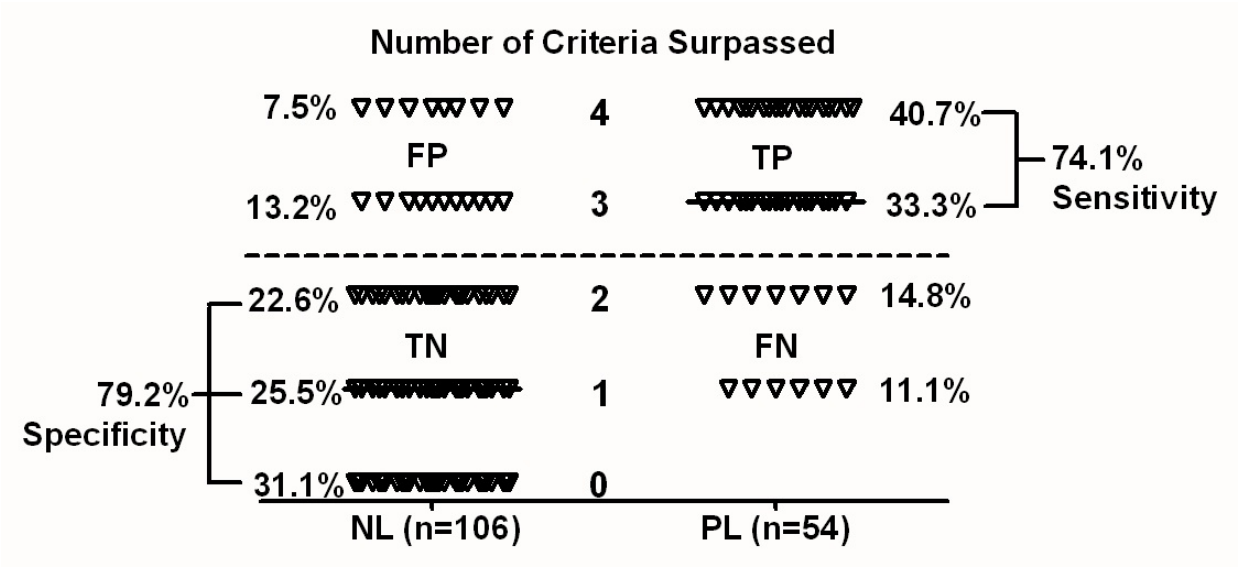


Figure 8.2. Distribution of NL- (n = 106) and PL-group (n = 54) ponies according to the number of cutoff values surpassed for criteria of prelaminitic metabolic syndrome. Determination of true positives (TP), true negatives (TN), false positives (FP), false negative (FN), sensitivity, and specificity are depicted.

Discussion

To the authors' knowledge, the study reported here is the first to apply specific proxies of insulin resistance to a nonhuman population to develop a data-based nonhuman metabolic syndrome that is a predisposition for disease in apparently healthy ponies. Determination of a possible genetic predisposition to laminitis is notable, especially with relevance to a phenotype in the form of a characteristic metabolic profile. To the authors' knowledge, for the first time, insulin resistance has been characterized in laminitis in ponies by a specific method, with compensated insulin resistance in apparently healthy ponies in which pasture-associated laminitis had been previously diagnosed and exaggerated compensated insulin resistance in ponies with clinical laminitis on day 1 or 2 of the disease. To the authors' knowledge, results of our study are also the first to indicate an association between an increase in pasture starch content and the new development of laminitis.

Insulin resistance develops with chronic adaptation to meals of grain and molasses (Treiber et al., 2005a), probably from the cumulative effects of repeated large fluctuations in glycemia and insulinemia after such meals. Results of our study suggested that insulin resistance may also develop as a chronic adaptation to gradually increasing starch content in spring pasture. Furthermore, an abrupt intake of a large amount of starch may mimic the starch overload model and lead to rapid fermentation in the cecum with production of trigger factors, which could contribute to insulin resistance and laminitis (Bailey et al., 2004a).

The actual increase of starch intake from March to May is probably underestimated by pasture proximate analysis. More pasture, particularly clover, was available in May and ponies and horses have a preference for legumes over grasses (LaCasha et al., 1999; Pearson et al., 2001).

Although fructan in pasture grass has been proposed as a causative factor for laminitis,[k,l] no significant ($P = 0.16$) difference in pasture contents of water-soluble carbohydrate, the proximate fraction consisting mainly of fructan in pasture, was detected between March and May. Moreover, no association between pasture fructan and laminitis such as that detected in the study reported here for starch has been reported.

Insulin resistance is a thrifty pattern of metabolism that spares glucose use and conserves energy (Hales and Barker, 1992). When the expression of a particular gene is associated with insulin resistance, that gene may also be regarded as thrifty (Neel, 1962). Many such thrifty genes have been identified in humans (Lindsay et al., 2002), and a dominant mode of inheritance has been suggested for metabolic syndrome (Kerem et al., 2001). Admittedly, a predisposition for laminitis in ponies would be expected to be polygenic, rather than the effect of a single or small number of genes. However, the results from this study support a mode of inheritance consistent with segregation of a major dominant gene or genes especially evident in females. Molecular characterization of such a major gene could provide a diagnostic screening test for the predisposition of laminitis.

Reported sex differences in the incidence of laminitis have been inconsistent among studies (Alford et al., 2001; Dorn et al., 1975; Polzer and Slater, 1996; USDA, 2000). In our study, laminitis was detected in only 1 male; however, all ponies in the PL group could be traced back to the single male ancestor. A possible model of inheritance may include nonaffected carrier males.

Partial suppression of the PL genotype also suggests an epigenetic threshold for expression (Junien et al., 2005). In particular, age, obesity, or an increase in dietary fructan or legume starch available in spring pasture are likely epigenetic triggers for the phenotypic expression of an underlying genetic predisposition and the appearance of clinical laminitis (Bailey et al., 2004a; Jeffcott and Field, 1985). Dietary triggers suggest an opportunity for intervention by dietetics and feeding management.

Obesity is associated with increased insulin resistance in horses (Hoffman et al., 2003a). In general, adipose tissue secretes proinflammatory adipocytokines, which influence insulin sensitivity (Lyon et al., 2003). Adipose tissue also influences the balance of circulating concentrations of nonesterified fatty acids and triglycerides, which tend to increase insulin resistance (Lewis et al., 2002; Yu et al., 2002). In humans, localized adipose tissue such as intra-abdominal fat has been particularly implicated in providing endocrine signals that disrupt glucose and insulin signalling (Abate et al., 1996; Montague and O'Rahilly, 2000). Specific fat deposits in ponies previously diagnosed with laminitis, including the cresty neck, may represent similarly metabolically active adipose tissue (Johnson et al., 2004b).

Hyperinsulinemia tends to sustain insulin-mediated glucose clearance, compensating for insulin insensitivity. By inference, hyperinsulinemia has been considered an indication of insulin insensitivity, albeit nonspecific and ambiguous (Kronfeld et al., 2005a). Euglycemia in all but 2 ponies (outliers) in the CL group indicates that hyperinsulinemia adequately compensates for tissue insulin insensitivity.

Another form of compensation, an increased use of fatty acids as an energy substrate, is suggested by detection of hypertriglyceridemia in PL-group ponies, particularly during pregnancy when energy demand was increased. Plasma concentrations of nonesterified fatty acids were not increased, almost certainly

attributable to rapid conversion to triglycerides by the liver (Watson, 1998). Hypertriglyceridemia can have adverse effects on the hepatic, renal, and cardiovascular systems (Jeffcott and Field, 1985), although these were not apparent in ponies in our study.

Changes detected in plasma concentrations of triglycerides and nonesterified fatty acids between March and May were compatible with a change from the use of stored fat as a major energy source during winter to increased use of soluble carbohydrates available in spring pastures. This metabolic switch-over would tend to exacerbate insulin resistance (Treiber et al., 2005a), increasing the risk of laminitis in the spring.

In our study, metabolic changes detected during lactation were consistent with results of other studies (Bell and Bauman, 1997; Hoffman et al., 2003b) in horses and other species. These changes suggest that the risk of laminitis would be decreased during lactation.

Cortisol is considered a thrifty hormone, stimulating gluconeogenesis and exacerbating insulin resistance to conserve glucose (Forhead and Dobson, 1997; Reeves et al., 2001). The hypothalamic-pituitary-adrenal axis regulates cortisol secretion, with posterior pituitary dysfunction manifesting in equines often as hyperinsulinemia and localized fat deposition (Beech and Garcia, 1985; Donaldson et al., 2004) similar to that observed in PL-group ponies in this study. However, unlike horses with posterior pituitary dysfunction, the PL-group ponies in this study had typical basal plasma concentrations of cortisol and glucose, many were young, and hirsuitism was not associated with PL. Similar inconsistencies from the classic clinical signs of posterior pituitary dysfunction have been detected in another study evaluating ponies with a history of laminitis (Beech and Garcia, 1985). Nevertheless, endocrine changes in the hypothalamic-pituitary axis, such as altered ACTH secretion (Donaldson et al., 2004) or peripheral hypercorticism (Johnson, 2002), have not been ruled out as possible factors of a thrifty disposition and increased risk of laminitis (Johnson, 2002).

Statistically derived proxies or surrogates of parameters of insulin resistance have been used extensively in human public health; however, to the authors' knowledge, this is the first direct application of such proxies or surrogates in an equine population. In our study, simple proxies enabled the assessment of insulin resistance in 160 ponies.

Subsequently, the minimal model was performed on 14 of the ponies described here and lower insulin sensitivity with increased β-cell responsiveness was observed in PL-group ponies, compared with NL-group ponies (Treiber et al., 2005c). Such results from a specific quantitative evaluation of the dynamic glucose and insulin system corroborate the estimation of insulin sensitivity and increased β-cell responsiveness parameters by use of RISQI and MIRG in the study reported here.

A RISQI in the lowest quintile of apparently healthy horses indicates the presence of insulin resistance in PL-group ponies (Treiber et al., 2005d). An MIRG in the highest quintile of apparently healthy horses characterizes the insulin resistance in PL-group ponies as compensated by increased β-cell secretory response (Treiber et al., 2005d). Effective compensation was further indicated by euglycemia in PL-group ponies.

In May, proxies continued to indicate compensated insulin resistance in PL-group ponies and exaggerated compensation in CL-group ponies. However, 4 of the 13 CL-group ponies and 1 PL-group pony had negative MIRG results because of extremely high basal insulin concentrations (> 157 mU/L) and were considered outliers ($P < 0.08$) for the aforementioned comparisons. Low MIRG indicates an inadequate insulin response to glucose stimulation and can occur when basal insulin secretion is low or extremely high. Low MIRG suggests an increased risk of hyperglycemia. Accordingly the 2 hyperglycemic ponies in the CL group were among these outlier ponies with low MIRG. Those 2 ponies had failed compensation for insulin resistance.

Results of the study reported here indicated that ponies predisposed to pasture-associated laminitis are metabolically distinct from ponies not at risk. This metabolic distinction is associated with insulin resistance, compensatory hyperinsulinemia, and

disturbed glucose and fat metabolism, all of which resonate with risk factors of metabolic syndromes in humans (Adult Treatment Panel III, 2002; WHO, 1999).

Evaluation of these differences from basal morning blood samples permitted determination of a prelaminitic metabolic syndrome that identifies ponies at high risk for developing laminitis. Determination of prelaminitic metabolic syndrome was used to predict development of clinical cases of pasture-associated laminitis in 11 of 13 ponies in May. An odds ratio of 10 and a total predictive power of 78% were quantitative assessments of the value of using prelaminitic metabolic syndrome to identify ponies requiring special management. Prelaminitic status may also influence economic decisions pertaining to pony breeding and trade. We have observed that qualities present in PL-group ponies have resulted in preference in the show ring, as breeding stock and at sale.

Within the design of the study reported here it was not possible to entirely rule out the possibility that the metabolic differences detected resulted from the original episode of laminitis. However the authors know of no such precedent because ponies did not have signs of pain, stress, or other chronic stimuli attributable to a previous episode of laminitis. One 16-year-old mare had had a single, severe episode of laminitis secondary to an infection in 1998 and was notably lame. This mare fulfilled only one criterion of the prelaminitic metabolic syndrome.

Other reports (Jeffcott and Field, 1985; Johnson et al., 2004b) have addressed insulin insensitivity in laminitis, but not the roles of increased pancreatic β-cell insulin secretion and fatty acid utilization. Results of the study reported here indicated that a genetic predisposition for laminitis was expressed partly by chronic compensated insulin resistance, hypertriglyceridemia, and characteristic fat deposition in ponies. During active laminitis, hyperinsulinemia and hypertriglyceridemia were exaggerated, with failed compensation in 2 CL-group ponies resulting in a diabetic-like state. Digestion of starch or fructan may also contribute to the release of additional trigger factors such as exotoxins, endotoxins, or amines, contributing to hoof failure directly (Bailey et al., 2004a; Johnson et al., 2004a; Kyaw-Tanner and Pollitt, 2004) and via exacerbation of

pre-existing insulin resistance.

Laminitis in horses is generally considered to involve a vascular component (Hinckley et al., 1996; Hood et al., 1978), reflecting current concepts of diabetes mellitus and cardiovascular disease in humans. Insulin is a vasoregulatory hormone, invoking vasodilation via nitric oxide through signaling pathways similar to those of insulin-mediated glucose metabolism as detected in human cell cultures (Zeng and Quon, 1996a). Insulin resistance is therefore expected to decrease the vasodilatory effects of insulin. In addition, concomitant hyperinsulinemia may signal other factors that result in vasoconstriction or endothelial damage including cytokines, growth factors, neurohormones, and endothelin-1 (DeFronzo and Ferrannini, 1991; Fonseca et al., 2004; Juan et al., 2004b).

Results of an in vitro study (Pass et al., 1998) in equids indicate that glucose deprivation of hoof-to-bone connective tissue results in separation. Insulin resistance can compromise glucose availability to insulin-dependent tissues. Keratinocytes of the equine lamina contain insulin-responsive glucose transporters and may therefore be partially regulated by insulin (Mobasheri et al., 2004).

The findings of the study reported here indicated that insulin resistance is a major metabolic and hormonal predisposing condition for laminitis. Ponies identified as metabolically and perhaps genotypically predisposed to laminitis can benefit from special management to avoid laminitis. Avoiding factors that contribute to obesity and insulin resistance, such as the moderation of dietary carbohydrates, particularly starch, may decrease changes in systemic insulin-signaling and reduce the risk of developing laminitis.

a. BD Vacutainer evacuated blood collection tube, Fisher Health Care, Chicago, Ill.
b. Non-esterified fatty acids, Wako Autokit, Richmond, VA.
c. Glucose Procedure #16-UV, Sigma Diagnostics, St Louis, Mo.
d. Triglyceride GPO Reagent, Sigma Diagnostics, St Louis, Mo.
e. Coat-A-Count Insulin, Diagnostic Products, Los Angeles, Calif.
f. Coat-A-Count Cortisol, Diagnostic Products, Los Angeles, Calif.

g. Dairy One Dairy Herd Improvement Association Laboratory, Ithaca, NY

h. Pollak JP, Egan K. LINEAGE Pedigree Analysis and Visualization Software, v 1.04 Cornell University, Ithaca, NY, 2001.

i. Splan RK, Kronfeld DS, Treiber KH, Hess TM, et al. Genetic predisposition for laminitis in ponies, in *Proceedings.* 19th Conference of the Equine Science Society, 2005; 219-220.

j. Intercooled Stata 8.0, Stata Corporation, College Station, Tex.

k. Longland AC, Cairns AJ, Humphreys MO. Seasonal and diurnal changes in fructan concentration in Lolium perenne: implications for the grazing management of equines predisposed to laminitis, in *Proceedings*. 16th Proceedings of the Equine Nutrition and Physiology Symposium, 1999; 258-259.

l. Pollitt CC, Kyaw-Tanner M, French KR. Equine laminits, in *Proceedings*. 49th Annual Convention of the American Association of Equine Practitioners 2003; 21-25.

SUMMARY AND CONCLUSIONS:

These studies demonstrated the following:

The minimal model can be applied during exercise to observe the immediate effects of exercise on glucose and insulin dynamics. The effects included increased insulin sensitivity and glucose effectiveness and decreased insulin secretion. Dietary adaptation influenced the metabolic response to exercise, with horses adapted to a feed high in hydrolyzable carbohydrates demonstrating a blunted change in insulin sensitivity.

Single injection tracer studies and compartmental analysis can provide important details on the distribution and transport of glucose to complement primed-infusion studies. These studies can also be applied during exercise to demonstrate increased glucose transport. Horses adapted to a feed high in hydrolyzable carbohydrates demonstrated increased utilization of glucose during exercise. Conversely, horses adapted to a feed utilizing fat and fiber as alternative energy sources spare glucose, presumably by upregulating the utilization of fat stores.

Simple proxy tests, as have been used in humans, can estimate measurements of insulin sensitivity and insulin secretory response in horses. Proxies allow for screening or when more specific tests are impractical. Reference quintiles for variables of the glucose and insulin system, and the results of related tests allow for comparison and standardization across laboratories and populations.

The minimal model has demonstrated specifically and quantitatively that ponies at risk for laminitis have decreased insulin sensitivity, increased insulin secretory response, and a lower disposition index than normal ponies. Similiarly, this specific quantitative test demonstrated lower insulin sensitivity in all ponies compared to horses. The results demonstrate a specific association between insulin resistance and the risk for laminitis.

A metabolic characterization of ponies at risk for pasture-associated laminitis further demonstrated insulin resistance in these ponies and identified several factors

which distinguish ponies at risk for laminitis. Combined, these factors resemble metabolic syndromes defined in humans, and constitute the first specific, standardized evidence of a metabolic syndrome in equines to aid in the identification of at risk individuals. Additionally, metabolic changes contributing to the risk of laminitis were associated with increased pasture non-structural carbohydrates.

Conclusions and application and future work

Glucose is a key energy metabolite for physiological function. Animals have evolved complex glucose regulation systems which respond to environmental and dietary signals, tissue demand, and available energy stores to ensure survival and continued performance. Understanding glucose regulation provides a foundation for advancing animal nutrition and management to optimize animal health and performance.

Whole animal models can be used to characterize the glucose space and the kinetics and dynamics of the glucose system without invasive procedures. These models aid in the comparison of glucose system parameters across species and physiological conditions such as exercise and metabolic disease. Simpler, standardized proxy tests allow for larger sampling schemes and repeated measures and have been used the study the epidemiology of metabolic syndrome in humans and now ponies.

Exercise has a significant effect on the circulation and utilization of glucose energy. Exercise-induced adaptations increase the availability of glucose energy to working skeletal muscle through increased tissue insulin sensitivity, increased insulin-independent glucose transport and increased endogenous glucose production.

Dietary energy source can influence the utilization of glucose. The impact of diet is most acute during periods of increased energy demand, such as exercise, when the efficiency of the system to mobilize, transport and metabolize energy may limit the body’s performance capacity.

Pasture-induced laminitis is a prevelant, critical disease which may be associated with metabolic dysfunction and metabolic syndromes observed in other species, including humans. Improved characterization of metabolic dysfunction and associated

disease states such as laminitis with the use of models and standardized tests, can aid the understanding of the underlying mechanisms. This understanding will facilitate the development of management to avoid laminitis in predisposed individuals.

Future work will hopefully elucidate the mechanisms uniting glucose regulation, exercise performance and metabolic disease. Controlled trials are also needed to evaluate the benefit of various types of exercise, management and nutrition on metabolism, exercise performance, and disease risk, to provide sound recommendations.

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APPENDIX: Additional tables and figures

Table A.1. (Chapter 2). Lactate data for individuals horses and diet groups during the incremental lactate test. After a 10 min warm-up at 1.8 m/s horses ran for two minutes at each speed interval. Sampling occured at the end of the two minutes.

A. Plasma lactate (mmol/L) for FF horses during the lactate test

	Horse #						Diet group	
Speed, m/s	1	3	5	7	9	11	Mean	SEM
1.8	0.58	0.59	0.86	0.68	0.82	0.75	0.71	0.05
2.7	0.60	0.71	0.58	0.53	0.71	0.54	0.61	0.03
3.2	0.70	0.89	0.65	0.53	0.73	0.50	0.66	0.06
3.6	0.72	1.03	0.62	0.50	0.65	0.49	0.67	0.08
4.1	0.77	1.02	0.67	0.50	0.61	0.46	0.67	0.08
4.5	1.12	1.10	0.95	0.51	0.74	0.46	0.81	0.12
5.0	1.54	1.55	1.23	0.56	0.97	0.51	1.06	0.19
5.4	1.73	1.53	1.82	0.81	1.20	0.92	1.33	0.17
5.9	2.09	2.03	2.35	0.91	3.11	0.94	1.90	0.35
6.3	2.22	2.76	3.00	1.26	4.21	1.22	2.44	0.46
6.8	2.89	3.21	4.17	1.64	3.98	1.69	2.93	0.44
7.2	4.41	4.95	6.31	2.19	4.38	2.26	4.08	0.65
7.7	5.80	7.05	7.64	3.22	5.51	3.56	5.46	0.73
8.1	7.61	9.26	10.27	4.30	7.26	6.08	7.46	0.88
8.6	10.72	12.78	14.52	7.00	9.27	8.64	10.49	1.13
9.0	14.47	17.61	18.34	10.24	13.34	12.30	14.38	1.27
9.5	17.07	21.56	22.53	13.01	17.45	15.31	17.82	1.49

B. Plasma lactate (mmol/L) for SS horses during the lactate test

	Horse #						Diet group	
Speed, m/s	2	4	6	8	10	12	Mean	SEM
1.8	0.94	0.52	1.04	0.95	0.72	0.72	0.81	0.08
2.7	1.15	1.00	1.11	0.53	0.50	0.47	0.79	0.13
3.2	0.88	1.06	1.47	0.60	0.52	0.55	0.84	0.15
3.6	0.91	1.11	1.55	0.61	0.54	0.58	0.88	0.16
4.1	0.96	1.09	1.65	0.62	0.62	0.67	0.93	0.16
4.5	1.27	1.27	1.65	0.72	1.14	1.31	1.23	0.12
5.0	1.66	1.57	2.58	0.80	1.12	1.67	1.56	0.25
5.4	1.97	2.43	3.21	2.09	1.76	1.75	2.20	0.23
5.9	2.67	1.94	4.07	2.20	2.18	2.07	2.52	0.33
6.3	4.19	2.36	5.36	2.96	2.48	2.88	3.37	0.48
6.8	7.61	3.62	6.99	3.12	3.91	4.26	4.92	0.77
7.2	10.1	3.42	8.35	3.72	4.21	6.18	5.99	1.12
7.7	15.78	4.31	9.95	4.76	5.33	10.91	8.51	1.85
8.1	21.81	6.85	11.23	7.59	6.31	14.66	11.41	2.45
8.6	26.04	10.41	14.8	9.43	9.36	19.17	14.87	2.73
9.0	30.9		18.12	12.90	14.1	19.74	19.15	3.19
9.5				15.88	20.2	20.97	19.02	1.58

Table A.2. (Chapters 2 & 3) Glucose, insulin and tracer enrichment data for individuals horses and diet groups during the FSIGT and single-injection glucose tracer tests performed during exercise or at rest. The FSIGT involved an iv glucose challenge of 600 mg/kg BW at 0 min and an iv insulin challenge of 0.010 IU/kg BW at 20 min. The glucose-tracer test involved an iv bolus of 100 µmol/kg BW [6,6-H^2] glucose at 0 min. For the exercise test, a resting baseline sample was taken, followed by a 25-min warm-up period (W). The 0 min sample of the exercising test was taken approximately 25-min after the onset of exercise. For the resting test, two resting baseline samples were collected at approximately 30 and <5 min before the glucose dose at 0 min. These samples were averaged to establish the baseline (0 min) value.

