ABSTRACT

Title of Document: EFFECT OF WEIGHT GAIN, DIET AND EXERCISE ON INSULIN SENSITIVITY IN THOROUGHBRED GELDINGS

Rachael Weaver Quinn, Doctor of Philosophy, 2007

Directed By: Associate Professor Thomas G. Hartsock
and
Assistant Professor Amy O. Burk
Department of Animal and Avian Sciences

Insulin sensitivity (SI) in horses is affected by diet, exercise and obesity and has been implicated in metabolic disease. The objectives of this research were to assess the impact of BW gain on SI utilizing two diets known to differentially impact glucose dynamics, evaluate the contribution of light exercise to overall SI and relate changes in SI to BCS to identify the threshold at which SI declines to a level consistent with an increased risk of metabolic disease. Fifteen mature Thoroughbred geldings (BW 516 ± 13 kg, BCS 4.3 ± 0.1) were fed to gain 90.8 kg on a diet high in fat and fiber (HF, n = 6) starch and sugar (HS, n = 9). To assess SI, frequently sampled i.v. glucose tolerance tests were performed before treatment initiation (CFMM), at the start (TXMM) and end (ENDMM) of weight gain and following a

period of minimal exercise. Using the minimal model of glucose dynamics, data from each test was used to estimate SI, glucose effectiveness (Sg) and the acute insulin response to glucose (AIRg). Final BW was 608 ± 12 kg and BCS was 7 ± 0.1. Diet had no effect on SI, AIRg or glucose effectiveness at CFMM. Within HF, SI, Sg and AIRg were unchanged at CFMM, TXMM and ENDMM. SI decreased at TXMM in HS (P = 0.05) and remained unchanged through ENDMM. SI in HS was lower than HF at TXMM (P = 0.01) and ENDMM (P = 0.07). At ENDMM, AIRg was higher in HS than HF (P = 0.01) and glucose effectiveness was reduced in both diets (P < 0.05). Following the minimal exercise period, SI decreased in HF (P = 0.03). These results indicate that diet may be more influential on SI than weight gain in mature Thoroughbred geldings. The higher SI in HF appears to be partially dependent on some level of physical activity. Because a BCS increase of 3 scores was not associated with a reduction in SI, the BCS where the perceived risk of metabolic disease is increased likely lies above that achieved in this study (BCS 7).

Key words: equine obesity, insulin sensitivity, minimal model of glucose dynamics

EFFECT OF WEIGHT GAIN, DIET AND EXERCISE ON INSULIN SENSITIVITY IN THOROUGHBRED GELDINGS

By

Rachael Weaver Quinn

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2007

Advisory Committee:
Associate Professor Thomas G. Hartsock, Co-Chair
Assistant Professor Amy O. Burk, Co-Chair
Professor Thomas Castonguay
Professor Richard Erdman
Associate Professor Niki C. Whitley

Acknowledgements

I can only imagine the path my life might have taken if I had slept through swine production in my senior year…Tom, you have been a wonderful advisor, mentor and friend and could not have prepared me better for my ascent into the 'real world.' I only hope I can be half the mentor to my students that you have been for me. Amy, through all of the trials and tribulations of starting this research program, I can honestly say it has been an honor to see this through to the end with you. Niki, you have been a valuable resource and friend and some of the best times I've had in graduate school have been spent at UMES getting beat up by the critters! Dr. C., I have enjoyed learning from you and have gained a new appreciation for the 'lowly' lab rat! And finally, Dr. Erdman, my most sincere thanks for your dedication to the equine research program. I feel as though you have made my program, and me, a priority within the department and I know we could not have done this without your support.

To Kelcey and Jess, my partners in poo – it is because of you that I have great memories to take away from this experience. We've seen each other at our very worst and very best, and because of that, we will be friends forever and I will miss you dearly. Special thanks to Tim Shellem for his tireless help during my MinMods and endless labwork and Janah Schreiber for her expert help as Unit manager. Thanks also to Dr. Angel for the use of her lab equipment and Erin Petersen for teaching me the ins and outs of ration formulation. I also must thank the countless undergraduate students who assisted with my study, especially Heather Scruggs, and of course, my boys, Major, Woody, Ninja, Cuppa, Ought Not, Carson, Mack, Sirius, William, Popeye, Grover, Mouse, Bubba, Harvey and Nordic (not Nemo!).