A. Glucose (mg/dL) for FF horses during the tracer test at rest								
	Horse #						Diet group	
Time	3	5	7	11	1	9	Mean	SEM
0	94	91.2	96.5	99.9	95.0	94.1	97.4	2.5
1	112.1	107.7	112.8	112.3	114.9	108.2	113.6	1.3
2	108.7	105.8	110.0	111.7	115.3	108.6	113.5	1.8
3	109.5	105.1	108.5	112.9	114.4	106.3	113.6	0.7
4	109.0	106.0	110.2	114.4	111.6	107.2	113.0	1.4
5	107.6	107.5	113.0	115.3	112	110.3	113.6	1.7
6	107.2	104.4	108.1	112.7	112.8	109.4	112.8	0
7	106.5	102.4	108.5	113.0	113.3	108.3	113.2	0.1
8	105.3	103.0	107.8	113.0	112.0	105.9	112.5	0.5
10	104.9	101.3	108.8	112.3	110.2		111.3	2
12	105.1	100.0	105.9	110.5	108.1	92.6	109.3	1.2
14	102.4	96.3	106.7	111.4	106.6	94.8	109.0	2.4
16	102.3	96.5	103.9	110.2	107.9	102.6	109.0	1.1
18	102.7	95.8	103.1	110.0	105.8	99.7	107.9	2.1
20	104.0	94.3	102.7	109.7	103.8	96.2	106.7	3
23	104.2	95.0	102.0	111.5	103.9	100.1	107.7	3.8
26	103.2	94.7	102.1	108.5	103.5	98.8	106.0	2.5
30	106.2	93.1	99.8	109.9	102.6	100.3	106.2	3.6
35	103.9	94.3	96.4	111.9	100.8	97.8	106.3	5.6
40	103.7	90.1	93.0	109.3	100.5	97.7	104.9	4.4
50	95.9	88.8	95.1	104.8	100.3	95.2	102.6	2.3
60	97.0	86.0	94.3	102.2	97.7	95.6	100	2.3
70	97.1	84.2	91.7	102.6	96.2	93.7	99.4	3.2
90	97.6	90.1	93.7	99.5	98.4	94.0	99.0	0.6
110	96.1	90.0	94.3	94.8	100.0	92.4	97.4	2.6
130	97.9	90.7	92.4	97.0	103.4	93.7	100.2	3.2
150	97.3	89	94.4	97.7	101.8	90.5	96.1	5.6

B. Glucose (mg/dL) for SS horses during the tracer test at rest								
	Horse #						Diet group	
Time	2	4	6	8	10	12	Mean	SEM
0	122.8	99.6	99.0	95.0	93.8	108.1	107.1	7.8
1	142.1	107.8	118.0	115.5	105.8	123.3	122.6	10.2
2	138.1	117.1	113.8	115.4	104.5	120.7	123.0	7.6
3	136.8	116.6	113.1	112.9	104.5	123.7	122.2	7.4
4	137.7	115.7	113.9	113.5	98.7	123.0	122.4	7.6
5	139.5	117.6	112.5	112.1	104.6	124.8	123.2	8.3
6	135.6	113.1	112.3	108.7	103.4	120.9	120.4	7.6
7	134.8	114.5	111.9	107.4	102.9	121.5	120.4	7.2
8	133.2	112.4	108.9	105.5	101.4	120.2	118.2	7.6
10	134.2	113.0	108.1	105.3	100.5	120.2	118.4	8.0
12	134.0	110.8	107.2	101.0	100.1	118.6	117.3	8.4
14	131.8	110.9	107.9	103.2	101.6	117.2	116.9	7.5
16	130.5	105.4	106.6	103.6	100.5	117.2	114.2	8.2
18	130.8	110.7	107.1	103.3	99.9	117.1	116.2	7.4
20	131.3	109.7	105.6	99.1	98.4	116.2	115.5	8.0
23	129.6	109.4	107.7	103.3	96.5	116.2	115.6	7.0
26	130.4	110.8	107.3	105.5	98.4	117.3	116.2	7.2
30	128.7	108.4	106.3	104.4	96.0	115.3	114.5	7.2
35	126.6	104.6	103.0	103.8	96.2	112.1	111.4	7.6
40	124.3	99.4	105.9	103.6	95.1	111.3	109.9	7.4
50	125.3	96.1	103.1	103.4	94.9	109.0	108.2	8.8
60	124.3	96.7	112.3	99.2	94.1	108.8	111.1	8.0
70	122.8	100.1	101.5	102.4	93.0	104.1	108.1	7.3
90	123.5	99.6	99.2	103.3	90.3	101.5	107.5	8.0
110	125.0	104.4	97.4	101.0	90.5	100.4	108.9	8.3
130	124.8	97.0	97.3	97.1	88.8	97.1	106.4	9.2
150	123.3	102.9	98.6	96.3	89.0	98.2	108.2	7.6

C. Glucose (mg/dL) for FF horses during the tracer test at exercise								
	Horse #						Diet group	
Time	1	3	5	8	9	11	Mean	SEM
0	100.2	94.4	97.5	93.2	97.4	101.9	97.4	1.3
1	116.6	112.4	114.1	110.9	109.6	115.4	113.2	1.1
2	114.9	109.6	114.6	111.4	109.3	114.3	112.4	1.1
3	110.6	109.9	114.7	111.7	111.5	115.1	112.3	0.9
4	111.9	105.6	112.1	107.0	106.9	111.7	109.2	1.2
5	111.8	105.6	110.4	106.6	104.7	111.3	108.4	1.3
6	109.1	103.2	107.8	101.9	104.5	110.2	106.1	1.4
7	106.6	101.3	106.4	98.7	103.5	108.7	104.2	1.5
8	104.4	102.0	105.3	96.6	101.4	108.1	103.0	1.6
10	102.7	97.5	100.7	89.0	98.6	104.1	98.8	2.2
12	96.5	94.3	96.5	85.1	97.6	101.3	95.2	2.2
14	91.9	94.3	96.1	83.8	96.9	99.8	93.8	2.3
16	89.2	94.5	97.0	83.0	95.7	99.2	93.1	2.4
18	90.8	94.3	96.8	84.9	96.7	99.6	93.9	2.2
20	92.0	96.9	97.9	87.0	99.3	101.2	95.7	2.2
23	95.8	95.6	98.0	89.2	99.8	102.1	96.7	1.8
26	98.9	94.7	97.6	91.0	100.5	101.0	97.3	1.6
30	98.0	95.7	99.1	95.3	99.0	99.2	97.7	0.7
35	97.8	95.5	95.9	97.6	100.0	100.9	98.0	0.9
40	98.7	95.8	97.6	97.6	100.4	100.7	98.5	0.8
50	98.3	95.9	95.8	95.7	101.4	100.3	97.9	1.0
60	95.7	96.0	96.1	95.8	101.7	100.4	97.6	1.1
70	99.6	93.5	95.7	91.2	98.6	100.6	96.5	1.5
90	93.1	91.1	91.5	89.1	101.6	98.2	94.1	2.0
110	92.9	88.0	91.6	82.6	98.2	101.2	92.4	2.7
130	96.5	83.7	91.2	78.3	94.6	96.6	90.2	3.1
150	89.0	77.7	92.3	73.1	88.4		84.1	3.7

D. Glucose (mg/dL) for SS horses during the tracer test at exercise								
	Horse #						Diet group	
Time	2	6	4	8	10	12	Mean	SEM
0	101.6	113.5	101.7	116.1	100.8	100.9	105.8	2.9
1	117.3	131.1	118.6	130.8	116.4	116.9	121.8	2.9
2	117.8	127.6	117.5	130.4	116.7	116.2	121.0	2.6
3	118.4	125.5	116.1	129.4	116.5	117.6	120.6	2.3
4	115.5	123.9	113.5	125.4	113.0	112.4	117.3	2.4
5	112.9	123.3	111.0	125.5	110.3	110.3	115.5	2.8
6	112.1	122.9	109.6	122.2	109.2	107.9	114.0	2.8
7	111.4	122.8	108.1	122.1	107.2	105.9	112.9	3.1
8	110.7	121.5	104.9	119.5	104.2	104.5	110.9	3.2
10	107.9	116.9	100.7	118.9	101.4	100.6	107.7	3.4
12	106.9	118.2	93.5	116.7	98.9	97.1	105.2	4.3
14	104.9	115.7	89.5	115.4	100.8	96.5	103.8	4.3
16	104.2	114.5	88.0	115.7	101.6	96.4	103.4	4.3
18	103.8	113.2	87.8	116.1	103.3	97.9	103.7	4.2
20	102.5	112.0	90.5	117.8	104.1	98.9	104.3	3.9
23	102.0	110.4	94.6	118.7	103.6	98.9	104.7	3.5
26	101.0	107.8	95.7	122.1	105.0	98.7	105.0	3.8
30	100.3	107.4	96.9	125.5	105.5	97.8	105.6	4.3
35	101.0	102.7	94.8	123.4	107.3	101.4	105.1	4.0
40	99.0	103.3	96.1	116.8	106.9	99.9	103.7	3.0
50	99.3	101.7	95.1	111.1	108.2	100.8	102.7	2.4
60	96.8	102.3	95.5	116.7	107.8	102.0	103.5	3.2
70	95.6	97.8	97.2	113.1	105.2	100.9	101.6	2.7
90	94.3	98.4	90.7	117.2	104.3	99.1	100.7	3.8
110	94.3	93.4	87.4	114.9	102.7	98.5	98.5	3.9
130	91.8	96.8	83.2	114.6	98.7	94.5	96.6	4.2
150	89.4	94.9	76.6	111.1	93.2	89.5	92.5	4.6

E. Glucose (mg/dL) for FF horses during the FSIGT at exercise								
	Horse #						Diet group	
Time	1	3	5	7	9	11	Mean	SEM
Rest	100 .60	88.12	96.37	104.71	89.56	103.14	97.08	2.86
W10	97.07			100.68			98.88	1.80
W15	92.25	91.61	91.28	97.98	87.18	84.54	90.81	1.89
W20	93.78	91.68	90.67	93.03	91.01	78.40	89.76	2.32
0	93.38	97.50	88.23	92.23	92.49	77.09	90.15	2.88
1	463.96		521.87	513.57	489.34	427.81	483.31	17.17
2	445.93	505.81	493.44	483.77	470.49	415.33	469.12	13.64
3	432.8	496.21	479.04	453.19	449.79	385.95	449.49	15.69
4	422.33	490.00	445.34	436.95	431.20	362.56	431.39	16.81
5	409.05	466.67	426.32	413.58	418.98	346.37	413.49	15.86
6	391.92	453.80	406.10	401.73	403.98	330.38	397.98	16.16
7	386.17	440.06	383.78	383.69	392.47	315.58	383.62	16.23
8	369.54	432.78	366.82	367.90	385.15	299.13	370.22	17.52
10	346.76	412.76	328.94	333.49	363.64	263.19	341.46	19.96
12	324.7	394.26	295.65	300.58	345.20	231.06	315.24	22.30
14	303 .73	374.81	266.39	263.90	320.05	202.29	288.53	23.95
16	285 .64	352.49	232.23	239.27	297.64	172.49	263.29	25.44
19	253 .32	321.37	197.18	201.53	273.69	138.88	230.99	26.45
22	232 .65	293.90	165.65	169.71	245.59	108.99	202.75	27.26
23	219 .34	283.35	153.07	160.25	237.95	98.05	192.00	27.42
24	214.4	270.18	142.20	149.57	226.46	90.31	182.18	26.98
26	195 .66	244.15	121.46	129.85	205.62	74.28	161.83	25.92
28	173 .43	212.51	102.36	108.30	188.00	61.04	140.94	24.04
30	162 .09	186.69	83.96	90.53	167.12	47.71	123.01	22.93
32	142 .38	160.48	69.35	75.11	147.57	40.08	105.83	20.54
35	120 .61	131.26	52.98	57.92	122.92	30.53	86.04	17.86
40	94.86	91.66	36.97	41.92	91.26	23.86	63.42	13.28
45	70.78	68.87	31.94	39.15	69.31	25.65	50.95	8.55
50	55.93	55.79	37.83	44.46	58.45	34.02	47.74	4.26
60	45.16	60.46	62.96	44.98	57.73	57.25	54.75	3.18
70	59.39	66.43	75.56	55.81	63.64	61.92	63.79	2.78
80	65.17	74.36	79.93	65.93	69.60	64.80	69.96	2.48
90	71.99	76.98	82.99	75.33	74.20	72.13	75.60	1.67
100	76.96	78.90	86.04	82.78	76.76	78.94	80.06	1.49
115	83.39	76.71	87.83	85.52	78.16	86.44	83.01	1.87
130	82.97	73.19	90.37	88.31	75.56	86.69	82.85	2.87
150	84.48	68.07	91.88	83.02	69.82	88.12	80.90	3.99

F. Glucose (mg/dL) for SS horses during the FSIGT at exercise								
	Horse #						Diet group	
Time	2	4	6	8	10	12	Mean	SEM
B	99.39	93.8	92.84	97.28	93.39	95.15	95.31	1.04
W10	104.67	104.67		.				
W15	95.06	107	99.37	105.29	97.9	98.14	100.46	1.9
W20	93.38	110.18	104.28	105.12	101.79	99.99	102.45	2.3
0	96.47	113.74	104.51	107.06	101.76	106.84	105.06	2.36
1	566.13	545.09	504.49	532.83	542.12	503	532.28	10.06
2	529.1	525.5	487.93	490.67	516.03	493.22	507.07	7.6
3	514.78	500.24	471.79	468.88	507.17	488.76	491.93	7.68
4	510.31	473.22	453.89	444.76	478.07	465.92	471.03	9.32
5	484.22	462.94	438.92	435.98	470.88	450.33	457.21	7.71
6	470.96	449.21	426.57	426.87	449.45	438.36	443.57	6.86
7	453.26	435.17	411.6	415.12	437.93	423.36	429.4	6.41
8	443.15	425.92	404.99	403.97	427.9	416.74	420.44	6.12
10	416.03	408.7	383.38	384.88	398.46	402.37	398.97	5.29
12	393.03	388.82	367.47	370.84	369.16	383.31	378.77	4.5
14	370.35	372.8	349.35	357.16	341.61	363.84	359.18	4.97
16	344.86	353.9	333.83	342.94	316.76	346.74	339.84	5.32
19	317.84	329.13	313.37	321.18	280.05	324.46	314.34	7.2
22	287.14	309.19	290.3	298.45	253.37	301.19	289.94	7.99
23	280.62	297.92	280.55	293.96	239.71	291.61	280.73	8.7
24	267.25	285.88	274.04	289.71	227.23	283.47	271.26	9.43
26	242.97	268.37	258.6	276.56	202.48	266.25	252.54	11.03
28	212.56	243.07	236.51	257.89	174.85	244.95	228.3	12.31
30	189.02	219.81	217	244.17	149.28	228.06	207.89	13.83
32	171.13	208.77	197.13	228.68	126.19	212.66	190.76	15.1
35	139.11	194.33	172.84	206.91	99.27	188.56	166.83	16.54
40	98.74	174.11	138.69	178.48	69.52	152.38	135.32	17.64
45	68.89	101.59	111.57	153.51	55.42	123.31	102.38	14.68
50	53.06	84.64	89.39	131.06	51.67	97.36	84.53	12.14
60	42.84	76.11	80.9	97.34	61.27	70.51	71.49	7.54
70	57.73	76.85	82.26	79.82	72.36	67.98	72.83	3.68
80	75.35	84.65	83.14	77.1	77.79	75.71	78.95	1.62
90	85.04	90.84	82.12	75.22	80.68	81.07	82.49	2.12
100	91.42	95.56	82.88	75.78	80.27	87.38	85.54	2.99
115	96.93	95.48	78.95	83.24	75.02	96.48	87.68	4
130	96.09	95.14	71.81	92.05	66.7	92.73	85.75	5.29
150	99.72	90.71	65.08	98.08	57.53	82.29	82.23	7.15

G. Glucose (mg/dL) for FF horses during the FSIGT at rest								
	Horse #						Diet group	
Time	1	3	5	7	9	11	Mean	SEM
-30	84.83	96.25	94.99	97.77	92.40	102.73	94.83	2.23
-5	82.66	93.33	98.56	94.06	90.85	92.34	91.96	1.96
0	83.75	94.79	96.77	95.91	91.62	97.53	93.40	1.92
1		543.08	546.27	524.15	545.87	491.97	530.27	8.5
2	496.06	520.10	514.71	513.02	508.33	477.68	504.98	5.83
3	487.07	499.88	497.36	490.16	480.62	473.73	488.13	3.7
4	460.86	478.26	480.80	470.88	458.50	458.99	468.05	3.73
5	457.00	474.13	466.46	450.26	450.73	444.93	457.25	4.13
6	460.54	454.47	448.60	435.75	443.97	440.16	447.25	3.43
7	435.19	439.73	439.20	419.19	418.15	431.21	430.44	3.59
8	417.22	435.63	431.25	411.64	407.57	416.24	419.92	4.14
10	395.35	420.67	408.71	390.20	389.99	400.86	400.96	4.46
12	375.61	405.18	388.85	370.74	376.98	383.17	383.42	4.61
14	355.98	390.72	367.45	346.08	362.73	373.66	366.10	5.72
16	347.85	373.06	356.44	327.27	352.86	351.20	351.45	5.51
19	308.70	355.70	334.82	312.35	335.27	332.33	329.86	6.42
22	294.23	342.59	321.34	296.51	324.29	319.79	316.46	6.81
23	286.06	345.80	309.61	290.64	318.17	309.27	309.92	7.99
24	281.14	333.07	302.63	286.61	313.79	305.05	303.71	7.01
26	265.91	325.98	289.25	277.72	304.76	295.01	293.10	7.84
28	252.66	313.88	279.92	260.39	296.86	277.75	280.24	8.45
30	247.86	301.96	265.62	254.17	289.00	274.32	272.15	7.7
32	235.92	289.34	254.67	246.92	278.34	260.22	260.90	7.4
35	205.96	279.01	235.90	235.30	262.11	240.20	243.08	9.36
40	196.37	261.14	211.69	212.93	248.14	190.50	220.13	10.58
45	187.15	246.60	192.30	196.53	230.94	179.66	205.53	9.99
50	177.55	235.55	176.75	187.49	219.62	165.68	193.77	10.27
60	141.31	210.61	158.21	172.74	191.02	133.83	167.95	10.98
70	128.31	181.71	142.94	159.33	177.07	121.41	151.79	9.34
80	116.56	174.32	127.25	145.57	163.97	107.33	139.16	9.94
90	105.16	160.36	116.94	127.24	150.71	97.17	126.26	9.34
100	97.96	153.78	103.95	113.82	133.18	93.38	116.01	8.68
115	84.78	134.95	89.83	107.92	113.07	73.11	100.61	8.35
130	77.99	120.33	78.17	96.97	100.89	70.22	90.76	6.99
150	68.99	93.92	70.98	80.34	82.73	68.05	77.50	3.76

H. Glucose (mg/dL) for SS horses during the FSIGT at rest								
	Horse #						Diet group	
Time	2	4	6	8	10	12	Mean	SEM
-30	97.78		93.37	101.28	94.98	99.05	97.29	1.15
-5	98.79	94.49		105.83	94.88	94.37	98.46	1.87
0	98.28	94.49	93.37	103.55	94.93	96.71	97.37	1.43
1	553 .22	516.72	460.30	518.94	549.32	514.20	519.19	13.61
2	527 .37	503.82	454.66	502.69	522.58	491.62	499.78	10.63
3	502 .68	478.15	439.84	476.97	503.18	450.80	474.69	10.62
4	492 .47	458.75	418.90	462.80	498.83	452.14	465.03	11.82
5	474 .55	448.13	418.97	456.43	473.75	448.04	454.34	8.33
6	455 .00	436.26	402.68	431.67	463.30	425.30	435.59	8.85
7	450 .26	422.13	399.96	422.62	453.17	412.96	427.79	8.51
8	439.75	405.46	391.27	407.12	444.61	401.83	416.91	8.7
10	416 .15	392.64	383.96	396.79	424.68	386.74	401.66	6.58
12	399.01	375.66	368.50	381.16	410.25	374.02	386.58	6.4
14	382 .26	352.47	363.89	367.82	389.23	358.92	372.42	4.67
16	370 .38	331.41	352.09	344.83	381.22	349.28	359.56	5.67
19	350 .02	301.70	342.60	329.06	359.90	333.24	342.96	4.56
22	326 .16	286.41	331.54	319.29	342.99	321.53	328.30	3.45
23	324 .27	269.06	329.90	315.78	339.97	318.11	325.60	3.56
24	319.58	267.59	324.05	310.06	334.52	313.49	320.34	3.5
26	307 .56	252.06	320.94	298.13	321.77	305.56	310.79	3.75
28	290 .93	240.19	305.14	291.15	309.28	301.78	299.65	3.03
30	275.33	225.82	296.06	283.24	297.00	294.92	289.31	3.5
32	266 .21	205.98	293.30	269.25	283.71		278.11	4.48
35	255 .10	188.13	285.06	254.45	268.64	278.51	268.35	5
40	228 .59	150.91	270.94	235.91	247.83	271.23	250.90	7.18
45	211 .25	130.01	255.80	216.82	231.59	259.02	234.89	7.99
50	203 .02	118.73	245.26	205.86	219.47	246.82	224.09	7.66
60	186 .11	99.78	233.42	185.62	203.50	222.20	206.17	7.81
70	166 .86	88.10	207.94	171.48	185.89	207.50	187.93	7.08
80	147 .24	82.91	195.00	152.62	173.15	187.35	171.07	7.64
90	133 .69			135.10	158.16	171.77	149.68	6.54
100	121 .90	76.05	173.21	118.38	143.58	149.91	141.39	8.16
115	107 .91	75.41	153.96	98.40	122.13	117.78	120.04	7.69
130	96.82	76.49	140.49	99.55	107.60	95.72	108.04	6.84
150	85.25	81.86	121.78	90.51	92.26	74.75	92.91	6.4

I. Insulin (mIU/L) for FF horses during the FSIGT at exercise								
	Horse #						Diet group	
Time	1	3	5	7	9	11	Mean	SEM
Rest	11.10	7.53	11.39	15.32	4.75	6.77	9.48	1.43
W5	9.88							
W15	6.81	7.26	3.59	8.86	2.69	3.08	5.38	0.96
W20	9.43	7.33	8.12	4.81	4.71	1.62	6.00	1.06
0	7.27	5.56	3.23	3.88	3.89	1.32	4.19	0.76
1	25.00		31.23	28.96	12.14	33.38	26.14	3.08
2	24.09	13.78	29.51	28.91	12.08	32.05	22.36	3.18
3	23.51	14.25	29.19	27.91	11.52	32.82	22.08	3.19
4	23.65	14.77	28.69	28.71	11.24	31.45	21.88	3.09
5	25.22	15.62	29.09	30.20	12.02	33.91	22.99	3.24
6	26.77	16.18	29.00	29.83	14.32	33.84	23.55	2.95
7	28.77	17.72	30.64	29.39	14.60	33.80	24.12	2.89
8	29.13	18.65	30.36	28.75	15.26	32.56	23.93	2.63
10		20.65	30.43	26.53	13.82	30.45	21.75	2.60
12	25.86	22.20	29.42	22.50	13.72	25.20	20.70	1.98
14	27.03	20.55	30.93	19.74	14.87	22.97	20.51	2.12
16	24.30	22.11	28.71	18.00	13.19	18.53	18.38	2.02
19	24.10	21.10	28.77	17.93	12.55	15.77	17.77	2.19
22	138.46	130.48	166.37	115.35	123.69	104.90	109.38	7.96
23	87.25	93.58	107.66	84.48	80.45	79.87	74.54	3.91
24	79.80	82.82	79.05	70.14	62.18	60.73	59.90	3.55
26	62.21	60.50	60.96	51.54	46.68	44.21	45.52	2.95
28	48.28	51.02	48.19	38.00	37.79	33.48	35.55	2.70
30	43.87	43.09	33.51	27.15	31.95	23.01	27.84	3.13
32	36.55	37.15	27.13	20.84	27.84	19.83	23.29	2.77
35	77.11	32.00	20.38	15.21	23.76	14.28	26.38	8.85
40	18.14	18.56	12.96	8.78	12.83	8.10	11.39	1.66
45	11.75	10.83	7.52	5.61	8.69	5.90	7.83	0.94
50	10.30	6.76	3.77	2.59	6.94	2.45	5.60	1.15
60	4.57	3.94	2.61	1.37	4.10	1.67	3.64	0.50
70	3.57	4.25	1.81	1.18	3.38	2.03	3.25	0.45
80	2.69	3.57	1.59	0.69	2.96	1.18	2.77	0.42
90	2.62	2.29	1.74	0.57	2.92	0.81	2.70	0.36
100	1.59	1.21	1.69	0.48	2.20	0.49	2.33	0.26
115	2.91	0.43	1.70	0.87	2.48	1.41	2.82	0.35
130	1.28	0.22	1.73	0.01	2.17	1.01	2.29	0.31
150	1.65	0.04	2.74	0.14	2.44	0.85	2.56	0.43

J. Insulin (mIU/L) for SS horses during the FSIGT at exercise								
	Horse #						Diet group	
Time	2	4	6	8	10	12	Mean	SEM
Rest	14.83	8.37	6.17	19.45	5.61	18.29	12.12	2.30
W15	7.48	4.77	4.39	20.98	4.28	15.12	9.50	2.60
W20	8.06	6.78	5.35	18.61	4.61	9.61	8.84	1.91
0	9.90	5.48	5.05	16.42	4.23	12.03	8.85	1.79
1	21.53	8.09	9.68	34.77	15.09	26.32	19.25	3.84
2	21.50	8.28	9.98	38.48	16.58	27.71	20.42	4.25
3	25.58	9.39	10.15	42.27	18.43	27.30	22.19	4.61
4	23.84	12.14	10.52	42.73	20.46	29.38	23.18	4.44
5	24.61	12.74	13.74	45.29	18.69	31.81	24.48	4.64
6	25.51	13.23	16.27	46.23	18.91	34.83	25.83	4.70
7	24.55	15.57	15.61	45.62	20.19	36.68	26.37	4.57
8	23.50	15.79	16.01	51.29	21.58	34.41	27.10	5.09
10	22.27	15.31	14.06	52.31	17.83	34.85	26.10	5.55
12	21.87	16.09	13.90	48.35	15.70	34.96	25.15	5.12
14	22.35	14.61	13.11	51.86	15.64	36.25	25.64	5.75
16	24.72	14.84	12.50	48.97	14.61	34.23	24.98	5.34
19	25.08	14.57	12.88	48.51	14.89	35.35	25.21	5.32
22	165.10	120.83	124.24	174.57	120.73	173.37	146.47	10.10
23	120.29	77.40	75.05	132.23	83.06	116.39	100.74	9.33
24	104.22	58.85	59.14	123.80	63.64	95.57	84.20	10.26
26	67.65	46.65	39.00	101.57	46.49	79.35	63.45	8.96
28	60.02	35.97	29.52	74.00	34.65	65.96	50.02	7.04
30	57.73	28.18	25.95	86.10	26.79	61.94	47.78	9.23
32	47.17	28.64	22.63	73.78	22.30	56.36	41.81	7.79
35	39.81	15.93	18.99	65.31	13.48	50.48	34.00	7.92
40	26.50	21.67	13.96	57.45	8.01	47.46	29.18	7.22
45	18.43	7.60	9.85	50.66	6.02	34.65	21.20	6.67
50	10.95	5.33	5.98	44.66	4.39	23.84	15.86	5.91
60	5.93	3.85	3.04	29.88	1.76	15.18	9.94	4.06
70	3.09	5.00	2.33	22.92	1.84	9.18	7.39	3.00
80	2.90	1.77	2.01	19.31	1.03	6.52	5.59	2.61
90	3.00	1.57	2.12	15.39	1.73	4.93	4.79	1.99
100	3.54	1.19	1.23	12.97	1.33	3.73	4.00	1.70
115	6.14	0.53	1.35	8.42	1.20	3.76	3.57	1.18
130	2.94	0.40	0.45	6.33	0.90	1.50	2.09	0.85
150	4.95	0.24	0.33	4.32	0.87	1.01	1.95	0.79

K. Insulin (mIU/L) for FF horses during the FSIGT at rest								
	Horse #						Diet group	
Time	1	3	5	7	9	11	Mean	SEM
-30	3.63	8.48	16.96	7.60	5.33	12.96	9.16	1.85
-5	8.88	12.58	11.40	6.01	5.87	9.34	9.01	1.02
0	6.25	10.53	14.18	6.81	5.60	11.15	9.09	1.27
1		26.87	46.65	73.48	33.48	25.04	41.10	7.30
2	48.77	25.85	46.40	81.07	31.43	26.91	43.40	7.78
3	49.31	29.64	49.13	95.07	33.35	30.97	47.91	9.22
4	48.69	31.32	46.20	93.65	37.01	30.51	47.90	8.81
5	39.67	30.58	46.81	96.98	38.11	38.71	48.48	9.06
6	47.73	30.92	50.81	94.20	38.54	36.73	49.82	8.55
7	56.58	32.59	47.52	96.34	40.88	40.74	52.44	8.55
8	58.33	31.25	47.42	94.84	40.42	37.76	51.67	8.60
10	59.70	32.59	45.39	83.86	43.80	38.83	50.69	6.92
12	61.31	31.99	46.45	69.08	42.78	38.73	48.39	5.25
14	62.94	29.75	43.35	59.50	36.34	37.95	44.97	4.98
16	72.95	29.12	48.60	50.98	35.57	33.50	45.12	6.02
19	66.84	28.87	47.87	43.16	33.97	33.46	42.36	5.17
22	725.46	126.95	1211.86	227.09	124.56	183.18	433.18	165.48
23	184.39	97.34	189.45	128.96	100.58	102.29	133.84	15.90
24	137.03	87.09	135.75	94.46	92.38	89.87	106.10	8.80
26	103.09	65.54	112.31	69.51	80.34	75.33	84.35	7.08
28	101.12	57.08	90.98	59.50	71.04	64.26	74.00	6.72
30	84.37	46.61	77.47	59.94	60.25	55.51	64.02	5.27
32	89.87	42.46	70.38	56.43	56.66	55.34	61.86	6.08
35	79.87	36.10	71.83	42.25	50.97	49.65	55.11	6.38
40	74.58	30.92	64.58	35.09	46.82	44.59	49.43	6.33
45	85.93	33.15	65.01	27.15	49.90	37.81	49.82	8.28
50	77.45	29.52	99.72	19.55	48.64	36.98	51.98	11.45
60	77.73	33.00	73.82	39.69	54.31	37.53	52.68	7.19
70	63.83	37.25	68.04	52.94	60.88	33.46	52.73	5.36
80	55.55	41.38	69.84	45.06	69.71	24.87	51.07	6.53
90	45.76	43.31	58.41	43.92	66.42	21.25	46.51	5.76
100	33.32	40.06	50.46	41.00	46.85	18.82	38.42	4.20
115	26.72	38.30	55.57	36.44	31.36	12.67	33.51	5.28
130	26.58	28.01	43.35	42.08	30.32	14.70	30.84	3.98
150	14.57	23.82	2 4.80	49.15	39.89	8.45	26.78	5.7

L. Insulin (mIU/L) for SS horses during the FSIGT at rest								
	Horse #						Diet group	
Time	2	4	6	8	10	12	Mean	SEM
Rest	7.83		11.96	16.32	3.70	17.35	11.43	2.10
-5	6.50	7.16		27.34	5.90	15.17	12.42	3.34
0	7.17	7.16	11.96	21.83	4.80	16.26	11.53	2.43
1	31.80	32.89	26.33	83.65	20.22	69.51	44.07	9.68
2	31.94	32.79	27.25	96.24	17.21	73.39	46.47	11.58
3	30.51	31.67	25.35	102.58	19.84	75.24	47.53	12.47
4	29.85	32.86	28.46	103.73	19.99	81.54	49.40	12.85
5	31.92	39.93	29.42	104.73	23.35	82.81	52.03	12.48
6	43.64	39.54	33.60	111.08	26.43	87.87	57.03	12.74
7	43.88	40.03	32.42	122.17	36.10	89.93	60.76	13.69
8	45.51	41.62	32.20	114.93	32.04	94.28	60.10	13.22
10	44.05	46.79	33.94	118.64	35.39	95.61	62.40	13.32
12	43.96	61.63	33.69	119.12	30.83	92.06	63.55	13.18
14	37.02	61.74	31.51	118.40	30.73	90.67	61.68	13.47
16	36.89	58.56	32.30	109.73	29.93	92.82	60.04	12.65
19	36.94	71.77	154.32	99.83	27.65	94.76	80.88	17.31
22	305.64	171.40	107.17	616.69	159.24	551.86	318.67	80.83
23	134.79	156.76	98.90	267.18	86.23	213.21	159.51	25.89
24	108.29	137.66	84.38	195.10	69.96	177.03	128.73	18.75
26	81.58	119.88	78.99	167.73	55.41	155.42	109.83	16.87
28	64.52	107.33	70.66	161.44	47.17	139.04	98.36	16.87
30	61.15	106.03	70.12	157.04	40.99	134.84	95.03	16.87
32	53.65	92.88	63.89	143.31	33.69		77.48	15.54
35	49.35	83.45	56.46	129.68	35.29	123.72	79.66	14.80
40	42.25	59.18	54.11	127.30	31.63	126.57	73.51	15.83
45	39.94	38.31	53.35	122.76	29.12	116.85	66.72	15.61
50	42.67	31.39	68.12	111.70	26.81	126.19	67.81	15.79
60	51.83	27.88	61.60	108.37	33.49	133.00	69.36	15.77
70	54.45	23.18	54.69	106.34	31.84	159.70	71.70	19.35
80	46.51	22.09	71.85	99.63	37.65	173.34	75.18	20.61
90	35.23			101.63	29.83	164.05	82.68	22.38
100	31.34	12.90	71.68	110.48	31.28	156.74	69.07	20.69
115	22.49	8.42	67.17	81.59	29.72	121.60	55.16	15.95
130	17.29	7.61	46.62	60.45	22.98	95.62	41.76	12.24
150	17.69	6.83	27.75	38.97	18.24	62.02	28.58	7.31

M. Insulin (mIU/L) for FF horses during the tracer test at exercise								
	Horse #						Diet group	
Time	1	3	5	7	9	11	Mean	SEM
Rest	9.29	9.09	8.95	5.6	4.06	6.67	7.28	0.89
w15	10.61	4.84	5.85	4.57	3.00	4.29	5.53	1.08
w25	13.20	6.86	5.36	3.92	3.89	3.99	6.20	1.48
10	11.56	5.90	6.50	5.49	4.54	3.44	6.24	1.15
30	6.09	4.58	5.76	3.20	4.33	2.05	4.34	0.62
40	8.54	3.67	5.17	4.08	3.31	3.20	4.66	0.83
70	8.54	2.31	4.07	2.99	3.11	1.78	3.80	1.00
150	3.68	0.53	1.90	1.37	1.58	2.97	2.01	0.47

N. Insulin (mIU/L) for SS horses during the tracer test at exercise								
	Horse #						Diet group	
Time	2	4	6	8	10	12	Mean	SEM
Rest	5.30	6.82	7.55	18.27	6.94	11.48	9.39	1.97
w15	8.54	5.02	6.81	12.41	3.71	10.68	7.86	1.36
w25	8.7	4.93	6.93	11.73	4.53	11.72	8.09	1.30
10	8.78	4.45	7.88	9.95	3.40	9.84	7.38	1.14
30	6.39	2.58	4.57	8.28	2.67	5.43	4.99	0.90
40	7.04	1.90	4.28	7.72	2.52	4.56	4.67	0.96
70	5.19	1.75	3.68	4.22	2.02	4.88	3.62	0.59
150	3.77	0.71	2.19	1.99	0.80	1.34	1.80	0.46

O. Insulin (mIU/L) for FF horses during the tracer test at rest								
	Horse #						Diet group	
Time	1	3	5	8	9	11	Mean	SEM
Rest	6.64	13.11	7.28	9.71	6.89	3.82	7.91	1.29
10	19.76	17.44	10.02	14.67	17.86	8.76	14.75	1.83
30	14.14	15.31	5.19	10.62	13.73	9.29	11.38	1.55
40	11.50	17.35	6.46	7.31	11.81	10.28	10.78	1.59
70	9.72	16.42	6.88	5.98	9.08	8.64	9.46	1.51
150	11.55	10.34	5.06	7.08	7.15	7.88	8.18	0.97

P. Insulin (mIU/L) for SS horses during the tracer test at rest								
	Horse #						Diet group	
Time	2	6	4	8	10	12	Mean	SEM
Rest	11.63	5.20	8.96	13.07	13.09	14.13	11.01	1.37
10	14.32	5.01	12.54	26.55	19.44	19.55	16.24	3.01
30	14.67	7.35	10.46	22.53	17.16	19.75	15.32	2.33
40	16.52	6.52	9.44	17.96	14.97	20.26	14.28	2.15
70	12.61	6.61	7.68	16.42	14.11	15.34	12.13	1.67
150	10.15	3.60	5.47	16.08	7.96	18.82	10.35	2.45

Q. Plasma % enrichment for FF horses during the FSIGT at rest								
	Horse #						Diet group	
Time	1	3	5	7	9	11	Mean	SEM
0	0.33	0.37	0.38	0.34	0.36	0.30	0.35	0.01
1	14.12	18.86	14.69	13.68	16.87	11.19	14.90	1.09
2	12.26	15.67	14.50	12.04	14.60	10.81	13.31	0.77
3	10.91	14.53	13.79	10.71	12.80	10.54	12.21	0.71
4	10.47	13.59	13.22	10.14	12.01	10.37	11.63	0.62
5	10.17	12.79	12.90	9.79	11.42	10.19	11.21	0.56
6	9.75	12.51	12.40	9.41	11.06	9.76	10.81	0.57
7	9.42	12.07	12.10	9.17	10.56	9.64	10.49	0.54
8	9.29	11.73	11.85	8.86	10.20	9.37	10.22	0.53
10	8.91	11.25	11.24	8.57	9.79	9.07	9.80	0.48
12	8.61	10.82	10.70	8.24	9.38	8.74	9.41	0.45
14	8.28	10.43	10.30	7.99	9.01	8.51	9.09	0.43
16	8.07	10.13	9.88	7.70	8.62	8.35	8.79	0.40
18	7.86	9.81	9.59	7.50	8.37	8.09	8.54	0.39
20	7.62	9.55	9.28	7.31	8.15	7.88	8.30	0.37
23	7.35	9.19	8.91	7.10	7.82	7.60	7.99	0.35
26	7.09	8.94	8.58	6.85	7.57	7.40	7.74	0.34
30	6.78	8.44	8.17	6.53	7.25	7.13	7.38	0.31
35	6.50	8.02	7.61	6.25	6.93	6.72	7.00	0.28
40	6.24	7.73	7.12	5.98	6.56	6.45	6.68	0.26
50	5.73	7.08	6.16	5.27	6.01	5.90	6.03	0.25
60	5.27	6.42	5.38	4.74	5.49	5.50	5.47	0.22
70	4.93	5.95	4.59	4.36	5.04	5.16	5.00	0.22
90	4.04	4.29	4.01	3.6	4.32	4.58	4.14	0.14
110	3.15	4.04	3.18	2.91	3.76	4.00	3.51	0.20
130	3.11	3.45	2.55	2.57	3.27	3.49	3.07	0.17
150	3.14	3.19	2.47	2.18	2.90	3.08	2.83	0.17

R. Plasma % enrichment for SS horses during the FSIGT at rest								
	Horse #						Diet group	
Time	2	4	6	8	10	12	Mean	SEM
0	0.38	0.57	0.38	0.33	0.34	0.35	0.39	0.04
1	21.67	18.87	14.03	15.42	11.54	13.98	15.92	1.51
2	20.89	16.99	13.07	13.54	11.20	12.56	14.71	1.46
3	20.64	14.10	12.62	12.01	10.90	11.05	13.55	1.50
4	18.99	13.17	11.81	11.49	10.50	10.62	12.76	1.31
5	17.92	12.15	11.60	10.87	10.48	10.05	12.18	1.19
6	16.69	11.48	11.44	10.42	9.72	9.56	11.55	1.08
7	15.88	10.96	11.10	10.05	9.45	9.27	11.12	1.00
8	15.55	10.54	10.96	9.75	9.28	8.97	10.84	0.99
10	14.76	9.88	10.44	9.31	8.83	8.56	10.30	0.94
12	13.84	9.44	10.09	8.88	8.38	8.27	9.82	0.85
14	13.14	8.98	9.78	8.58	8.24	7.96	9.45	0.78
16	12.35	8.59	9.52	8.33	7.97	7.76	9.09	0.70
18	12.10	8.39	9.26	8.02	7.78	7.53	8.85	0.70
20	11.72	8.16	9.04	7.85	7.55	7.33	8.61	0.67
23	11.30	7.77	8.80	7.53	7.31	7.13	8.31	0.64
26	10.37	7.41	8.77	7.25	7.06	6.87	7.95	0.56
30	9.82	7.06	8.31	6.89	6.79	6.63	7.58	0.51
35	9.11	6.62	7.94	6.54	6.52	6.44	7.20	0.45
40	8.78	6.24	7.58	6.22	6.27	6.12	6.87	0.44
50	7.66	5.67	6.90	5.61	5.80	5.69	6.22	0.35
60	7.01	5.24	6.40	5.09	5.38	5.29	5.74	0.32
70	6.51	4.78	5.91	4.60	5.09	4.93	5.30	0.30
90	5.14	4.14	5.09	3.91	4.52	4.41	4.53	0.20
110	4.45	3.60	4.43	3.34	4.06	3.98	3.98	0.18
130	3.76	3.20	3.89	2.95	3.67	3.63	3.52	0.15
150	3.05	2.89	3.38	2.61	3.34	3.15	3.07	0.12

S. Plasma % enrichment for FF horses during the FSIGT at exercise								
	Horse #						Diet group	
Time	1	3	5	7	9	11	Mean	SEM
0	0.33	0.33	0.35	0.36	0.35	0.30	0.34	0.01
1	14.68	17.47	16.05	15.36	15.05	13.99	15.43	0.49
2	13.58	13.71	13.80	12.98	13.63	11.62	13.22	0.34
3	12.56	12.68	12.67	11.84	12.30	10.68	12.12	0.32
4	11.51	11.80	12.04	11.10	11.82	9.84	11.35	0.33
5	11.16	11.31	11.46	10.60	10.90	9.66	10.85	0.27
6	10.62	10.85	11.11	10.19	10.69	9.27	10.46	0.27
7	10.32	10.52	10.73	9.82	10.27	9.05	10.12	0.25
8	10.05	10.19	10.45	9.54	9.89	8.78	9.82	0.24
10	9.50	9.65	9.86	8.91	9.10	8.34	9.23	0.23
12	9.32	9.07	9.37	8.17	8.49	7.97	8.73	0.25
14	9.24	8.45	8.81	7.37	7.71	7.55	8.19	0.31
16	7.86	7.86	8.30	6.65	7.07	7.19	7.49	0.25
18	7.09	7.29	7.71	6.06	6.61	6.81	6.93	0.23
20	6.40	6.82	7.20	5.43	6.19	6.35	6.40	0.25
23	5.65	6.29	6.75	4.86	5.88	5.82	5.87	0.26
26	5.28	5.84	6.36	4.62	5.27	5.48	5.48	0.24
30	4.96	5.17	5.70	4.43	4.87	5.12	5.04	0.17
35	4.23	4.68	5.27	3.87	4.02	4.61	4.45	0.21
40	3.85	4.20	4.79	3.58	3.71	4.26	4.06	0.18
50	3.19	3.41	4.08	2.27	2.78	3.68	3.24	0.26
60	2.71	2.75	3.45	1.85	2.14	3.08	2.66	0.24
70	2.21	2.32	2.93	1.56	1.88	2.66	2.26	0.20
90	1.67	1.61	2.24	1.11	1.41	2.00	1.67	0.17
110	1.17	1.18	1.64	0.84	1.01	1.48	1.22	0.12
130	0.82	0.96	1.19	0.67	0.58	1.20	0.90	0.11
150	0.66	0.79	0.90	0.57	0.48		0.68	0.07

T. Plasma % enrichment for SS horses during the FSIGT at exercise								
	Horse #						Diet group	
Time	2	4	6	8	10	12	Mean	SEM
0	0.38	0.34	0.34	0.50	0.34	0.34	0.37	0.03
1	19.92	13.83	12.13	12.68	14.10	13.09	14.29	1.16
2	18.45	12.57	10.34	10.42	12.47	11.01	12.54	1.25
3	16.91	11.49	9.30	9.38	11.59	10.17	11.47	1.16
4	16.23	10.91	8.75	8.86	10.89	9.64	10.88	1.14
5	15.05	10.39	8.28	8.53	10.42	9.21	10.31	1.02
6	14.59	10.05	7.90	8.21	10.09	8.89	9.95	1.00
7	14.06	9.64	7.61	7.99	9.59	8.52	9.57	0.96
8	13.25	9.38	7.34	7.81	9.36	8.38	9.25	0.87
10	12.13	8.82	6.90	7.38	8.77	7.90	8.65	0.76
12	11.44	8.29	6.55	6.99	8.07	7.47	8.13	0.71
14	10.46	7.83	6.21	6.36	7.45	6.93	7.54	0.64
16	10.22	7.18	5.89	5.99	6.77	6.34	7.07	0.66
18	9.96	6.42	5.59	5.60	6.24	5.61	6.57	0.69
20	9.14	5.84	5.26	5.19	5.82	5.44	6.12	0.62
23	8.44	5.00	5.08	4.71	5.31	4.95	5.58	0.58
26	7.18	4.46	4.65	4.19	4.94	4.58	5.00	0.45
30	6.60	3.93	4.22	3.87	4.39	4.15	4.53	0.42
35	5.77	3.58	3.80	3.79	3.88	3.55	4.06	0.35
40	5.10	3.14	3.38	3.67	3.49	3.25	3.67	0.30
50	3.47	2.41	2.74	3.18	2.78	2.66	2.87	0.16
60	2.44	1.93	2.20	2.45	2.31	2.21	2.26	0.08
70	1.77	1.50	1.92	2.10	1.93	1.89	1.85	0.08
90	1.21	1.04	1.36	1.39	1.37	1.37	1.29	0.06
110	0.68	0.74	1.06	1.02	1.02	1.01	0.92	0.07
130	0.58	0.60	0.80	0.76	0.68	0.77	0.70	0.04
150	0.38	0.53	0.65	0.63	0.56	0.65	0.57	0.04

Table A.3. (Chapter 2 and 3) Proximate analysis of pasture and supplements (SS & FF; fed as twice daily 1 kg meals). Analysis was performed by Dairy One DHIA Forage Testing Laboratory, Ithaca, NY. Data are summarized as means ± SE.

	Pasture		SS		FF	
	Mean	SEM	Mean	SEM	Mean	SEM
% DM	24.02	2.17	87.43[a]	0.20	91.08[b]	0.39
% CP	18.31	0.92	15.42	0.83	14.02	0.52
% ADF	34.98	1.02	14.70[a]	0.84	32.58[b]	0.61
% NDF	62.40	1.21	24.20[a]	1.22	45.07	1.07
% NFC[4]	11.64	1.10	52.23[a]	2.14	26.40	0.99
% NSC[3]	10.16	0.90	44.73[a]	2.06	13.07	0.76
% Starch	2.13	0.51	35.98[a]	2.54	4.87	0.79
% Sugar	8.02	0.83	8.78	0.50	8.15	0.29
% C Fat	3.28	0.28	3.33[a]	0.14	10.60	0.83
% Ash	10.55	0.67	7.22	0.56	8.44	0.13
% Ca	0.48	0.02	1.51	0.19	1.59	0.05
% P	0.35	0.02	0.69	0.07	0.66	0.06
Ca:P	1.39	0.09	2.16	0.13	2.50	0.16
% Mg	0.20	0.01	0.23	0.02	0.22	0.01
% K	2.94	0.23	1.33	0.07	1.44	0.05
% Na	0.01	0.00	0.25	0.02	0.24	0.03
ppm Fe	466	102	356[c]	42	496[d]	28
ppm Zn	25.92	3.65	129.83	10.55	115.00	7.30
ppm Cu	4.50	0.66	48.17	4.00	43.83	3.82
Zn:Cu	6.14	0.84	2.73	0.15	2.68	0.18
ppm Mn	54.08	5.67	79.83	4.27	96.00	10.19
ppm Molybdenum	1.01	0.12	1.4	0.42	1.15	0.31
% Cl ion			0.50	0.05	0.52	0.02
Mcal/kg DM	2.29	0.02	3.03[c]	0.05	2.85[d]	0.04
Mcal/kg as fed	0.55	0.05	2.65	0.04	2.59	0.05
Mcal/d as fed			5.30	0.09	5.19	0.09

[3] Non-structural carbohydrates: NSC = suger + starch
[4] Non-fiber carbohydrates: NFC = 100 - water - ash - CP - fat - NDF.
[a,b] Supplement means differ ($P < 0.01$)
[c,d] Supplement means differ ($P < 0.05$)

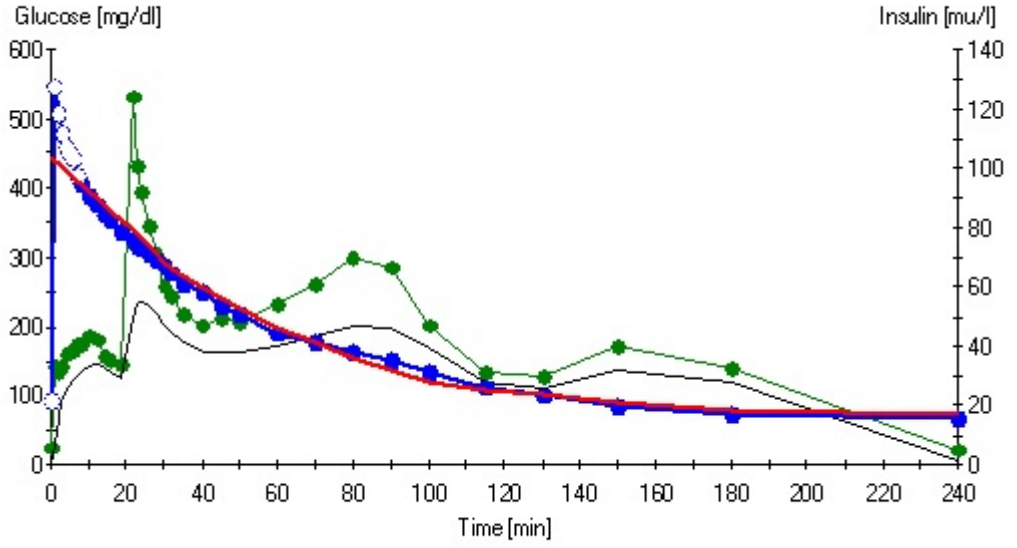
Table A.4. (Chapter 3) Comparison of 2 and 3 phase exponential fits for tracer glucose curves at rest (R) and during exercise (E) in trained Arabian geldings adapted to diets rich in sugar and starch (SS) or fat and fiber (FF). Residual autocorrelation was based on the Durbin Watson Statistic. Models with maximum parameter fractional standard deviation $\geq$ 0.50 were considered unresolved. The 3 phase exponential fit was preferred if this fit was more likely correct according to the AICc, eliminated autocorrelation of the residuals, and all parameters were resolved.

			AICc Probability		Residual		Maximum		Preferred
Horse	Test	Diet	of Best Fit		Autocorrelation		Parameter FSD		Model
			2 terms	3 terms	2 terms	3 terms	2 terms	3 terms	# terms
1	R	FF	0.06	0.94	yes	no	0.12	0.91	2
2	R	SS	0.05	0.95	yes	no	0.07	0.82	2
3	R	FF	0.95	0.05	yes	yes	0.14	21.1	2
4	R	SS	0.01	0.99	yes	no	0.07	0.28	3
5	R	FF	0.24	0.76	yes	no	0.08	0.91	2
6	R	SS	0.95	0.05	no	no	0.14	0.48	2
7	R	FF	0.01	0.99	no	no	0.11	0.37	2
8	R	SS	0.01	0.99	yes	no	0.08	0.37	3
9	R	FF	0.38	0.62	no	no	0.09	1.11	2
10	R	SS	0.01	0.99	no	yes (neg)	0.10	0.40	2
11	R	FF	0.05	0.95	yes	no	0.15	0.39	3
12	R	SS	0.01	0.99	no	no	0.10	0.66	2
1	E	FF	0.99	0.01	yes	yes	0.07	1.20	2
2	E	SS	0.96	0.04	no	no	0.33	20.4	2
3	E	FF	0.07	0.93	no	no	0.07	0.83	2
4	E	SS	0.95	0.05	yes	yes	0.05	1.63	2
5	E	FF	0.98	0.02	no	no	0.07	4.26	2
6	E	SS	0.01	0.99	yes	no	0.05	0.66	2
7	E	FF	0.96	0.04	yes	yes	0.06	0.57	2
8	E	SS	0.95	0.05	yes	yes	0.14	0.97	2
9	E	FF	0.99	0.01	yes	yes	0.15	0.40	2
10	E	SS	0.99	0.01	yes	yes	0.09	0.66	2
11	E	FF	0.99	0.01	no	no	0.04	1.32	2
12	E	SS	0.99	0.01	no	yes	0.06	14.1	2

Figure A.1. (Chapter 2) Examples of results from MinMod Milleniuum from one horse at rest (A) and exercise (B)

A).Rest

Parameters Name	Value	FSD	Units
AIRg	304.03		[mu.l^-1.min]
DI	420.64	0.033599	[]
SI	1.3836	0.033602	[(mu/l)^-1.min^-1]
Sg	0.0097714	0.043914	[min^-1]
GB	81.791		[mg/dl]
IB	5.598		[mu/l]
P(2)	0.46195	0.20917	[min^-1]
P(3)	6.3914E-05	0.22422	[(mu/l)^-1.min^-2]
G(0)	441.95	0.009505204	[mg/dl]
GEZI	0.0089968	0.050528	[min^-1]
Beta-Cell function	107.25		[mu/mM]
Insulin Resistance	1.1305		[mM.mu/l^2]
Rsquared	99.11		[%]



B). Exercise

Parameters Name	Value	FSD	Units
AIRg	87.67		[mu.l^-1.min]
DI	1437.4	0.0072291	[]
SI	16.395	0.0072291	[(mu/l)^-1.min^-1]
Sg	0.029763	0.012563	[min^-1]
GB	82.588		[mg/dl]
IB	3.8906		[mu/l]
P(2)	0.067136	0.026555	[min^-1]
P(3)	0.00011007	0.023928	[(mu/l)^-1.min^-2]
G(0)	493.8	0.00642613	[mg/dl]
GEZI	0.023384	0.016184	[min^-1]
Beta-Cell function	71.504		[mu/mM]
Insulin Resistance	0.79337		[mM.mu/l^2]
Rsquared	99.41		[%]

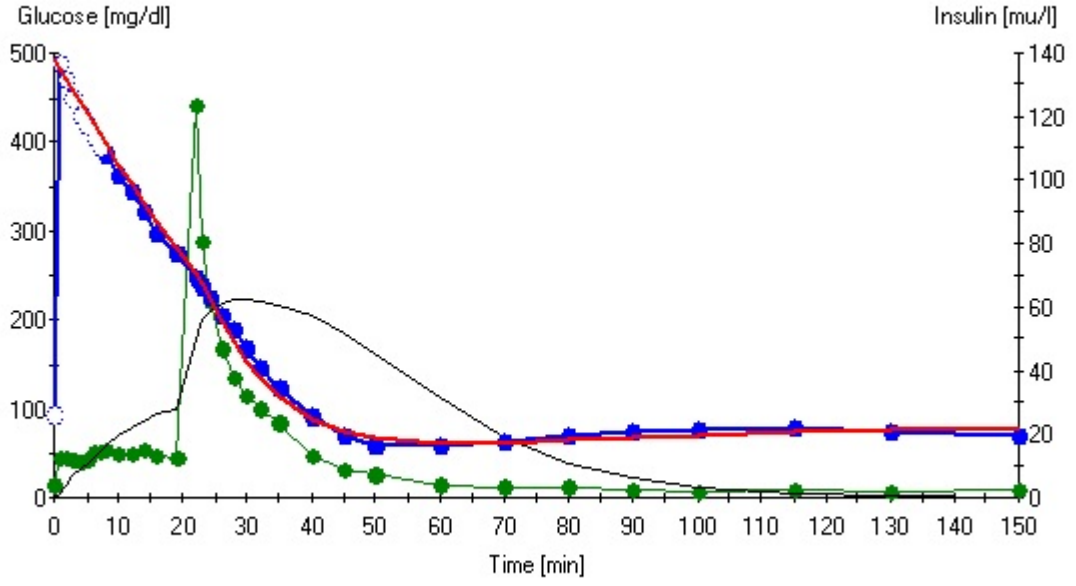


Figure A.2. (Chapter 3) Example of WINSAAM code used to fit tracer curves and determined macrio- and microparameters for a two- (A) or three- (B) compartment model.

A). Two-compartment Model

```
A SAAM31
2      10
C Insert Control lines 2,3,4 here as needed
C DATA: %ENRICH * GLUCOSE MG/DL = TRACER MG/DL
C       TRACER MG/DL * 10 DL/L * 1/DOSE MG
C       = TRACER FRACTION OF DOSE / L
C P(97)=TRACER DOSE MG
C P(98)=BW KG
C P(99)=BASAL GLUCOSE MG/DL
C P(7)=V1 ML/KG BW
C P(8)=Q1 MG/KG BW
C P(9)=K11
C P(10)=K22
C P(14)=K12K21
C P(18)=K12MIN
C P(19)=K12MAX
C P(20)=K21MIN
C P(21)=K21MAX
C P(26)=K01MAX
C P(27)=K02MAX
C P(40)=Q2 MIN MG/KG BW
C P(41)=Q2 MAX
C P(48)=V2 MIN ML/KG BW
C P(49)=V2 MAX
C P(56)=VD MIN
C P(57)=VD MAX
C P(60)=R12 MG/MIN PER KG BW
C P(61)=R21 MIN
C P(62)=R21 MAX
C P(67)=R01 R02 MAX = HPO
C P(75)=% VARIABILITY K12, K21, V2, Q2
C P(80)=% VARIABILITY VD
H PAR
C Insert model parameters here
  P(4)    0.1        0.0001       1
  P(5)    0.01       0.0001       1
  K(81)   0.01       0.0001       0.03
  K(82)   0.01       0.0001       0.03
  p(1)=-p(4)
  p(2)=-p(5)
  P(97)=8780
  P(98)=482.7
```

```
  P(99)=93.96
  P(7)=(1000/(K(81)+K(82)))/P(98)
  P(8)=P(7)*P(99)*0.01
  P(9)=(P(1)*K(81)+P(2)*K(82))/(K(81)+K(82))
  P(10)=P(1)+P(2)-P(9)
  P(14)=(K(81)+K(82))/((K(81)/(P(10)-P(1))**2)+
  (K(82)/(P(10)-P(2))**2))
  P(18)=P(14)/(-P(9))
  P(19)=-P(10)
  P(20)=P(14)/(-P(10))
  P(21)=-P(9)
  P(26)=-P(9)-P(20)
  P(27)=-P(10)-P(18)
  P(40)=-(P(8)*P(14))/(P(10)*P(19))
  P(41)=-(P(8)*P(14))/(P(10)*P(18))
  P(48)=(P(7)*P(14))/(P(10)**2)
  P(49)=(-P(7)*P(21))/P(10)
  P(56)=P(7)+P(48)
  P(57)=P(7)+P(49)
  P(60)=-(P(8)*P(14))/P(10)
  P(61)=-P(10)*P(40)
  P(62)=-P(10)*P(41)
  P(67)=P(8)*((P(14)/P(10))-P(9))
  P(75)=100*P(19)/P(18)-100
  P(80)=100*P(57)/P(56)-100
H DAT
X G(1)=K(81)*EXP(-P(4)*T)+K(82)*EXP(-P(5)*T)
C Insert data values here
101 G(1)                    fsd=0.02
        4        0.0164135785876993
        5        0.015219451025057
        6        0.0148258712984055
        7        0.0141912471526196
        8        0.0136300592255125
       10        0.0130020956719818
       12        0.0125060791571754
       14        0.0117345660592255
       16        0.0113740683371298
       18        0.011039854214123
       20        0.0108753724373576
       23        0.0104664669703872
       26        0.0100775783029613
       30        0.00976265660592255
       35        0.0090578354214123
       40        0.00869623633257404
       50        0.00733793479498861
       60        0.00668911987471526
       70        0.00617657744874715
       90        0.00436399202733485
```

```
110        0.00402324601366743
130        0.00344093451025057
150        0.00312921839407745
```

B). Three-compartment model

```
A SAAM31
2      10
C Insert Control lines 2,3,4 here as needed
C DATA: %ENRICH * GLUCOSE MG/DL = TRACER MG/DL
C       TRACER MG/DL * 10 DL/L * 1/DOSE MG
C       = TRACER FRACTION OF DOSE / L
C P(1)=K1
C P(2)=K2
C P(3)=K3
C P(97)=TRACER MG
C P(98)=BW KG
C P(99)=BASAL GLUCOSE MG/DL
C P(7)=V1 ML/KG BW
C P(8)=Q1 MG/KG BW
C P(9)=K11
C P(10)=K22 CAT
C P(11)=K22 MAM
C P(12)=K33 CAT
C P(13)=K33 MAM
C P(14)=K12K21 CAT %/MIN
C P(15)=K12K21 MAM
C P(16)=K13K31 MAM
C P(17)=K23K32 CAT
C P(18)=K12MIN CAT
C P(19)=K12MAX CAT
c P(20)=K21MIN CAT
C P(21)=K21MAX CAT
C P(22)=K23MIN CAT
C P(23)=K23MAX CAT
C P(24)=K32MIN CAT
C P(25)=K32MAX CAT
C P(26)=K01MAX CAT
C P(27)=K02MAX CAT
C P(28)=K03MAX CAT
C P(29)=K12MIN MAM
C P(30)=K12MAX MAM
C P(31)=K21MIN MAM
C P(32)=K21MAX MAM
C P(33)=K13MIN MAM
C P(34)=K13MAX MAM
C P(35)=K31MIN MAM
```

```
C P(36)=K31MAX MAM
C P(37)=K01MAX MAM
C P(38)=K02MAX MAM
C P(39)=K03MAX MAM
C P(40)=Q2MIN CAT MG/KG
C P(41)=Q2MAX CAT
C P(42)=Q3MIN CAT
C P(43)=Q3MAX CAT
C P(44)=Q2MIN MAM
C P(45)=Q2MAX MAM
C P(46)=Q3MIN MAM
C P(47)=Q3MAX MAM
C P(48)=V2MIN CAT ML/KG
C P(49)=V2MAX CAT
C P(50)=V3MIN CAT
C P(51)=V3MAX CAT
C P(52)=V2MIN MAM
C P(53)=V2MAX MAM
C P(54)=V3MIN MAM
C P(55)=V3MAX MAM
C P(56)=VDMIN CAT
C P(57)=VDMAX CAT
C P(58)=VDMIN MAM
C P(59)=VDMAX MAM
C P(60)=R12    CAT MG/KG /MIN
C P(61)=R21MIN CAT
C P(62)=R21MAX CAT
C P(63)=R23MIN CAT
C P(64)=R23MAX CAT
C P(65)=R32MIN CAT
C P(66)=R32MAX CAT
C P(67)=R01R02R02 MAX CAT = HPO CAT
C P(68)=R12    MAM
C P(69)=R21MIN MAM
C P(70)=R21MAX MAM
C P(71)=R13    MAM
C P(72)=R31MIN MAM
C P(73)=R31MAX MAM
C P(74)=R01R02R03 MAX MAM = HPO MAM
C P(75)=%VAR CAT K12, K21, V2, Q2
C P(76)=%VAR CAT K23, K32
C P(77)=%VAR MAM K12, K21, V2, Q2
C P(78)=%VAR MAM K13, K31, V3, Q3
C P(79)=%VAR CAT         V3, Q3
C P(80)=%VAR MAM&CAT      VD
H PAR
C Insert model parameters here
  P(4)    0.3         0.0001        1
  P(5)    0.03        0.0001        1
```

```
P(6)    1.001       1.0001      10
K(81)   0.01        0.0001      0.03
K(82)   0.01        0.0001      0.03
K(83)   0.01        0.0001      0.03
p(1)=-p(4)
p(2)=-p(5)
P(3)=-LOG(P(6))
P(97)=8780
P(98)=482.7
P(99)=93.96
P(7)=(1000/(K(81)+K(82)+k(83)))/P(98)
P(8)=P(7)*P(99)*0.01
P(9)=(P(1)*K(81)+P(2)*K(82)+P(3)*K(83))/
(K(81)+K(82)+K(83))
P(91)=(P(3)*P(2)*K(81)+K(82)*P(1)*P(3)+
K(83)*P(2)*P(1))/(K(81)+K(82)+K(83))
P(14)=P(91)-p(2)*P(1)-P(3)*(P(1)+P(2))-
P(9)*(P(9)-P(1)-P(2)-P(3))
P(12)=-(P(2)*P(3)*P(1)-P(9)*P(91))/P(14)
P(10)=-P(12)-(P(9)-P(1)-P(2)-P(3))
P(92)=P(1)+P(2)+P(3)-P(9)
P(11)=(P(92)-(((P(92)**2)-4*P(91))**2)**0.25)/2
P(13)=P(92)-P(11)
P(15)=(P(9)*P(91)-P(11)*(P(9)*P(92)+P(91)-P(1)*P(2)-
P(2)*P(3)-P(1)*P(3))-P(1)*P(2)*P(3))/(P(13)-P(11))
P(16)=P(9)*P(92)+P(91)-P(1)*P(2)-P(2)*P(3)-
P(1)*P(3)-P(15)
P(17)=-P(91)-P(10)*(-P(12))
P(18)=-P(14)/P(9)
P(19)=-P(10)+P(17)/P(12)
P(20)=P(14)/P(19)
P(21)=-P(9)
P(25)=-P(10)+P(14)/P(9)
P(22)=P(17)/P(25)
P(23)=-P(12)
P(24)=-P(17)/P(12)
P(26)=-P(20)-P(9)
P(27)=-P(10)-P(18)-P(24)
P(28)=-P(22)-P(12)
P(32)=(P(16)/P(13))-P(9)
P(29)=P(15)/P(32)
P(30)=-P(11)
P(31)=-P(15)/P(11)
P(36)=(P(15)/P(11))-P(9)
P(33)=P(16)/P(36)
P(34)=-P(13)
P(35)=-P(16)/P(13)
P(37)=-P(31)-P(35)-P(9)
P(38)=-P(11)-P(29)
```

```
  P(39)=-P(33)-P(13)
  P(43)=(P(8)*P(21)*P(25))/(P(10)*P(12)-P(22)*P(25))
  P(41)=(-P(8)*P(21)-P(22)*P(43))/P(10)
  P(42)=(P(8)*P(20)*P(24))/(P(10)*P(12)-P(23)*P(24))
  P(40)=(-P(8)*P(20)-P(23)*P(42))/P(10)
  P(44)=-(P(8)*P(15))/(P(11)*P(30))
  P(45)=-(P(8)*P(15))/(P(11)*P(29))
  P(46)=-P(8)*P(16)/(P(13)*P(34))
  P(47)=-P(8)*P(36)/(P(13))
  P(48)=100*P(40)/P(99)
  P(49)=100*P(41)/P(99)
  P(50)=100*P(42)/P(99)
  P(51)=100*P(43)/P(99)
  P(52)=(P(7)*P(15))/(P(11)**2)
  P(53)=(-P(7)*P(32))/P(11)
  P(54)=(P(7)*P(16))/(P(13)**2)
  P(55)=(-P(7)*P(36))/P(13)
  P(56)=P(7)+P(48)+P(50)
  P(57)=P(7)+P(49)+P(51)
  P(58)=P(7)+P(52)+P(54)
  P(59)=P(7)+P(53)+P(55)
  P(60)=-P(9)*P(8)-(-(P(8)*(K(81)+K(82)+
  K(83)))/(K(81)/P(1)+K(82)/P(2)+K(83)/P(3)))
  P(61)=P(20)*P(8)
  P(62)=P(21)*P(8)
  P(63)=P(42)*P(23)
  P(64)=P(43)*P(22)
  P(65)=-P(12)*P(42)
  P(66)=-P(12)*P(43)
  P(67)=P(8)*P(26)
  P(68)=-(P(8)*P(15))/P(11)
  P(69)=-P(11)*P(44)
  P(70)=-P(11)*P(45)
  P(71)=-(P(8)*P(16))/P(13)
  P(72)=-P(13)*P(46)
  P(73)=-P(13)*P(47)
  P(74)=P(8)*((P(15)/P(11))+(P(16)/P(13))-P(9))
  P(75)=100*P(19)/P(18)-100
  P(76)=100*P(23)/P(22)-100
  P(77)=100*P(30)/P(29)-100
  P(78)=100*P(34)/P(33)-100
  P(79)=100*P(51)/P(50)-100
  P(80)=100*P(57)/P(56)-100
H DAT
c G(1)=K(81)*EXP(-P(1)*T)+K(82)*EXP(-P(2)*T)
x G(1)=K(81)*EXP(-P(4)*T)+K(82)*EXP(-P(5)*T)+
  K(83)*EXP(-(LOG(P(6)))*T)
C Insert data values here
101 G(1)                    fsd=0.02
```

c	1	0.0236105506833713
c	2	0.0189437699316629
c	3	0.0176596810933941
	4	0.0164135785876993
	5	0.015219451025057
	6	0.0148258712984055
	7	0.0141912471526196
	8	0.0136300592255125
	10	0.0130020956719818
	12	0.0125060791571754
	14	0.0117345660592255
	16	0.0113740683371298
	18	0.011039854214123
	20	0.0108753724373576
	23	0.0104664669703872
	26	0.0100775783029613
	30	0.00976265660592255
	35	0.0090578354214123
	40	0.00869623633257404
	50	0.00733793479498861
	60	0.00668911987471526
	70	0.00617657744874715
	90	0.00436399202733485
	110	0.00402324601366743
	130	0.00344093451025057
	150	0.00312921839407745

Figure A.3. (Chapter 3) Three-compartment catenary (top) and mammillary (bottom) models with bounds for parameters of glucose kinetics in horses adapted to fat and fiber (FF, n=6) or sugar and starch (SS, n=6) horses during rest (R) and exercise (E). Parameters bounds are reported as median minimum value to median maximum value with CV in parentheses. Parameters units are min^{-1} unless otherwise indicated. The poor precision of the three-compartment model is demonstrated by the wide ranges and CV for parameters.

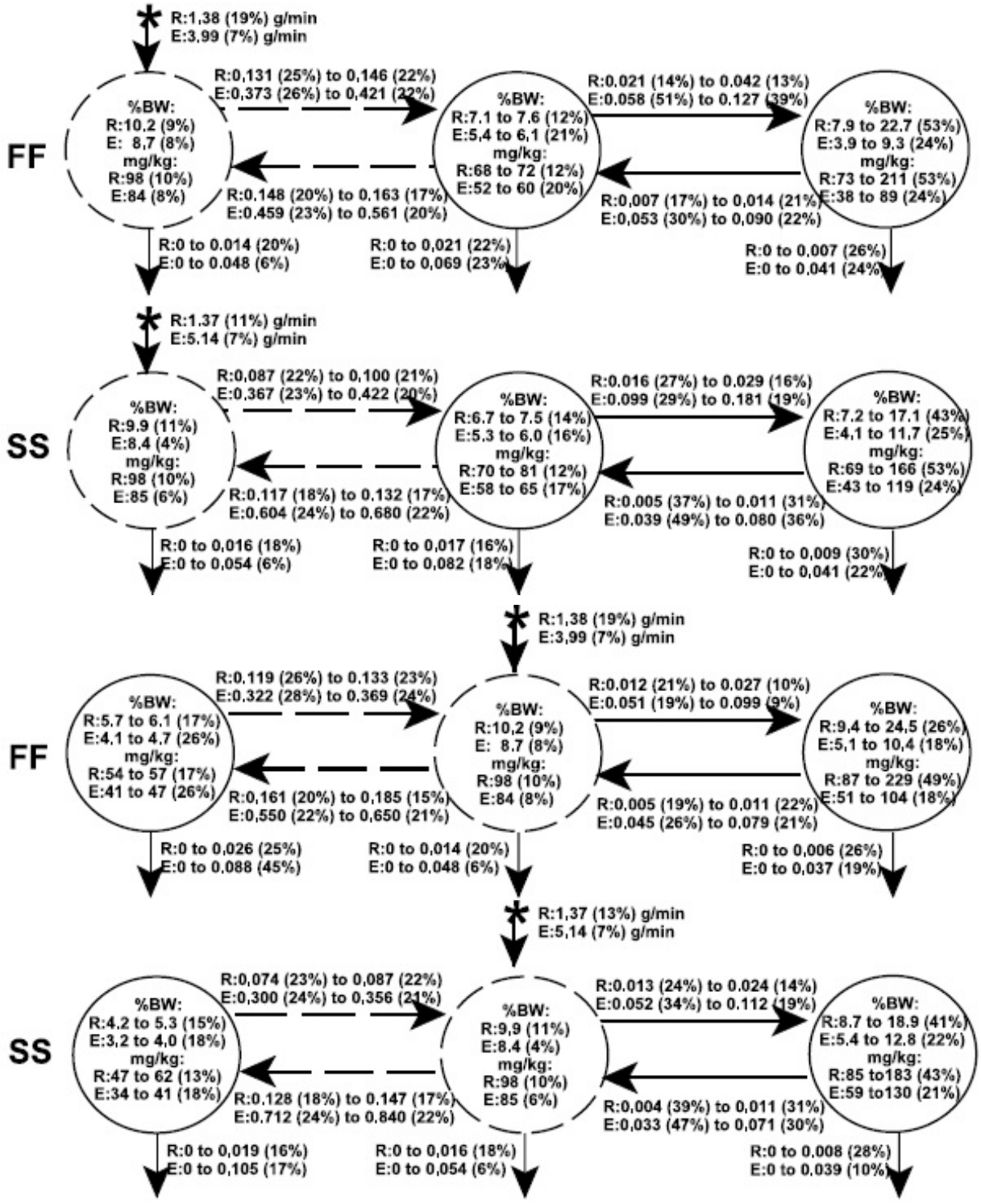


Table A.5 (Chapter 7) Population information, minimal model parameters and glucose and insulin concentrations for individual ponies and study groups undergoing FSIGT tests. The FSIGT involved an iv glucose challenge of 300 mg/kg BW at 0 min and an iv insulin challenge of 0.020 IU/kg BW at 20 min. Groups were either ponies who had never had laminitis (NL) or ponies which had been previously diagnosed with laminitis (PL).

A. Population information and minimal model parameters for ponies

Pony	Breed	Laminitis history	Age, y	Body Condition Score	Minimal Model Parameters AIRg	DI	SI x 10^4	Sg
1	Welsh	NL	5	6.5	420.5	85.0	0.2022	0.0102
2	Dartmoor	NL	6	7.5	506.6	126.3	0.2493	0.0072
3	Welsh	PL	6	7	643.8	70.6	0.1097	0.0073
4	Dartmoor	PL	6	7	686.5	32.6	0.0475	0.0090
5	Welsh	NL	4	6.75	661.6	407.6	0.6160	0.0195
6	Dartmoor	PL	9	6.5	515.1	463.3	0.8995	0.0156
7	Welsh	PL	21	6	1238.1	297.1	0.2399	0.0069
8	Dartmoor	PL	21	7	371.5	15.5	0.0417	0.0155
9	Welsh	NL	4	7	304.1	134.7	0.4428	0.0232
10	Dartmoor	NL	4	7.5	246.0	107.9	0.4388	0.0057
11	Welsh	PL	8	8	1807.4	60.3	0.0334	0.0273
12	Dartmoor	NL	5	8	289.0	174.8	0.6050	0.0126
13	Welsh	PL	7	7	931.0	80.2	0.0861	0.0102
14	Dartmoor	NL	4	7.5	1417.0	231.2	0.1631	0.0117

B. Glucose (mg/dL) for NL ponies during the minimal model									
	Pony #							NL	
Time, min	1	2	12	9	10	14	5	Mean	SEM
-5	118.59	111.15	102.99	110.28	119.05	115.21	113.14	112.92	2.09
0	117.85	115.10	103.71	112.35	121.94	116.11	111.88	114.13	2.16
1	363.01	362.90	379.21	378.82	390.25	407.49	368.47	378.59	6.10
2	337.31	343.41	351.41	351.63	388.01	361.10	353.39	355.18	6.17
3	333.35	307.56	343.30	331.48	357.31	361.34	340.52	339.26	6.78
4	329.69	295.46	332.09	326.29	355.05	339.00	332.12	329.95	6.77
5	324.15	295.07	321.73	337.20	351.64	327.29	326.16	326.17	6.48
6	309.02	294.89	315.24	324.15	346.38	323.37	315.52	318.36	5.98
7	302.93	311.23	310.19	310.17	334.10	310.09		313.12	4.37
8	298.36	295.97	303.00	299.96	330.03	304.50	302.60	304.91	4.33
10	290.67	300.35	296.13	289.29	322.11	293.66	291.51	297.67	4.31
12	285.09	267.61	284.35	282.57	310.65	291.40	281.27	286.13	4.91
14	281.95	272.55	279.56	283.19	302.64	277.29	274.93	281.73	3.76
16	273.61	265.31	277.16	272.59	296.41	283.26	261.17	275.64	4.42
19	269.94	237.20	265.25	266.93	295.74	265.79	249.22	264.29	6.89
22	263.45	243.71	260.29	249.30	291.97	260.93	243.42	259.01	6.33
23	262.69	271.09	258.52	249.44	288.15	247.95	239.58	259.63	6.17
24	258.21	217.41	256.63	240.94	286.34	252.70	235.73	249.70	8.14
25	257.06	267.06	252.09	247.61	285.02	246.17	231.49	255.21	6.44
27	255.80	269.35	243.86	229.43	277.99	240.51	222.59	248.50	7.68
30	245.98	258.57	226.03	219.40	272.07	236.96	208.30	238.18	8.48
35	239.32	239.41	205.47	192.13	259.95	229.78	189.28	222.19	10.17
40	233.50	237.96	187.42	177.72	245.49	220.15	174.24	210.92	11.47
50	223.68	222.25	169.08	157.38	234.68	211.46	154.58	196.16	13.02
60	213.54	206.66	162.25	148.04	231.78	204.19	140.69	186.73	13.51
70	210.08	195.10	152.13	139.74	227.00	194.38	132.85	178.75	13.94
80	202.80	192.00	144.76	134.64	216.14	187.80	125.76	171.98	13.65
100	189.23	167.84	129.62	131.83	207.66	177.96	114.58	159.82	13.17
120	172.03	153.39	121.76	124.36	199.49	161.71	111.47	149.17	11.97
150	153.24	144.20	114.74	121.43	186.77	148.60	105.30	139.18	10.51
180	138.91	121.74	106.25	116.49	168.35	132.44	102.96	126.73	8.49
210	126.71	111.15	102.52	103.56	152.22	111.71	97.49	115.05	7.14
240	118.39	103.74	103.56	101.16	138.20	104.66	96.37	109.44	5.43

C. Glucose (mg/dL) for PL ponies during the minimal model									
	Pony #							PL	
Time, min	3	4	6	7	11	13	8	Mean	SEM
-5	91.16	119.53	108.15	95.41	106.37	109.50	118.39	106.93	4.02
0	93.84	118.39	107.12	109.21	104.02	113.08	126.89	110.36	3.99
1	317.58	374.96	377.51	326.63	338.10	375.22	337.52	349.64	9.65
2	316.50	358.30	359.45	316.37	333.54	350.97	347.07	340.31	6.96
3	302.33	341.06	342.82	306.88	314.81	326.96	343.75	325.51	6.68
4	303.31	330.42	333.57	290.53	298.86	303.05	334.36	313.44	7.04
5	292.13	318.47	343.72	284.07	291.23	311.96	327.33	309.84	8.25
6	289.17	313.81	324.87	276.46	283.77	303.08	317.22	301.20	6.97
7	282.24	309.26	318.07	274.56	275.57	295.50		292.53	7.43
8	272.16	298.65	305.34	265.17	274.81	289.18	313.99	288.47	6.96
10	261.23	293.60	294.08	261.03	262.02	282.28	303.92	279.74	6.89
12	253.04	287.30	286.77	254.02	256.01	272.63	300.01	272.82	7.19
14	247.40	280.01	277.69	249.57	247.54	267.24	276.36	263.68	5.69
16	243.93	277.85	270.76	249.53	246.77	261.65	282.68	261.88	5.92
19	233.77	237.20	261.91	235.10	234.61	259.56	291.54	250.52	8.23
22	219.38	270.01	253.10	235.24	230.57	252.96	287.27	249.79	8.92
23	223.86	268.25	252.06	227.26	229.86	251.58	277.48	247.19	7.94
24	222.93	271.78	249.94	225.14	229.62	248.16	284.49	247.44	8.98
25	223.31	266.90	244.36	220.75	226.33	243.02	283.19	243.98	8.92
27	216.01	261.00	236.30	221.40	223.43	245.52	278.70	240.34	8.70
30	208.07	246.96	226.02	214.74	218.72	237.73	275.98	232.60	8.82
35	205.52	242.44	201.93	203.34	215.35	233.41	265.18	223.88	9.07
40	200.25	240.91	190.61	197.29	209.81	226.93	260.89	218.10	9.77
50	190.23	230.60	159.58	173.95	203.96	222.91	256.76	205.42	12.82
60	185.47	220.67	140.09	162.19	194.83	221.06	250.04	196.33	14.26
70	184.83	215.54	128.78	157.64	190.55	207.09	247.28	190.24	14.68
80	176.58	206.51	118.82	137.08	185.40	199.91	240.56	180.69	15.73
100	171.87	198.40	103.31	121.38	177.28	188.84	240.33	171.63	17.56
120	166.99	190.89	92.28	109.94	169.33	176.77	241.31	163.93	18.88
150	154.70	179.35	82.50	94.87	156.38	166.52	235.01	152.76	19.50
180	132.60	138.91	82.50	89.16	148.55	169.36	231.59	141.81	19.05
210	121.30	126.71	86.16	82.64	139.06	118.80	241.95	130.94	20.11
240	118.90	118.39	94.12	85.00	132.26	112.93	229.33	127.27	18.05

D. Insulin (mIU/L) for NL ponies during the minimal model									
	Pony #							NL	
Time, min	1	2	5	9	10	12	14	Mean	SEM
-15	39.99	43.52	20.45	27.79	21.70	73.23	52.10	39.83	7.12
-5	37.81	53.90	30.39	31.38	22.64	19.28	56.12	35.93	5.43
0	38.90	48.71	25.42	29.58	22.17	19.28	54.11	34.03	5.10
1	84.00	52.60	93.00	64.68	44.34	47.81	142.38	75.54	13.12
2	85.18	84.35	91.04	62.14	46.01	46.47	166.55	83.10	15.58
3	83.63	77.98	92.79	58.49	50.09	48.19	171.53	83.24	16.08
4	80.95	96.01	97.12	56.23	48.24	47.24	209.76	90.79	21.39
5	80.07	105.38	97.25	62.41	54.15	50.37	215.90	95.08	21.64
6	87.64	117.01	103.40	67.56	50.66	51.36	229.13	100.97	23.39
7	88.12	120.35	100.46	62.36	48.91	51.01	233.91	100.73	24.35
8	81.39	127.00	92.64	61.18	46.63	52.88	233.75	99.35	24.68
10	78.36	125.19	91.59	59.53	44.24	50.38	211.31	94.37	22.12
12	75.24	123.36	72.29	57.70	46.51	52.89	189.80	88.26	19.45
14	75.82	125.14	80.22	57.72	45.93	52.56	167.69	86.44	16.81
16	76.93	130.63	73.01	64.24	47.31	49.14	168.91	87.17	17.21
19	71.84	133.77	74.00	63.05	49.61	49.53	138.77	82.94	14.25
22	259.20	268.55	263.07	236.63	276.67	255.08	332.56	270.25	11.40
23	204.93	347.48	226.57	193.67	205.24	213.45	279.65	238.71	21.03
24	174.13	145.84	196.07	167.40	158.14	179.03	249.78	181.48	12.87
25	153.27	267.20	169.21	163.88	147.99	176.50	233.32	187.34	17.04
27	145.14	259.29	152.44	143.79	128.01	146.15	231.62	172.35	19.32
30	130.50	232.71	130.17	116.13	102.08	121.88	199.78	147.61	18.44
35	97.21	243.48	107.43	101.77	78.22	102.21	189.25	131.37	23.00
40	86.20	184.58	95.45	82.95	67.82	83.15	166.54	109.53	17.43
50	90.68	178.81	81.08	72.28	54.67	75.54	153.38	100.92	17.54
60	99.62	176.50	94.74	70.70	51.22	74.33	145.67	101.83	16.79
70	96.37	147.76	88.76	68.07	56.59	69.72	172.74	100.00	16.57
80	111.02	155.44	70.17	68.26	59.51	73.11	170.71	101.17	17.21
100	122 .68	172.23	72.93	69.72	60.64	65.95	195.78	108.56	21.13
120	117.45	178.84	82.15	71.27	68.05	53.04	170.08	105.84	19.25
150	126.18	161.47	36.70	78.36	62.54	43.55	159.87	95.53	20.10
180	100.72	104.44	66.01	50.64	58.88	34.59	152.57	81.12	15.31
210	74.78	85.92	43.79	43.70	61.88	31.74	140.02	68.83	13.87
240	57.79	69.92	29.73	40.22	53.78	22.18	164.36	62.57	18.08

E. Insulin (mIU/L) for PL ponies during the minimal model									
	Pony #							PL	
Time, min	3	4	6	7	8	11	13	Mean	SEM
-15	10.32	50.67	30.73	23.80	39.84	35.73	59.53	35.80	6.21
-5	16.55	52.50	31.43	31.28	43.30	82.20	70.26	46.79	8.78
0	13.44	51.58	31.08	27.54	41.57	58.96	64.89	41.30	6.98
1	86.15	118.48	74.39	126.45	51.79	301.04	144.79	129.01	31.15
2	79.97	117.26	69.56	128.81	52.46	270.57	133.10	121.68	27.44
3	78.37	112.87	76.01	143.23	57.01	248.04	150.01	123.65	24.61
4	73.87	125.61	78.5	138.56	79.84	243.78	151.01	127.31	22.76
5	75.58	128.68	87.11	149.06	84.73	216.19	175.73	131.01	19.96
6	89.2	123.88	97.91	155.75	90.72	253.62	182.00	141.87	22.85
7	86.59	132.21	94.87	184.54	92.07	241.56	182.71	144.94	22.47
8	83.79	128.87	94.53	184.50	104.74	241.00	168.01	143.63	21.53
10	76.01	124.28	90.22	196.89	99.31	231.24	176.11	142.01	22.53
12	73.21	128.39	101.97	191.19	96.29	207.24	173.54	138.83	19.65
14	67.66	130.57	95.65	185.22	102.81	213.79	158.59	136.33	19.77
16	62.78	116.26	99.27	178.96	96.79	215.21	130.20	128.50	19.76
19	56.56	135.26	95.07	185.02	83.72	219.82	160.35	133.68	22.20
22	210.46	353.23	318.79	400.59	282.35	460.6	394.49	345.79	31.50
23	182.40	306.74	256.25	364.12	244.33	435.52	330.84	302.89	31.72
24	156.85	259.01	238.99	308.61	205.90	362.87	199.80	247.43	26.49
25	149.52	260.88	228.03	292.55	205.11	343.58	246.43	246.58	23.51
27	126.11	233.97	216.34	292.53	163.77	348.91	279.32	237.28	29.07
30	111.44	214.41	188.38	272.44	151.01	299.96	247.14	212.11	25.36
35	95.10	190.77	140.37	263.64	122.75	318.31	222.45	193.34	30.42
40	82.11	184.28	142.09	270.75	116.25	301.20	207.15	186.26	30.27
50	74.86	178.18	111.94	297.17	90.44	302.69	194.14	178.49	35.41
60	80.99	160.25	102.25	299.13	73.74	321.35	211.14	178.41	38.62
70	86.87	172.03	104.54	331.13	75.4	338.92	213.76	188.95	41.99
80	92.57	188.29	90.21	306.69	81.69	343.59	242.68	192.25	41.15
100	116.69	209.66	66.36	226.51	110.35	369.81	299.04	199.77	41.48
120	108.02	239.56	48.81	201.98	115.98	377.47	256.74	192.65	42.05
150	92.24	228.31	34.96	122.24	109.38	380.76	247.16	173.58	44.7
180	87.82	224.91	21.90	82.03	132.97	335.77	298.2	169.09	44.91
210	101.17	208.25	19.68	39.27	153.79	330.85	258.14	158.74	43.34
240	77.11	174.50	20.76	21.89	162.95	280.66	157.3	127.88	35.49

Table A.6. (Chapter 8) Data from pasture-kept ponies and pony-crosses collected between 8 and 10 am in March (winter pasture) and May (spring pasture).

	Pony #	1	2	3	4	5	6	7	8	9	10	11	12
	Breed	Dartmoor	Cross	Welsh	Dartmoor	Welsh	Welsh	Welsh	Welsh	Welsh	Welsh	Cross	Dartmoor
	Age, y	5		12	8	6	10	19	18	7	8	21	7
	Gender	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare
	Pregnant March	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
	Lactating May	Yes	No		No	Yes		Yes	Yes	Yes	Yes	No	Yes
	Pregnant May	No	No		No	No		No	No	No	No	Yes	No
	Previous Laminitis	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
March	Body Condition	6.75	5.25	6.5	7	5.5	5.5	6.5	6	6.5	6	6.5	7.5
	Insulin, mIU/L	30.83	13.75	212.38	27.34	7.75	13.18	19.78	20.88	15	16.3	11.37	10.26
	Glucose, mg/dL	102.6	111.88	116.42	107.38	84.15	83.21	100.16	91.66	93.98	85.56	92.81	101.7
	Triglyceride, mg/dL	89.6	37.88	92.27	370.42	162.4	34.13	103.6	47.32	152.99	101.07	82.37	94.46
	Cortisol, mIU/L	3.1	5.71	6.63	2.92	6.06	9.12	3.74	5.13	3.64	4.37	4.29	3.54
	Non-esterified fatty acids, mEq/L	0.54	0.72	0.3	0.41	0.58	0.24	0.39	0.17	0.5	0.84	0.57	0.72
	RISQI	0.18	0.27	0.07	0.19	0.36	0.28	0.22	0.22	0.26	0.25	0.30	0.31
	MIRG	9.5	4.96	-82.27	8.35	4.88	7.39	7.50	8.85	6.76	8.27	5.61	4.55
May	Body Condition	6.5				6.5		6.5	5.25	6.5	6	5	7
	Insulin, mIU/L	18.26	7.98		1.43	2.58		9.15	1.14	5.06	7.13	33.9	5.9
	Glucose, mg/dL	89.57	96.54		86.36	79.9		92.75	77.12	84.65	87.87	100.64	97.97
	Triglyceride, mg/dL	27.84	31.79		20.96	23.1		26.21	22.86	25.11	19.66	46.18	30.69
	Cortisol, mIU/L	2.4	5.89		8.38	9		8.14	7.15	7.39	6.97	8.7	3.22
	Non-esterified fatty acids, mEq/L	0.28	0.23		0.25	0.11		0.07	0.13	0.2	0.06	0.13	0.38
	RISQI	0.23	0.35		0.84	0.62		0.33	0.94	0.44	0.37	0.17	0.41
	MIRG	8.36	4.06		1.64	2.51		4.77	1.78	3.55	4.3	10.22	3.19
Criteria Passed	Body Condition	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
	Triglyceride	Yes	No	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes
	RISQI	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	MIRG	Yes	No	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No
	Total # of criteria passed	4	1	3	4	1	2	4	3	4	4	4	3
	PLMS	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes
	Hirsutism	No	No	No	No	No	No	No	No	No	No	No	No

	Pony #	13	14	15	16	17	18	19	20	21	22	23	24
	Breed	Welsh	Dartmoor	Welsh	Welsh	Welsh	Welsh	Cross	Welsh	Connemara	Dartmoor	Dartmoor	Welsh
	Age, y	10	9	8	20	9	15	6	25	13	20	18	6
	Gender	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare
	Pregnant March	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes
	Lactating May	Yes	No	Yes	Yes	Yes	Yes	Yes			No	No	Yes
	Pregnant May	No	No	No	No	No	No	No			No	No	No
	Previous Laminitis	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes
March	Body Condition	7	8	6.75	6.5	7	7.75	5.5	3.5	6	6.75	6.5	6.25
	Insulin, mIU/L	4.51	15.15	18.82	15.51	13.39	11.28	9.91	26.62	3.12	11.61	4.21	14.15
	Glucose, mg/dL	80.14	95.21	90.4	85.75	103.37	95.7	109.22	117.63	94.7	84.94	73.15	92.52
	Triglyceride, mg/dL	188.71	64.29	83.24	154.06	83.21	143.15	90.29	39.36	60.17	117.5	737.01	90.6
	Cortisol, mIU/L	4.05	3.6	3.81	2.68	3.99	5.13	4.67	3.07	4.99	1.5	2.61	5.3
	Non-esterified fatty acids, mEq/L	0.91	0.4	0.75	0.83	0.94	0.91	0.25	0.13	0.58	0.6	1.07	1.14
	RISQI	0.47	0.26	0.23	0.25	0.27	0.30	0.32	0.19	0.57	0.29	0.49	0.27
	MIRG	3.57	6.68	8.42	7.95	5.42	5.33	4.01	7.26	2.17	6.51	3.96	6.63
May	Body Condition	7	7.5	6.5	5.5	7	7.5	6.25			6		6.75
	Insulin, mIU/L	17.19	64.1	15.08	7.7	4.42	24.54	0			7.08	5.11	5.34
	Glucose, mg/dL	88.88	101	86.38	90.07	84.58	93.39	88.46			97.16	80.87	79.6
	Triglyceride, mg/dL	24.95	95.99	24.76	19.05	22.78	17.98	16.71			56.64	16.18	21.08
	Cortisol, mIU/L	11.73	14.11	5.86	5.49	7.7	10.09	9.12			7.57	5.2	5.03
	Non-esterified fatty acids, mEq/L	0.2	0.11	0.06	0.14	0.1	0.1	0.1			0.61	0.2	0.11
	RISQI	0.24	0.12	0.26	0.36	0.48	0.20				0.38	0.44	0.43
	MIRG	8.10	10.43	7.70	4.38	3.24	9.55	0.86			3.68	3.84	4.07
Criteria Passed	Body Condition	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes
	Triglyceride	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
	RISQI	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes
	MIRG	No	Yes	Yes	Yes	No	No	No	Yes	No	Yes	No	Yes
	Total # of criteria passed	2	4	4	4	3	3	2	2	2	4	2	4
	PLMS	No	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	No	Yes
	Hirsutism	No	No	No	No	No	No	No	No	No	No	No	No

	Pony #	25	26	27	28	29	30	31	32	33	34	35	36
	Breed	Welsh	Dartmoor	Welsh	Cross	Cross	Welsh	Welsh	Welsh	Dartmoor	Welsh	Welsh	Welsh
	Age, y	8	12	7	16	8	9	21	6	9	13	24	18
	Gender	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare	Mare
	Pregnant March	Yes	No	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes
	Lactating May	Yes	No	No	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes
	Pregnant May	No	No	No	No	No	No	No	No	No	No	No	No
	Previous Laminitis	Yes	Yes	Yes (CL)	Yes	No	Yes	Yes (CL)	Yes	Yes	Yes	Yes	No
March	Body Condition	6.5	7.25	6.5	4.5	4.5	6.25	6.5	5.75	7	7.25	5.75	5.75
	Insulin, mIU/L	8.46	12.26	37.16	6.3	3.45	17.07	51.65	19.75	11.4	57.3	17.2	39.43
	Glucose, mg/dL	88.6	85.3	102.96	89.44	87.87	103.6	111.6	97.5	104.56	108.1	97.3	104.03
	Triglyceride, mg/dL	380.33	54.01	194.43	258.95	78.75	257.2	58.16	44.8	28.18	47.33	82.3	41.53
	Cortisol, mIU/L	4.09	5.98	5.45	2.21	4.79	6.21	5.13	6.88	5.44	3.36	4.77	4.87
	Non-esterified fatty acids, mEq/L	0.56	0.26	0.43	0.89	0.62	0.72	0.65	0.64	1.34	0.47	0.61	0.49
	RISQI	0.34	0.29	0.16	0.40	0.54	0.24	0.14	0.23	0.3	0.13	0.24	0.16
	MIRG	4.82	6.74	10.29	3.82	2.59	6.45	9.79	7.79	4.73	10.04	7.10	10.35
May	Body Condition	6.5	7.5	6.5	4.25	4.75	6.75	6.5	6.5	7	7	6.5	5.5
	Insulin, mIU/L	12.4	165.46	73.66	0.58	4.12	0.9	224.21	51	11.72	13.36	46.2	4.19
	Glucose, mg/dL	82.52	99.3	98.11	82.85	77.7	73.99	145.81	92.95	90.44	83.44	89.52	71.12
	Triglyceride, mg/dL	29.26	87.06	83.33	18.26	14.18	27.57	74.12	79.29	51.69	31.88	98.62	21.43
	Cortisol, mIU/L	5.46	18.36	8.3	3.27	10	3.49	9.98	13.72	7.42	4.44	10.07	3.88
	Non-esterified fatty acids, mEq/L	0.03	0.34	0.12	0.1	0.16	0.14	0.04	0.07	0.17	0 .13	0.1	0.3
	RISQI	0.28	0.08	0.12	1.31	0.49	1.05	0.07	0.14	0.29	0.27	0.15	0.49
	MIRG	7.16	-46.17	9.28	1.27	3.53	1.74	-71.71	12.70	5.96	7.43	13.37	4.14
Criteria Passed	Body Condition	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	No	No
	Triglyceride	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes	No
	RISQI	No	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	MIRG	No	Yes	Yes	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes
	Total # of criteria passed	2	3	4	1	1	4	4	2	2	3	3	2
	PLMS	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes	Yes	No
	Hirsutism	No	No	No	No	No	No	No	No	No	No	No	No

	Pony #	37	38	39	40	41	42	43	44	45	46	47	48
	Breed	Dartmoor		Welsh	Cross	Welsh	Dartmoor	Cross	Cross	Dartmoor	Cross	Welsh	Dartmoor
	Age, y	5	6	17	2	8	7	3	18	16	3	3	3
	Gender	Mare	Mare	Mare	Gelding	Mare	Mare	Mare	Mare	Mare	Mare	Gelding	Mare
	Pregnant March	Yes	No	No		No	No	No	No	No	No		No
	Lactating May	Yes		No		No	No	No		No	No		No
	Pregnant May	No		No		No	No	No		No	No		No
	Previous Laminitis	Yes	Yes	Yes (CL)	No	Yes	No	No	No	Yes (CL)	No	No	No
March	Body Condition	6.5	6.5	7.5	5	6	7.25	5	5.5	7	5	7	6
	Insulin, mIU/L	31.05	61.82	9.8	7.65	14.64	8.86	3.26	16.2	27.89	15.59	47.39	18.04
	Glucose, mg/dL	108.82	113.69	84.34	100.17	99.2	96.72	89.73	87.5	96.72	100.17	107.62	114.73
	Triglyceride, mg/dL	205.98	66.46	29.31	281.05	92.29	203.45	49.65	39.88	88.41	21.82	44.66	108.4
	Cortisol, mIU/L	3.11	6.35	4.73	5.84	6.04	3.01	5.78	6.52	3.98	3.9	6.36	7.43
	Non-esterified fatty acids, mEq/L	0.5	0.13	0.16	0.31	0.24	0.31	0.5	0.48	0.58	0.13	0.37	0.55
	RISQI	0.18	0.13	0.32	0.36	0.26	0.34	0.55	0.25	0.19	0.25	0.15	0.24
	MIRG	8.78	9.06	5.80	3.73	6.14	4.38	2.42	7.95	9.79	6.34	10.28	5.83
May	Body Condition	6.5		7.5		7	7.5	6	6	6.5	6	6.5	7
	Insulin, mIU/L	12.71		52.58	1.94	20.84	12.17	11.08		190.61	15.86	19.27	32.04
	Glucose, mg/dL	89.04		86.31	88.58	96.85	92.23	95.08		80.9	93.27	93.69	114.87
	Triglyceride, mg/dL	64.39		48.4	24.84	29.14	70.94	35.74		69.85	41.8	32.5	88.44
	Cortisol, mIU/L	5.29		14.03	14.4	11.03	6.52	8.42		15.58	10.81	7.94	16.82
	Non-esterified fatty acids, mEq/L	0.24		0.16	0.06	0.05	0.18	0.04		0.06	0.05	0.06	0.05
	RISQI	0.28		0.14	0.72	0.22	0.29	0.30		0.07	0.25	0.23	0.18
	MIRG	6.48		14.17	1.83	8.15	5.96	5.31		-100.81	7.12	8.11	8.29
Criteria Passed	Body Condition	Yes	Yes	Yes	No	Yes	Yes	No	No	Yes	No	Yes	Yes
	Triglyceride	Yes	Yes	No	Yes	Yes	Yes	No	No	Yes	No	No	Yes
	RISQI	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes
	MIRG	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes
	Total # of criteria passed	4	4	3	1	4	2	0	2	4	2	3	4
	PLMS	Yes	Yes	Yes	No	Yes	No	No	No	Yes	No	Yes	Yes
	Hirsutism	No	No	Yes	No	No	No	No	No	No	No	No	No

	Pony #	49	50	51	52	53	54	55	56	57	58	60	61
	Breed	Welsh	Cross	Welsh	Welsh	Welsh	Welsh	Cross	Welsh	Welsh	Welsh	Welsh	Welsh
	Age, y	6	4	9	6	5		3	12	20	4	3	3
	Gender	Mare	Mare	Mare	Mare	Gelding	Mare	Mare	Mare	Mare	Mare	Gelding	Gelding
	Pregnant March	No	No	No	No		No	No	No	No	No		
	Lactating May	No	No	No	No		No	No	No	No			
	Pregnant May	No	No	No	No		No	No	No	No			
	Previous Laminitis	Yes	No	Yes	Yes (CL)	No	No	No	Yes (CL)	Yes (CL)	No	No	No
March	Body Condition	6	5	7.5	7.75	7.25	6	4.5	6.75	6	5.5	5.5	5.5
	Insulin, mIU/L	18.53	8.03	48.06	38.64	29.16	17.69	15.33	52.12	66.71	20.82	8.06	7.32
	Glucose, mg/dL	98.63	89.78	90.33	98.91	93.21	103.35	90.54	89.13	98.79	87.42	84.14	92.54
	Triglyceride, mg/dL	63.57	37.05	57.62	41.64	61.99	48.42	28.82	39.9	47.75	41.81	87.72	111.28
	Cortisol, mIU/L	9.54	5.04	6.75	2.96	5.5	5.89	5.22	5.4	9.43	3.39	4.37	7.73
	Non-esterified fatty acids, mEq/L	0.28	0.58	0.27	0.23	0.27	0.14	0.25	0.18		0.56	0.42	0.54
	RISQI	0.23	0.35	0.14	0.16	0.19	0.24	0.26	0.14	0.12	0.22	0.35	0.37
	MIRG	7.33	4.54	13.24	11.05	10.59	6.64	7.26	13.51	10.41	9.48	5.03	4.05
May	Body Condition	7	6	7	7			6.25	6.5	6.25	6.75	6	6.5
	Insulin, mIU/L	28.16	22.86	28.34	157.08		5.49	15.93	39.97	39.38		24.49	20.31
	Glucose, mg/dL	101.07	100.79	95.76	92.08		94.12	98.2	88.26	89.09	97.37	86.57	93.92
	Triglyceride, mg/dL	57.77	34.77	20.2	78.03		32.62	21.93	32.92	38.8	51.67	74.73	61.79
	Cortisol, mIU/L	16.86	16.77	13.34	9.19		12	14.6	11.98	5.78		6.27	9.29
	Non-esterified fatty acids, mEq/L	0.17	0.04	0.24	0.08		0.07	0.04	0.15	0.15	0.03	0.04	0.04
	RISQI	0.19	0.21	0.19	0.08		0.43	0.25	0.16	0.16		0.20	0.22
	MIRG	9.24	8.18	10.03	-42.52		3.21	6.62	13.21	12.97		10.69	8.38
Criteria Passed	Body Condition	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	No
	Triglyceride	Yes	No	Yes	No	Yes	No	No	No	No	No	Yes	Yes
	RISQI	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
	MIRG	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
	Total # of criteria passed	4	0	4	3	4	3	2	3	3	2	1	1
	PLMS	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	No	No	No
	Hirsutism	No	No	No	No	No	No	No	No	No	No	No	No

	Pony #	62	63	64	65	66	67	68	69	70	71	72
	Breed	Dartmoor	Dartmoor	Dartmoor	Welsh	Welsh	Cross	Welsh	Cross	Welsh	Welsh	Dartmoor
	Age, y	4	3	4	3	26	27	18	3	6	3	5
	Gender	Gelding	Mare	Gelding	Gelding	Mare	Mare	Mare	Gelding	Mare	Mare	Mare
	Pregnant March		No			No	No	No		No	No	No
	Lactating May		No			No	No	No		No	No	No
	Pregnant May		No			No	No	No		No	No	No
	Previous Laminitis	No	No	No	No	Yes (CL)	Yes	No	No	Yes (CL)	No	Yes (CL)
March	Body Condition	7	5.5	6.75	6	5.5	5.25	7.25	5	7	6.5	7
	Insulin, mIU/L	11.83	3.51	8.7	20.83	5.51	9.97	17.77	3.59	13.88	5.54	28.95
	Glucose, mg/dL	97.5	90.72	96.19	87.97	83.67	102.88	95.76	85.13	90.35	86.02	110.38
	Triglyceride, mg/dL	37.53	103.55	47.73	21.97	104.74	29.82	43.72	26.95	40.65	60.26	49.47
	Cortisol, mIU/L	3.5	4.6	2.33	8.19	2.77	6.02	5.06	6.13	8.93	5.43	5.23
	Non-esterified fatty acids, mEq/L	0.42	0.39	0.41	0.33	0.26	0.63	0.48	0.28	0.35	0.45	0.74
	RISQI	0.29	0.53	0.34	0.22	0.43	0.32	0.24	0.53	0.27	0.42	0.19
	MIRG	5.38	2.50	4.36	9.40	3.84	4.38	7.43	2.79	6.77	3.69	8.30
May	Body Condition	6.75		6.75	6.25	6	6	6.75	5.5	6.5	6.75	7
	Insulin, mIU/L	29.72	8.89	11.76	16.32	75.14	52.9	83.01	4.75	19.96	20.2	79.55
	Glucose, mg/dL	96.05	87.21	91.91	87.45	92.24	92.94	85.57	94.51	87.13	90.05	103.52
	Triglyceride, mg/dL	69.14	69.78	55.48	62.67	51.38	1 5.2	7 0.9	14.35	32.76	45.08	82.1
	Cortisol, mIU/L	7.83	8.74	4.98	8.9	7.22	18.65	13.03	10.99	11	9.36	11.94
	Non-esterified fatty acids, mEq/L	0.04	0.04	0.03	0.06	0.05	0.02	0.06	0.28	0.36	0.05	0.14
	RISQI	0.18	0.34	0.29	0.25	0.12	0.14	0.11	0.46	0.22	0.22	0.11
	MIRG	10.24	5.12	5.84	8.00	9.81	12.67	8.51	2.88	9.26	8.89	7.32
Criteria Passed	Body Condition	Yes	No	Yes	Yes	No	No	Yes	No	Yes	Yes	Yes
	Triglyceride	No	Yes	No	No	Yes	No	No	No	No	Yes	No
	RISQI	Yes	No	No	Yes	No	Yes	Yes	No	Yes	No	Yes
	MIRG	No	No	No	Yes	No	No	Yes	No	Yes	No	Yes
	Total # of criteria passed	2	1	1	3	1	1	3	0	3	2	3
	PLMS	No	No	No	Yes	No	No	Yes	No	Yes	No	Yes
	Hirsutism	No	No	No	No	No	No	No	No	No	No	No

	Pony #	73	74	75	76	77	78	79	80	81	82	83	84
	Breed	Welsh	Welsh	Cross	Cross	Welsh	Cross	Welsh	Welsh	Cross	Welsh	Welsh	Welsh
	Age, y	6		3	5	6	22	3	6	5	7	4	21
	Gender	Mare	Mare	Mare	Mare	Mare	Mare	Gelding	Gelding	Mare	Mare	Gelding	Mare
	Pregnant March	No	No	No	No	Yes	No			No	No		No
	Lactating May	No		No	No	Yes	No			No	No		No
	Pregnant May	No		No	No	No	No			No	No		No
	Previous Laminitis	Yes	No	No	No	Yes	No	No	No	No	Yes (CL)	No	Yes (CL)
March	Body Condition	6.5	5	5.5	5.5	5	5.75	5.5	7.75	6	7.25	5	5.75
	Insulin, mIU/L	34.35	40.13	5.33	9.02	6.32	5.41	5.78	16.14	6.94	11.89	11.13	7.44
	Glucose, mg/dL	95.37	86.2	91.1	91.42	94.35	90.32	83.71	93.61	91.97	84.18	89.54	82.74
	Triglyceride, mg/dL	40.92	38.98	18.96	24.65	111.89	106.96	42.49	39.68	43.34	30.72	44.21	59.26
	Cortisol, mIU/L	5.84	2.77	8.89	5.78	8.45	6.04	8.68	8.73	13.15	4.75	10.47	6.14
	Non-esterified fatty acids, mEq/L	0.32	0.22	0.3	0.32	0.55	0.57	0.46	0.52	0.79	0.41	0.56	0.36
	RISQI	0.17	0.16	0.43	0.33	0.40	0.43	0.42	0.25	0.38	0.29	0.30	0.37
	MIRG	11.11	13.71	3.30	4.82	3.54	3.37	3.97	7.17	3.93	6.72	5.82	4.87
May	Body Condition	7.5		6	6.5	5.5	6.5		7.75	6	7.5	6	6.5
	Insulin, mIU/L	44.84		16.8	21.69	19.55	24.6		21.47	6.99	32.99	17.54	298.74
	Glucose, mg/dL	100.89		94.2	95.27	84.9	94.58		86.33	92.95	87.07	92.39	191.23
	Triglyceride, mg/dL	51.43		17.52	43.04	33.99	49.8		52.88	56.03	75.73	65.81	67.93
	Cortisol, mIU/L	12.13		8.68	16.28	9.45	11.85		11.45	11.04	11.63	10.23	10.32
	Non-esterified fatty acids, mEq/L	0.09		0.04	0.05	0.09	0.04		0.08	0.08	0.06	0.04	0.16
	RISQI	0.15		0.24	0.21	0.23	0.20		0.22	0.38	0.17	0.24	0.06
	MIRG	11.17		7.31	8.57	9.51	9.39		9.87	3.89	12.50	7.76	-110.16
Criteria Passed	Body Condition	Yes	No	No	No	No	No	No	Yes	Yes	Yes	No	No
	Triglyceride	No	No	No	No	Yes	Yes	No	No	No	No	No	Yes
	RISQI	Yes	Yes	No	No	No	No	No	Yes	No	Yes	Yes	No
	MIRG	Yes	Yes	No	No	No	No	No	Yes	No	Yes	Yes	No
	Total # of criteria passed	3	2	0	0	1	1	0	3	1	3	2	1
	PLMS	Yes	No	No	No	No	No	No	Yes	No	Yes	No	No
	Hirsutism	No	Yes	No	No	No	No	No	No	No	No	No	No

	Pony #	85	86	87	88	89	90	91	92	93	94	95	96
	Breed	Welsh	Cross	Welsh	Dartmoor	Welsh	Welsh	Welsh	Welsh	Welsh	Cross	Welsh	Welsh
	Age, y	5	3	7	3	4	28	3	4	4	4	4	4
	Gender	Mare	Gelding	Mare	Mare	Gelding	Mare	Gelding	Mare	Mare	Mare	Mare	Gelding
	Pregnant March	No		No	No		No		No	No	No	No	
	Lactating May	No		No	No		No		No	No		No	
	Pregnant May	No		No	No		No		No	No		No	
	Previous Laminitis	Yes	No	Yes	No	No	Yes	No	No	No	No	No	No
March	Body Condition	7	5	7.26	6.75	6.5	6.5	6.25	6.5	6.75	6	5.5	6.25
	Insulin, mIU/L	13.67	3.12	12.29	6.74	18.55	19.68	6.67	13.88	38.6	7.98	13.44	3.15
	Glucose, mg/dL	86.35	94.05	91.18	107.87	89.91	94.59	87.47	90.74	120.01	90.82	97.31	83.61
	Triglyceride, mg/dL	82.69	41.77	35.55	70.09	29.58	104.58	50.85	90.41	67.54	46.55	23.23	32.56
	Cortisol, mIU/L	9.6	14.08	6.06	5.79	7.38	4.92	6.24	8.15	7.26	9.25	11	6.62
	Non-esterified fatty acids, mEq/L	0.53	0.74	0 .45	0.59	33.84	0.31	0.25	0.55	0.25	1.05	0.75	0.5
	RISQI	0.27	0.57	0.29	0.39	0.23	0.23	0.39	0.27	0.16	0.35	0.27	0.56
	MIRG	7.17	2.20	6.10	3.06	8.40	8.12	4.12	6.73	8.45	4.44	5.93	2.64
May	Body Condition	7.75	6	7.5	6.5	6.75	6.5	6.5	7	6.75			7
	Insulin, mIU/L	50.31	1.98	44	19.24	38.47	59.95	25.36	46.74	18.45		23.18	7.42
	Glucose, mg/dL	89.26	92.29	93.95	90.81	89.95	92.89	93.24	98.88	96.23		100.05	89.57
	Triglyceride, mg/dL	84.15	38.55	54.25	48.69	41.62	88.93	85.55	54.48	101.67		54.8	44.51
	Cortisol, mIU/L	16.61	16.62	11.98	14.76	8.46	19.31	11.48	10.77	8.99		11.45	12.48
	Non-esterified fatty acids, mEq/L	0.13	0.07	0.08	0.04	0.05	0.04	0.05	0.11	0.16		0.05	0.04
	RISQI	0.14	0.71	0.15	0.23	0.16	0.13	0.20	0.15	0.23		0.21	0.37
	MIRG	13.50	1.74	12.34	8.49	12.68	12.25	9.77	11.57	7.57		8.34	4.30
Criteria Passed	Body Condition	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
	Triglyceride	Yes	No	No	Yes	No	Yes	No	Yes	Yes	No	No	No
	RISQI	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	No
	MIRG	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes	No	Yes	No
	Total # of criteria passed	4	0	3	2	3	4	1	4	4	1	2	1
	PLMS	Yes	No	Yes	No	Yes	Yes	No	Yes	Yes	No	No	No
	Hirsutism	No	No	No	No	No	Yes	No	No	No	No	No	No

	Pony #	97	98	99	100	101	102	103	104	105	106	107
	Breed	Welsh	Cross	Dartmoor	Cross	Dartmoor	Dartmoor	Dartmoor	Cross	Welsh	Welsh	Dartmoor
	Age, y	3	3	5	10	7	5	3	27	26	32	27
	Gender	Gelding	Gelding	Mare	Mare	Gelding	Gelding	Gelding	Mare	Mare	Mare	Mare
	Pregnant March			No	No				No	No	No	No
	Lactating May			No	No				No	No	No	
	Pregnant May			No	No				No	No	No	
	Previous Laminitis	No	No	No	No	No	No	No	No	Yes	Yes	No
March	Body Condition	4.75	5	6.75	6	7.26	7.5	7	6.5	5.75	6.75	5
	Insulin, mIU/L	8.98	2.87	19.15	8.39	14.45	15.07	5.62	17.4	15.29	9.82	3.24
	Glucose, mg/dL	90.13	91.03	111.66	57.23	99.62	95.47	94.95	94.25	89.91	85.92	100.51
	Triglyceride, mg/dL	19.89	27.06	56.85	18.78	69.03	36.87	41.77	20.49	25.44	16.67	20.96
	Cortisol, mIU/L	8.21	5.87	8.97	8.68	4.33	6.96	7.09	9.02	8.6	9.63	4.48
	Non-esterified fatty acids, mEq/L	0.44	0.48	0.91	0.74	0.81	0.87	0.89	0.72	0.67	0.74	0.62
	RISQI	0.33	0.59	0.23	0.35	0.26	0.26	0.42	0.24	0.26	0.32	0.56
	MIRG	4.91	2.19	6.30	10.30	6.05	6.63	3.22	7.49	7.32	5.65	2.04
May	Body Condition	5.5	6	7	6.25	7.5	7.75	6.75	7	6	6.5	
	Insulin, mIU/L	21.27	5.25	12.93	15.46	36.08	41.18	9.3	14.66	36.28	6.79	
	Glucose, mg/dL	95.83	96.31	91.42	103.55	97.24	103.42	107.13	96.04	88.16	81.82	
	Triglyceride, mg/dL	54.24	22.94	76.14	22.29	71.64	68.12	48.64	41.68	45.73	34.19	
	Cortisol, mIU/L	9.95	15.4	8.38	16.21	14.57	9.82	9.29	16.19	9.67	9.55	
	Non-esterified fatty acids, mEq/L	0.08	0.17	0.05	0.06	0.12	0.1	0.05	0.04	0.07	0.13	
	RISQI	0.22	0.44	0.28	0.25	0.17	0.16	0.33	0.26	0.17	0.38	
	MIRG	8.39	3.00	6.31	6.01	11.03	10.58	3.93	6.44	12.78	4.63	
Criteria Passed	Body Condition	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No
	Triglyceride	No	No	No	No	Yes	No	No	No	No	No	No
	RISQI	No	No	Yes	No	Yes	Yes	No	Yes	Yes	Yes	No
	MIRG	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No
	Total # of criteria passed	0	0	3	2	4	3	1	3	2	3	0
	PLMS	No	No	Yes	No	Yes	Yes	No	Yes	No	Yes	No
	Hirsutism	No	No	No	No	No	No	No	No	No	No	No

	Pony #	108	109	110	111	112	113	114	115	116	117	118
	Breed	Cross	Dartmoor	Welsh	Dartmoor	Dartmoor	Welsh	Welsh	Welsh	Welsh	Welsh	Dartmoor
	Age, y	3	23	3	10	4	4	3	6	7	4	4
	Gender	Gelding	Mare	Stallion	Stallion	Stallion	Stallion	Stallion	Stallion	Stallion	Stallion	Stallion
	Pregnant March		No									
	Lactating May		No									
	Pregnant May		No									
	Previous Laminitis	No	Yes (CL)	No	No	No	No	No	No	No	No	No
March	Body Condition	5.5	4.5	5.5	6.75	6.75	6.25	6	7	6	7	6.5
	Insulin, mIU/L	14.73	62.96	6.12	22.27	8.02	7.74	6.01	14.9	8.97	19.11	5.85
	Glucose, mg/dL	89.01	92.13	91.33	102.77	98.05	97.07	100.7	99.95	87.74	99.15	98.05
	Triglyceride, mg/dL	23.54	65.64	23.2	52.47	19.65	41.84	34.53	80.29	58.95	40.21	64.44
	Cortisol, mIU/L	8.88	2.27	5.05	5.63	8.67	7.66	6.98	6.59	4.29	7.37	4.68
	Non-esterified fatty acids, mEq/L	0.53	0.46	0.66	0.5	0.67	0.69	0.6	0.61	0.56	0.7	0.61
	RISQI	0.26	0.13	0.40	0.21	0.35	0.36	0.41	0.26	0.33	0.23	0.41
	MIRG	7.23	12.07	3.63	7.82	3.99	3.94	3.10	6.15	5.11	7.43	3.16
May	Body Condition	5.75	5.5		7.25					6.5		
	Insulin, mIU/L	11.37	63.58		17.97	2.58	2.34			14.84		
	Glucose, mg/dL	86.52	85.79		117.32	102.75	102.46			84.42		
	Triglyceride, mg/dL	37.3	78.79		71.93	20.13	17.53			29.07		
	Cortisol, mIU/L	5.89	5.74		5.2	10.17	6.02			4.75		
	Non-esterified fatty acids, mEq/L	0.04	0.08		0.79	0.48	0.48			0.03		
	RISQI	0.30	0.13		0.24	0.62	0.65			0.26		
	MIRG	6.23	13.35		5.64	1.72	1.64			7.89		
Criteria Passed	Body Condition	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Triglyceride	No	Yes	No	No	No	No	No	Yes	Yes	No	Yes
	RISQI	Yes	Yes	No	Yes	No	No	No	Yes	No	Yes	No
	MIRG	Yes	Yes	No	Yes	No	No	No	Yes	No	Yes	No
	Total # of criteria passed	2	3	0	3	1	1	1	4	2	3	2
	PLMS	No	Yes	No	Yes	No	No	No	Yes	No	Yes	No
	Hirsutism	No	Yes	No	No	No	No	No	No	No	No	No

	Pony #	119	120	121	122	123	124	125	126	127	128	129
	Breed	Welsh	Welsh	Dartmoor	Welsh	Welsh	Welsh	Welsh	Welsh	Dartmoor	Cross	Welsh
	Age, y	24	5	20	21	5	17	25	20	8	2	2
	Gender	Stallion	Stallion	Stallion	Stallion	Stallion	Stallion	Stallion	Stallion	Stallion	Gelding	Gelding
	Pregnant March											
	Lactating May											
	Pregnant May											
	Previous Laminitis	No	No	No	Yes	No	No	No	No	No	No	No
March	Body Condition	5.5	6	4.5	7	7	6.5	5.5	6	5.75	5	5
	Insulin, mIU/L	7.81	15.21	2.72	22.84	18.3	10.69	14.94	17.96	6.45	5.67	18.03
	Glucose, mg/dL	82.56	97.8	86.28	95.57	93.88	90.97	81.24	89.52	103.03	97.98	108.51
	Triglyceride, mg/dL	29.16	46.11	31.39	66.39	62.05	56.66	18.46	22.29	22.33	48.66	36.7
	Cortisol, mIU/L	7.4	10.27	2.03	7.36	6.68	4.87	4.69	8.48	5.83	7.47	11.7
	Non-esterified fatty acids, mEq/L	0.69	0.84	0.61	0.59	0.57	0.66	0.69	0.68	0.69	0.59	0.67
	RISQI	0.36	0.26	0.61	0.21	0.23	0.31	0.26	0.24	0.39	0.42	0.24
	MIRG	5.06	6.44	2.30	8.83	7.80	5.52	8.42	8.27	3.16	3.10	6.28
May	Body Condition				7	7.5		3			5.5	6
	Insulin, mIU/L				23.75	16.09	9.16	0.52			0.5	9.7
	Glucose, mg/dL				102.66	91.93	94.56	80.17			87	100.82
	Triglyceride, mg/dL				70.59	27	28.35	14.57			23.34	29.8
	Cortisol, mIU/L				9.89	8.79	3.66	3.48			13.05	16.73
	Non-esterified fatty acids, mEq/L				0.66	0.1	0.26	0.19			0.16	0.05
	RISQI				0.21	0.25	0.33	1.39			1.41	0.32
	MIRG				8.17	7.35	4.64	1.31			1.14	4.42
Criteria Passed	Body Condition	No	Yes	No	Yes	Yes	Yes	No	Yes	No	No	No
	Triglyceride	No	No	No	Yes	Yes	No	No	No	No	No	No
	RISQI	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	No	Yes
	MIRG	No	Yes	No	Yes	Yes	No	Yes	Yes	No	No	Yes
	Total # of criteria passed	0	3	0	4	4	2	2	3	0	0	2
	PLMS	No	Yes	No	Yes	Yes	No	No	Yes	No	No	No
	Hirsutism	No	No	No	No	No	No	No	No	No	No	No

	Pony #	130	131	132	133	134	135	136	137	138	139	140
	Breed	Welsh	Dartmoor	Welsh	Dartmoor	Dartmoor	Dartmoor	Cross	Welsh	Welsh	Welsh	Cross
	Age, y	2	2	2	2	2	2	2	2	2	2	2
	Gender	Mare	Gelding	Mare	Gelding	Mare	Mare	Gelding	Mare	Mare	Mare	Mare
	Pregnant March	No		No		No	No		No	No	No	No
	Lactating May	No		No		No	No		No	No	No	No
	Pregnant May	No		No		No	No		No	No	No	No
	Previous Laminitis	No	No	No	No	No	No	No	No	No	No	No
March	Body Condition	5.5	5.25	5	4.75	5	5.5	5.5	4.75	5	5	5.75
	Insulin, mIU/L	5.24	7.4	14.83	5.62	3.72	7.32	5.21	6.08	7.72	8.3	4.05
	Glucose, mg/dL	91.47	103.61	103.21	95.77	85.92	89.17	85.93	90.31	90.51	91.17	93.41
	Triglyceride, mg/dL	88.19	33.72	38.94	101.73	89.08	102.56	30.97	47.63	40.26	53.4	38.95
	Cortisol, mIU/L	9.3	3.7	8.66	9.24	5.46	5.14	4.44	7.8	3.76	6.26	8.5
	Non-esterified fatty acids, mEq/L	0.69	0.57	0.73	0.7	0.66	0.93	0.58	0.62	0.56	0.63	0.62
	RISQI	0.44	0.37	0.26	0.42	0.52	0.37	0.44	0.41	0.36	0.35	0.50
	MIRG	3.24	3.47	5.86	3.18	2.82	4.28	3.54	3.67	4.36	4.55	2.63
May	Body Condition	6.25		6.25		6	6.5	5.5	6	6	5.5	5
	Insulin, mIU/L	13.17	2.83	12.28	0.11	6.5	4.61	4.59	3.6	8.54	13.77	5.75
	Glucose, mg/dL	91.93	94.16	95.48	85.26	102.31	93.28	95.37	91.24	90.34	96.7	93.04
	Triglyceride, mg/dL	45.19	29.27	21.1	37.97	25.37	45.61	19.75	32.61	21.61	22.49	39.84
	Cortisol, mIU/L	8.81	6.25	10.93	8.37	8.32	11.23	14.62	9.44	10.68	12.28	11.83
	Non-esterified fatty acids, mEq/L	0.05	0.22	0.04	0.1	0.03	0.07	0.03	0.04	0.04	0.17	0.02
	RISQI	0.28	0.59	0.29	3.02	0.39	0.47	0.47	0.53	0.34	0.27	0.42
	MIRG	6.35	2.07	5.70	0.96	3.21	2.87	2.77	2.52	4.71	6.09	3.37
Criteria Passed	Body Condition	No	No	No	No	No	No	No	No	No	No	No
	Triglyceride	Yes	No	No	Yes	Yes	Yes	No	No	No	No	No
	RISQI	No	No	Yes	No	No	No	No	No	No	No	No
	MIRG	No	No	Yes	No	No	No	No	No	No	No	No
	Total # of criteria passed	1	0	2	1	1	1	0	0	0	0	0
	PLMS	No	No	No	No	No	No	No	No	No	No	No
	Hirsutism	No	No	No	No	No	No	No	No	No	No	No

	Pony #	141	142	143	144	145	146	147	148	149	150	151
	Breed	Welsh	Cross	Welsh	Cross	Dartmoor	Welsh	Welsh	Connemara	Cross	Welsh	Welsh
	Age, y	2	2	2	2	2	2	2	2	2	2	2
	Gender	Mare	Mare	Gelding	Gelding	Mare	Gelding	Gelding	Mare	Gelding	Mare	Mare
	Pregnant March	No	No			No			No		No	No
	Lactating May	No	No			No			No		No	No
	Pregnant May	No	No			No			No		No	No
	Previous Laminitis	No	No	No	No	No	No	No	No	No	No	No
March	Body Condition	6	4.75	6	5.25	5.75	5.25	5.5	5.5	4.75	4.75	6
	Insulin, mIU/L	3.68	3.91	9.82	6.46	7.67	5.84	6.47	5.27	1.92	5.24	6.99
	Glucose, mg/dL	87.16	90.56	99.45	91.17	91.15	89.15	84.71	83.11	85.27	92.76	80.13
	Triglyceride, mg/dL	49.53	19.89	50.07	25.72	67.21	44.45	72.68	40.03	31.69	38.86	41.1
	Cortisol, mIU/L	8.26	7.14	5.23	14.39	4.28	10.6	7.96	8.41	4.49	7.3	6.87
	Non-esterified fatty acids, mEq/L	0.65	0.62	0.61	0.73	0.66	0.73	0.75	0.67	0.55	0.56	0.66
	RISQI	0.52	0.51	0.32	0.39	0.36	0.41	0.39	0.44	0.72	0.44	0.38
	MIRG	2.74	2.69	4.55	3.78	4.29	3.63	4.23	3.76	1.93	3.17	4.89
May	Body Condition	6.5	5.5	6.5	5.5	6	5.5	6.25	6	5	5.25	6.5
	Insulin, mIU/L	5.86	4.54	3.45	4.92	11.96	20.68	4.99	7.8	1.31	3.96	8.04
	Glucose, mg/dL	91.86	101.39	90.43	90.26	106.8	93.78	86.84	89.23	88.57	92.42	91.86
	Triglyceride, mg/dL	49.52	15.99	35.18	13.61	43.41	46.56	50.79	17.46	26.65	35.52	37.54
	Cortisol, mIU/L	13.17	11.99	12.23	22.3	9.08	10.48	11.91	7.27	11.59	11.55	12.22
	Non-esterified fatty acids, mEq/L	0.04	0.1	0.16	0.06	0.03	0.04	0.03	0.14	0.11	0.03	0.04
	RISQI	0.41	0.47	0.54	0.45	0.29	0.22	0.45	0.36	0.87	0.50	0.35
	MIRG	3.48	2.52	2.48	3.16	4.76	8.50	3.38	4.49	1.52	2.63	4.39
Criteria Passed	Body Condition	Yes	No	Yes	No	No	No	No	No	No	No	Yes
	Triglyceride	No	No	No	No	Yes	No	Yes	No	No	No	No
	RISQI	No	No	Yes	No	No	No	No	No	No	No	No
	MIRG	No	No	No	No	No	No	No	No	No	No	No
	Total # of criteria passed	1	0	2	0	1	0	1	0	0	0	1
	PLMS	No	No	No	No	No	No	No	No	No	No	No
	Hirsutism	No	No	No	No	No	No	No	No	No	No	No

	Pony #	152	153	154	157	158	159	160	161	162	163
	Breed	Welsh	Welsh	Welsh	Cross	Welsh	Welsh	Welsh	Cross	Welsh	Welsh
	Age, y	2	2	2	2	1	1	1	1	1	1
	Gender	Gelding	Mare	Mare	Gelding	Colt	Colt	Colt	Colt	Colt	Colt
	Pregnant March		No	No							
	Lactating May		No	No							
	Pregnant May		No	No							
	Previous Laminitis	No	No	No	No	No	No	No	No	No	No
March	Body Condition	6	5. 5	5. 5	5	5	5	5.75	5	6	5
	Insulin, mIU/L	5.98	6.78	9.38	5.61	2.61	5.32	3.2	1.42	5.4	2.97
	Glucose, mg/dL	89.06	86.7	95.33	88.45	101.54	92.22	82.39	87.44	106.51	100.73
	Triglyceride, mg/dL	78.33	75.2	50.56	55.91	38.05	20.81	140.76	23.63	26.21	34.69
	Cortisol, mIU/L	8.7	9.22	8.5	9.35	6.43	6.46	7.03	7.29	10.4	6.6
	Non-esterified fatty acids, mEq/L	0.66	0.86	0.53	0.92	0.56	0.68	0.76	0.62	0.67	0.59
	RISQI	0.41	0.38	0.33	0.42	0.62	0.43	0.56	0.84	0.43	0.58
	MIRG	3.70	4.23	4.67	3.57	1.76	3.23	2.73	1.60	2.66	1.93
May	Body Condition	6.25	4.75	6.25	5		5			5	4.75
	Insulin, mIU/L	12.96	0.88	4.58	7.78	0.74	6.92	2.74		0.7	0.46
	Glucose, mg/dL	97.15	89.3	87.18	94.2	92.45	90.82	92.3		97.54	91.76
	Triglyceride, mg/dL	32.34	22.6	46.65	40.69	20.74	18.36	30.97		22.16	26.57
	Cortisol, mIU/L	13.56	9.43	11.79	16.41	9.16	6. 6	8.74		9.06	7.89
	Non-esterified fatty acids, mEq/L	0.04	0	0.02	0.15	0.06	0.05	0.17		0.04	0.11
	RISQI	0.28	1.07	0.47	0.36	1.16	0.38	0.60		1.20	1.47
	MIRG	5.78	1.29	3.17	4.13	1.15	4.00	2.09		1.05	1.03
Criteria Passed	Body Condition	Yes	No	No	No	No	No	No	No	Yes	No
	Triglyceride	Yes	Yes	No	No	No	No	Yes	No	No	No
	RISQI	No	No	No	No	No	No	No	No	No	No
	MIRG	No	No	No	No	No	No	No	No	No	No
	Total # of criteria passed	2	1	0	0	0	0	1	0	1	0
	PLMS	No	No	No	No	No	No	No	No	No	No
	Hirsutism	No	No	No	No	No	No	No	No	No	No

Abbreviations: RISQI - reciprocal of the square root of insulin; MIRG - modified insulin -to-glucose ratio; PLMS - pre-laminitic metabolic syndrome. Criteria passed indicates that the pony was indicated at risk for laminitis according to the respective variable.

Table A.7. (Chapter 6). Basal resting glucose and insulin concentrations, proxies for insulin sensitivity and insulin secretory response and minimal model parameters for 46 Thoroughbred and Arabian horses and one hyperlipidemic/laminitic pony (Hlpony). All horses were maintained on pasture and supplemented twice daily with feeds rich in fat and fiber (FF) or sugar and starch (SS).

				Insulin sensitivity proxies				Insulin secretory response proxies			Minimal model parameters			
Type	Diet	Basal insulin (mIU/L)	Basal glucose (mg/dL)	RISQI	GIR	HOMA	QUICKI	MIRG	HOMA BC%	IGR	SI x 104	Sg	DI	AIRg
Arabian	FF	8.68	92.17	0.339	10.62	35.55	0.344	4.63	5.95	0.094	0.22	0.0284	95	427
Arabian	SS	10.67	98.83	0.306	9.27	46.86	0.331	4.88	5.95	0.108	1.73	0.0219	497	288
Arabian	FF	10.05	91.45	0.315	9.1	40.86	0.337	5.23	7.07	0.11	2.81	0.0174	526	187
Arabian	SS	7.77	94.14	0.359	12.12	32.5	0.349	4.13	4.99	0.083	3.31	0.0281	961	291
Arabian	FF	11.4	96.57	0.296	8.47	48.91	0.329	5.3	6.79	0.118	1.09	0.0237	344	315
Arabian	SS	9.06	93.1	0.332	10.27	37.5	0.342	4.71	6.02	0.097	1.17	0.0119	201	171
Arabian	FF	10.67	100.31	0.306	9.4	47.55	0.33	4.78	5.72	0.106	2.21	0.0059	1752	792
Arabian	SS	17.89	100.42	0.236	5.61	79.82	0.307	6.97	9.56	0.178	1.45	0.0195	1174	808
Arabian	FF	5.31	90.59	0.434	17.07	21.36	0.373	3.31	3.85	0.059	1.56	0.0076	473	304
Arabian	SS	5.76	94.16	0.417	16.35	24.09	0.366	3.32	3.7	0.061	1.43	0.0133	288	201
Arabian	FF	8.06	100.33	0.352	12.45	35.93	0.344	3.87	4.32	0.08	3.92	0.0222	858	219
Arabian	SS	16.73	95.93	0.244	5.73	71.33	0.312	7.1	10.16	0.174	1.52	0.0089	976	643
Weanling	SS	4.99	119.15	0.448	23.88	26.42	0.36	2.16	1.78	0.042	2.51	0.006	308	123
Weanling	FF	3.68	120.33	0.521	32.7	19.68	0.378	1.73	1.28	0.031	2.84	0.0073	317	112
Weanling	SS	4.78	123.42	0.457	25.82	26.22	0.361	2	1.58	0.039	2.78	0.0076	465	164
Weanling	FF	1.78	115.43	0.75	64.85	9.13	0.432	1.2	0.68	0.015	5.94	0.0078	359	60
Weanling	FF	2.97	112.51	0.58	37.88	14.85	0.396	1.65	1.2	0.026	3.93	0.0076	408	103
Weanling	SS	5.07	122.39	0.444	24.14	27.58	0.358	2.1	1.71	0.041	1.76	0.0082	314	179
Weanling	FF	5.04	123.35	0.445	24.47	27.63	0.358	2.07	1.67	0.041	2.95	0.0085	334	113
Weanling	SS	10.36	123.25	0.311	11.9	56.75	0.322	3.52	3.44	0.084	2.54	0.0073	612	241
Weanling	SS	7.65	114.14	0.362	14.92	38.81	0.34	3.11	2.99	0.067	1.94	0.0101	216	111
Weanling	FF	5.57	121.05	0.424	21.73	29.97	0.354	2.28	1.92	0.046	3.1	0.0062	452	146
Weanling	FF	4.56	124.86	0.468	27.38	25.3	0.363	1.9	1.47	0.037	2.76	0.0074	476	173
Weanling	SS	4.52	123.96	0.47	27.42	24.9	0.364	1.91	1.48	0.036	2.15	0.0056	253	114

				Insulin sensitivity proxies				Insulin secretory response proxies			Minimal model parameters			
Type	**Diet**	**Basal insulin (mIU/L)**	**Basal glucose (mg/dL)**	**RISQI**	**GIR**	**HOMA**	**QUICKI**	**MIRG**	**HOMA BC%**	**IGR**	**SI x 104**	**Sg**	**DI**	**AIRg**
Gelding	FF	11.51	109.06	0.295	9.48	55.79	0.323	4.5	5.00	0.106	0.3	0.011	123	406
Gelding	FF	8.29	93.41	0.347	11.27	34.42	0.346	4.39	5.45	0.089	0.14	0.0296	30	218
Gelding	FF	8.79	104.55	0.337	11.89	40.84	0.337	3.9	4.23	0.084	1.28	0.0292	292	228
Gelding	FF	43.29	103.71	0.152	2.4	199.54	0.274	10.67	21.27	0.417	0.35	0.0163	273	789
Gelding	SS	13.03	96.27	0.277	7.39	55.75	0.323	5.88	7.83	0.135	1.4	0.0152	412	294
Gelding	FF	26.75	106.76	0.193	3.99	126.93	0.289	8.31	12.23	0.251	0.49	0.0129	205	423
Gelding	SS	12.86	100.53	0.279	7.82	57.46	0.321	5.48	6.85	0.128	0.19	0.0227	57	306
Gelding	SS	9.24	119.65	0.329	12.95	49.14	0.329	3.36	3.26	0.077	0	0.0152	0	101
Gelding	SS	14.97	115.31	0.258	7.7	76.72	0.309	5.06	5.72	0.13	1.35	0.0072	210	156
Gelding	SS	11.74	96.67	0.292	8.23	50.44	0.327	5.41	6.97	0.121	2.04	0.0188	415	203
Pregnant	FF	3.4	94.95	0.542	27.9	14.36	0.398	2.29	2.13	0.036	2.57	0.0009	422	164
Pregnant	FF	9.87	94.32	0.318	9.56	41.36	0.337	4.93	6.3	0.105	0.43	0.01	134	309
Pregnant	FF	10.77	92.12	0.305	8.55	44.1	0.334	5.45	7.4	0.117	2.5	0.0129	897	359
Pregnant	SS	4.34	79.68	0.48	18.36	15.37	0.394	3.51	5.2	0.054	4.01	0.0117	769	191
Pregnant	SS	7.58	90.28	0.363	11.91	30.41	0.353	4.32	5.56	0.084	1.11	0.0024	323	292
Pregnant	FF	1.1	87.62	0.953	79.55	4.29	0.504	1.43	0.89	0.013	5.59	0.0072	1109	198
Pregnant	FF	3.36	90.28	0.546	26.9	13.47	0.403	2.44	2.46	0.037	4.77	0.0094	720	151
Pregnant	SS	7.23	85.55	0.372	11.83	27.5	0.358	4.52	6.42	0.085	1.57	0.009	403	257
Pregnant	FF	2.76	88.54	0.602	32.09	10.86	0.419	2.23	2.16	0.031	4.88	0.0072	849	174
Pregnant	SS	6.66	77.14	0.388	11.59	22.82	0.369	5.01	9.41	0.086	0.87	0.0145	243	280
Pregnant	SS	4.63	73.19	0.465	15.82	15.05	0.395	4.22	9.09	0.063	1.87	0.0156	1312	351
Pregnant	SS	8.9	88.41	0.335	9.94	34.95	0.345	5.02	7.00	0.101	0.73	0.01	198	272
HLpony	-	101.3	170.7	0.099	1.69	768.53	0.236	0.07	18.81	0.593	0.09	0.0137	3	35

Abbreviations:
RISQI = Reciprocal of the square root of insulin; GIR = glucose-to-insulin ratio; HOMA = Homeostasis model assessment; QUICKI = Quantitative insulin sensitivity check index; MIRG = Modified insulin-to-glucose ratio; HOMA-BC%= Percent HOMA-beta-cell function; IGR = insulin-to-glucose ratio; SI = minimal model insulin sensitivity index; Sg = minimal model glucose effectiveness; DI = minimal model disposition index; AIRg = minimal model acute insulin response to glucose

Table A.8. (Chapter 2 and 3) Variables and parameters of lactate threshold, minimal model and kinetics test in trained Arabian geldings at rest and during exercise.

A. Minimal Model test

		Horse											
	Trial	1	3	5	7	9	11	2	4	6	8	10	12
Feed		FF	FF	FF	FF	FF	FF	SS	SS	SS	SS	SS	SS
Glucose Dose (mg)	Rest	515	580	575	550	564	471	724	540	492	588	519	601
	Exercise	510	597	533	561	568	475	763	553	505	604	520	586
Insulin Dose (IU)	Rest	4.3	4.8	4.8	4.6	4.7	3.9	6	4.5	4.1	4.9	4.3	5
	Exercise	4.3	4.9	4.4	4.7	4.7	4	6.4	4.6	4.2	5	4.3	4.9
SI x 10^4, min^{-1} per mIU/L	Rest	0.546	2.408	1.055	2.121	1.384	3.903	3.446	3.303	1.249	0.852	1.496	0.646
	Exercise	16.6	14.373	19.629	29.63	16.4	36.33	17.608	15.910	15.261	5.398	20.908	7.841
Sg, min^{-1}	Rest	0.0280	0.0191	0.0271	0.0069	0.0098	0.0220	0.0228	0.0288	0.0108	0.0215	0.0143	0.0102
	Exercise	0.0352	0.0199	0.0395	0.0474	0.0298	0.0502	0.0430	0.0299	0.0227	0.0201	0.0448	0.0202
AIRg, mIU/L·min	Rest	406.1	187.2	315.33	792.27	304	219.14	287.78	290.88	171.46	807.81	201	642.83
	Exercise	179.1	97.778	252.67	236.75	87.67	2 98	130.59	66.358	76.009	268.66	138.4	186.2
DI x 10^4	Rest	221.5	450.71	332.79	1680.5	420.6	855.4	991.63	960.7	214.15	688.25	300.7	414.98
	Exercise	2973	1405.3	4959.6	7015.1	1437	10827	2299.5	1055.8	1160	1450.2	2893	1459.9
Minimal fit, R^2	Rest	99.37	99.35	99.7	99.64	99.11	99.67	99.47	99.88	97.93	99.7	98.63	96
	Exercise	97.77	99.2	97.65	97	99.41	98.41	96.73	98.04	99.5	99.58	96.72	99.31

B. Kinetics observational parameters and rate constants													
	Trial	1	3	5	7	9	11	2	4	6	8	10	12
Feed		FF	FF	FF	FF	FF	SS	SS	SS	SS	SS	SS	SS
Tracer Dose (g)	Rest	7.49	8.78	8.02	8.17	8.31	6.84	9.82	7.89	7.55	8.46	7.29	8.45
	Exercise	7.82	8.6	7.82	8.26	8.2	7.06	10.51	8.33	7.25	8.51	8.15	8.56
A1, eigenvector	Rest	0.0098	0.0097	0.0114	0.0074	0.0116	0.0070	0.0163	0.0177	0.0072	0.0095	0.0098	0.0100
	Exercise	0.0112	0.0108	0.0118	0.0130	0.0092	0.0105	0.0073	0.0113	0.0093	0.0094	0.0100	0.0093
A2, eigenvector	Rest	0.0083	0.0104	0.0074	0.0091	0.0099	0.0109	0.0164	0.0103	0.0107	0.0089	0.0088	0.0097
	Exercise	0.0117	0.0104	0.0121	0.0092	0.0129	0.0114	0.0176	0.0089	0.0102	0.0113	0.0114	0.0082
a1, eigenvalue	Rest	-0.0625	-0.0917	-0.0462	-0.0968	-0.1568	-0.0449	-0.1203	-0.1840	-0.0603	-0.0819	-0.0474	-0.1934
	Exercise	-0.1647	-0.1692	-0.1738	-0.1899	-0.2491	-0.1617	-0.3419	-0.1296	-0.1230	-0.3237	-0.1976	-0.1480
a2, eigenvalue	Rest	-0.0057	0.0077	-0.0079	-0.0095	-0.0082	-0.0066	-0.0108	-0.0080	-0.0070	-0.0079	-0.0049	-0.0072
	Exercise	-0.0247	-0.0219	-0.0193	-0.0269	-0.0289	-0.0174	-0.0366	-0.0270	-0.0217	-0.0249	-0.0251	-0.0216
Turnover rate constant$_1$ (k11), min^{-1}	Rest	-0.0364	-0.0483	-0.0312	-0.0488	-0.0886	-0.0216	-0.0655	-0.1191	-0.0283	-0.0461	-0.0273	-0.1016
	Exercise	-0.0933	-0.0971	-0.0953	-0.1224	-0.1208	-0.0863	-0.1263	-0.0845	-0.0701	-0.1604	-0.1058	-0.0890
Turnover rate constant$_2$ (k22), min^{-1}	Rest	-0.0319	-0.0511	-0.0230	-0.0575	-0.0764	-0.0300	-0.0657	-0.0729	-0.0389	-0.0437	-0.0249	-0.0990
	Exercise	-0.0916	-0.0940	-0.0977	-0.0944	-0.1572	-0.0928	-0.2521	-0.0721	-0.0747	-0.1881	-0.1168	-0.0805
k12k21	Rest	0.0008	0.0018	0.0003	0.0019	0.0055	0.0003	0.0030	0.0072	0.0007	0.0014	0.0005	0.0087
	Exercise	0.0049	0.0054	0.0060	0.0064	0.0118	0.0052	0.0194	0.0026	0.0026	0.0221	0.0074	0.0040
k12, min^{-1}	Rest	0.0220 to 0.0319	0.0365 to 0.0511	0.0112 to 0.0230	0.0386 to 0.0575	0.0619 to 0.0764	0.0162 to 0.0300	0.0459 to 0.0657	0.0606 to 0.0729	0.0241 to 0.0389	0.0297 to 0.0437	0.0165 to 0.0249	0.0852 to 0.0990
	Exercise	0.0525 to 0.0961	0.0558 to 0.0940	0.0625 to 0.0977	0.0527 to 0.0944	0.0976 to 0.1572	0.0602 to 0.0928	0.1532 to 0.2521	0.0307 to 0.0721	0.0365 to 0.0747	0.1380 to 0.1881	0.0701 to 0.1168	0.0446 to 0.0805
k21, min^{-1}	Rest	0.0251 to 0.0364	0.0344 to 0.0483	0.0152 to 0.0312	0.0328 to 0.0488	0.0718 to 0.0886	0.0117 to 0.0216	0.0457 to 0.0655	0.0990 to 0.1191	0.0175 to 0.0283	0.0313 to 0.0461	0.0181 to 0.0273	0.0874 to 0.1016
	Exercise	0.0510 to 0.0933	0.0576 to 0.0971	0.0610 to 0.0953	0.0683 to 0.1224	0.0750 to 0.1208	0.0560 to 0.0863	0.0768 to 0.1263	0.0360 to 0.0845	0.0343 to 0.0701	0.1176 to 0.1604	0.0634 to 0.1058	0.0494 to 0.0890
k01max, min^{-1}	Rest	0.0112	0.0139	0.0160	0.0161	0.0169	0.0099	0.0197	0.0201	0.0108	0.0147	0.0092	0.0142
	Exercise	0.0423	0.0395	0.0344	0.0541	0.0458	0.0303	0.0496	0.0485	0.0358	0.0428	0.0424	0.0397
k02max, min^{-1}	Rest	0.0098	0.0147	0.0118	0.0189	0.0145	0.0138	0.0198	0.0123	0.0148	0.0140	0.0084	0.0138
	Exercise	0.0436	0.0382	0.0352	0.0417	0.0595	0.0325	0.0990	0.0414	0.0381	0.0502	0.0468	0.0359

C. Kinetics compartment masses (Q), volumes (V) and transport rates (R)													
	Trial	1	3	5	7	9	11	2	4	6	8	10	12
Feed		FF	FF	FF	FF	FF	SS	SS	SS	SS	SS	SS	SS
Q1, mg/kg	Rest	127.4	96.7	109.6	130.5	95.7	148.6	69.6	78.0	126.5	116.6	145.1	108.4
	Exercise	101.6	94.3	94.8	92.7	97.3	119.9	70.5	108.0	145.7	119.5	104.9	122.4
Q2, mg/kg	Rest	100.5 to 145.4	65.1 to 91.3	72.3 to 148.3	74.4 to 110.8	89.8 to 110.9	57.7 to 106.8	48.4 to 69.4	106.1 to 127.6	57.0 to 92.1	83.7 to 123.0	105.1 to 158.9	95.6 to 111.1
	Exercise	53.9 to 98.6	57.8 to 97.3	59.2 to 92.5	67.1 to 120.3	46.5 to 74.8	72.4 to 111.6	21.5 to 35.3	53.9 to 126.4	66.9 to 136.8	74.7 to 101.9	56.9 to 95.0	75.0 to 135.3
V1, mL/kg	Rest	134.2	102.9	120.1	135.1	101.7	148.8	56.7	82.1	134.9	117.1	134.2	109.4
	Exercise	101.4	99.8	97.2	99.5	100.0	117.7	69.4	107.8	128.4	102.9	104.1	121.4
V2, mL/kg	Rest	105.8 to 153.1	69.3 to 97.2	79.3 to 162.7	77.0 to 114.7	95.5 to 117.9	57.8 to 106.9	39.4 to 56.5	111.6 to 134.3	60.7 to 98.1	84.0 to 123.5	97.2 to 147.0	96.6 to 112.2
	Exercise	53.8 to 98.4	61.2 to 103.1	60.7 to 94.8	72.0 to 129.0	47.7 to 76.8	71.1 to 109.4	21.1 to 34.8	53.8 to 126.2	59.0 to 120.5	64.3 to 87.7	56.5 to 94.3	74.4 to 134.2
V_{total}, mL/kg	Rest	240.0 to 28.7	172.2 to 200.1	199.4 to 282.8	212.1 to 249.9	197.2 to 219.6	206.6 to 255.7	96.1 to 113.1	193.7 to 216.4	195.6 to 233.0	201.1 to 240.6	231.4 to 281.1	206.0 to 221.6
	Exercise	155.2 to 199.8	161.0 to 202.9	157.9 to 192.1	171.5 to 228.5	147.7 to 176.8	188.7 to 227.1	90.5 to 104.1	161.6 to 234.0	187.4 to 249.0	167.2 to 190.6	160.6 to 198.4	195.7 to 255.5
R12, mg/min	Rest	3.20	3.33	1.66	4.28	6.87	1.73	3.18	7.73	2.22	3.65	2.62	9.471
	Exercise	5.18	5.43	5.78	6.33	7.30	6.72	5.41	3.89	5.00	1 4.06	6.65	6.043
R21min	Rest	3.20 to 4.64	3.33 to 4.67	1.66 to 3.41	4.28 to 6.37	6.87 to 8.48	1.73 to 3.20	3.18 to 4.55	7.73 to 9.30	2.22 to 3.58	3.65 to 5.37	2.62 to 3.96	9.47 to 11.01
	Exercise	5.18 to 9.47	5.43 to 9.15	5.78 to 9.04	6.33 to 11.35	7.30 to 11.76	6.72 to 10.35	5.41 to 8.91	3.89 to 9.12	5.00 to 10.21	14.06 to 19.17	6.65 to 11.10	6.04 to 10.90
Endogenous Glucose Production, mg/min	Rest	1.43	1.34	1.75	2.10	1.61	1.47	1.37	1.57	1.37	1.72	1.34	1.53
	Exercise	4.30	3.72	3.26	5.02	4.45	3.63	3.50	5.23	5.21	5.11	4.45	4.86

D. Kinetics 'non-compartmental' parameters													
	Trial	1	3	5	7	9	11	2	4	6	8	10	12
Feed		FF	FF	FF	FF	FF	SS	SS	SS	SS	SS	SS	SS
Clearance rate, mL/min per kg	Rest	1.508	1.428	1.918	2.169	1.714	1.473	1.118	1.650	1.455	1.726	1.241	1.548
	Exercise	4.289	3.940	3.340	5.382	4.574	3.561	3.44	5.223	4.594	4.401	4.411	4.817
Mean residence $time_{system}$, min	Rest	159.2 to 190.6	120.6 to 140.1	104.0 to 147.5	97.8 to 115.2	115.0 to 128.1	140.3 to 173.6	85.9 to 101.1	117.4 to 131.1	134.4 to 160.1	116.5 to 139.4	186.4 to 226.5	133.02 to 143.12
	Exercise	36.2 to 46.6	40.9 to 51.5	47.3 to 57.5	31.9 to 42.5	32.3 to 38.7	53.0 to 63.8	26.3 to 30.3	30.9 to 44.8	40.8 to 54.2	38.0 to 43.3	36.4 to 45.0	40.64 to 53.05
Mean residence $time_1$, min	Rest	89.0	72.0	62.6	62.3	59.3	101.0	50.7	49.8	92.7	67.8	108.1	70.7
	Exercise	23.6	25.3	29.1	18.5	21.9	33.0	20.2	20.6	28.0	23.4	23.6	25.2
Mean residence $time_2$,min	Rest	70.2 to 191.6	48.5 to 68.1	41.4 to 84.8	35.5 to 52.9	55.7 to 68.8	39.3 to 72.6	35.3 to 50.5	67.6 to 81.4	41.7 to 67.4	48.7 to 71.6	78.3 to 118.4	62.36 to 72.46
	Exercise	12.5 to 23.0	15.5 to 26.2	18.2 to 28.4	13.4 to 24.0	10.4 to 16.8	20.0 to 30.7	6.1 to 10.1	10.3 to 24.2	12.8 to 26.2	14.6 to 19.9	12.8 to 21.4	15.44 to 27.86
Turnover $time_1$, min	Rest	27.49	20.69	32.1	20.47	11.28	46.39	15.28	8.40	35.31	21.70	36.61	9.85
	Exercise	10.72	10.30	10.49	8.17	8.28	11.59	7.92	11.84	14.28	6.23	9.45	11.23
Turnover $time_2$, min	Rest	31.37	19.55	43.47	17.39	13.08	33.34	15.23	13.73	25.70	22.89	40.1	10.1
	Exercise	10.41	10.64	10.23	10.60	6.36	10.78	3.97	13.86	13.40	5.32	8.56	12.42
Turnover $rate_1$,mg/min per kg	Rest	4.63	4.67	3.41	6.37	8.48	3.20	4.55	9.30	3.58	5.37	3.96	11
	Exercise	9.47	9.15	9.04	11.35	11.75	10.35	8.91	9.12	10.21	19.17	11.1	10.9
Turnover $rate_2$,mg/min per kg	Rest	3.20 to 4.63	3.33 to 4.67	1.66 to 3.41	4.28 to 6.37	6.87 to 8.48	1.73 to 3.20	3.18 to 4.55	7.73 to 9.30	2.22 to 3.58	3.65 to 5.37	2.62 to 3.96	9.47 to 11.00
	Exercise	5.18 to 9.47	5.43 to 9.15	5.78 to 9.04	6.33 to 11.35	7.30 to 11.75	6.72 to 10.35	5.41 to 8.91	3.89 to 9.12	5.00 to 10.21	14.06 to 19.17	6.65 to 11.10	6.04 to 10.90

E. General properties of individuals and tests

			Horse											
	Study	Trial	1	3	5	7	9	11	2	4	6	8	10	12
Feed			FF	FF	FF	FF	FF	FF	SS	SS	SS	SS	SS	SS
Age, y			12	14	6	13	13	13	12	11	5	13	8	16
Body Condition			5	6	5	5	6	4	7	5	4	6	6	5
Date	MinMod	Rest	10/30	09/29	09/30	11/03	10/22	11/04	11/06	10/01	10/01	11/05	10/23	10/27
	MinMod	Exercise	09/29	10/30	10/31	10/22	11/03	10/23	09/30	10/21	11/04	10/27	11/05	11/06
	Kinetics	Rest	39039	11/10	11/11	11/25	11/13	11/21	11/26	11/22	11/12	11/26	11/18	11/20
	Kinetics	Exercise	39030	12/04	11/21	11/12	11/25	11/13	11/20	11/11	11/19	11/18	12/02	12/03
BW (kg)	0 wk adapt		418	489	433	455	481	395	555	437	385	483	433	474
	4 wk adapt		425	483	422	452	467	397	571	455	407	470	436	486
	MinMod	Rest	429	479	435	458	470	393	604	450	410	490	433	501
	MinMod	Exercise	427	498	445	467	474	395	578	461	421	504	434	488
	Kinetics	Rest	412	483	441	449	457	376	540	434	416	466	401	465
	Kinetics	Exercise	430	473	430	455	451	388	578	458	399	468	448	471
Breakpoint,m/s	Lactate		6.30	6.29	5.92	6.35	6.70	6.70	5.85	7.12	5.04	6.72	6.64	6.28
Slope 1 of broken line	Lactate		0.23	0.33	0.34	0.19	0.44	0.14	0.30	0.28	0.35	0.36	0.46	0.35
Slope 2	Lactate		2.64	3.74	3.36	2.94	3.46	2.97	4.87	3.57	2.03	2.83	5.42	3.00
Speed run, m/s	Kinetics & Minmod	Exercise	3.7	3.9	3.8	4.2	4.3	4.1	3.4	4.0	3.3	4.1	4.0	3.5
% of Lactate threshold run	Kinetics & Minmod	Exercise	58.2	62.6	64.2	65.5	64.1	60.7	58.9	56.4	65.7	60.5	60.6	55.5
Start Time (am)	MinMod	Rest	9:25	10:27	10:00	9:15	9:21	9:04	9:00	~10	~10	9:15	9:25	9:15
	MinMod	Exercise	10:27	9:25	9:07	9:21	9:15	9:25	10:00	9:20	9:04	9:15	9:15	9:00
	Kinetics	Rest	0.38	9:41	9:00	9:53	9:14	8:52	9:02	9:04	9:03	9:02	9:43	9:09
	Kinetics	Exercise	0.403		8:52	9:03	9:53	9:14	9:09	9:00	9:07	9:43		
°C at 10 am	MinMod	Rest	9	15.3	12.9	13.6	13.2	13.3	17.6	11.7	11.7	19.6	9.2	16.5
	MinMod	Exercise	15.3	9	8.3	13.2	13.6	9.2	12.9	15.6	13.3	16.5	19.6	17.6
	Kinetics	Rest	17	3	6	1	6	16	0	13	8	0	8	9
	Kinetics	Exercise	3	-1	16	8	4	6	9	6	17	5	1	1
Basal heartrate, bpm	MinMod	Rest	60	41	56	48	44			44			48	52
	MinMod	Exercise	64	48	38	56	36	48	56	38	40	44	36	60
	Kinetics	Rest	37	44	32	41	43	34	49	.	39	42	37	42
	Kinetics	Exercise	40	43	39	42	41	36	53	36	36	44	41	43

VITAE

Kimberly (Kibby) Hoffer Treiber graduated from the North Carolina School of Science and Mathematics in 1997 and received her B.S. in Biology and B.A. in Ancient/Medieval History from the University of North Carolina at Chapel Hill in 2001. She recieved an MS in Animal and Poultry Science in 2003, focusing on equine nutrition, growth, and insulin resistance. 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 animals and science as a primary source for inspiration.