Finally, to my friends and family – you have believed in me from the beginning and always could see the light at the end of the tunnel, even when I could not. Jim, to you I say 'greenish brown female sheep' and of course, you were right…

'Corpulence is not only a disease unto itself but the harbinger of others'

-Hippocrates

Table of Contents

Rachael Weaver Quinn, Doctor of Philosophy, 2007 i

Acknowledgements ii

Table of Contents iii

List of Tables vii

List of Figures ix

CHAPTER 1: INTRODUCTION AND OBJECTIVES 1

Introduction 1
Objectives 2
CHAPTER 2: LITERATURE REVIEW 4

PART I: EQUINE BODY COMPOSITION 4
Equine obesity 4
Characterization of equine body composition 6
Weight manipulation in the adult horse 10
Weight loss 11
Weight gain 12
PART II: INSULIN ACTION AND CAUSES OF RESISTANCE 14
Insulin action 15
Release 15
Arrival and action 16
Insulin resistance 20
Impaired insulin sensitivity 20
Impaired insulin effectiveness 23
The role of the β-cell 23
The role of the central nervous system 25
Adiponectin 28
Oxidative stress 29
Equine insulin resistance 31
Dietary impacts on insulin sensitivity 35
Onset of insulin resistance 36
PART III: EQUINE DIABETES AND METABOLIC SYNDROME 38
Equine diabetes 38
The equine Metabolic Syndrome 40
PART IV: MODULATION OF INSULIN SENSITIVITY THROUGH INTERVENTION 44
Diet and exercise 44
Pharmaceutical intervention 46

PART V: MODERN TECHNIQUES FOR THE QUANTIFICATION OF INSULIN SENSITIVITY 48
Simple techniques 48
Oral and intravenous glucose tolerance tests 49
Modern methods: the euglycemic-hyperinsulinemic clamp 50
Modern methods: the minimal model of glucose dynamics 51
Defining the equations 54
Equation 1 55
Equation 2 56
Limitations to the minimal model 57
CHAPTER 3: MANUSCRIPT I 60

Effect of weight gain on glucose and insulin dynamics, leptin and measures of oxidative stress in Thoroughbred geldings 60
Abstract 61
Introduction 63
Materials and methods 64
Results 73
Discussion 82
Literature Cited 87
CHAPTER 4: MANUSCRIPT II 94

Effect of weight gain, diet and exercise on insulin sensitivity in Thoroughbred geldings 94
Abstract 95
Introduction 97
Materials and methods 98
Results 107
Discussion 112
Literature Cited 120
GENERAL SUMMARY AND CONCLUSIONS 125

APPENDIX 128

LITERATURE CITED 134

Vitae 159

Rachael Weaver Quinn, Doctor of Philosophy, 2007 i

Acknowledgements i

Table of Contents ii

List of Tables iv

List of Figures vi

CHAPTER 1: INTRODUCTION AND OBJECTIVES 1

Introduction 1
Objectives 2
CHAPTER 2: LITERATURE REVIEW 4

PART I: EQUINE BODY COMPOSITION 4
Equine obesity 4
Characterization of equine body composition 6
Weight manipulation in the adult horse 10
Weight loss 11
Weight gain 12
PART II: INSULIN ACTION AND CAUSES OF RESISTANCE 14
Insulin action 15
Release 15
Arrival and action 16
Insulin resistance 20
Impaired insulin sensitivity 20
Impaired insulin effectiveness 23
The role of the β-cell 23
The role of the central nervous system 25
Adiponectin 28
Oxidative stress 29
Equine insulin resistance 31
Dietary impacts on insulin sensitivity 35
Onset of insulin resistance 36
PART III: EQUINE DIABETES AND METABOLIC SYNDROME 38
Equine diabetes 38
The equine Metabolic Syndrome 40
PART IV: MODULATION OF INSULIN SENSITIVITY THROUGH INTERVENTION 44
Diet and exercise 44
Pharmaceutical intervention 46
PART V: MODERN TECHNIQUES FOR THE QUANTIFICATION OF INSULIN SENSITIVITY 48
Simple techniques 48
Oral and intravenous glucose tolerance tests 49
Modern methods: the euglycemic-hyperinsulinemic clamp 50
Modern methods: the minimal model of glucose dynamics 51
Defining the equations 54
Equation 1 55
Equation 2 56
Limitations to the minimal model 57
CHAPTER 3: MANUSCRIPT I 60

Effect of weight gain on glucose and insulin dynamics, leptin and measures of oxidative stress in Thoroughbred geldings 60
Abstract 61
Introduction 63
Materials and methods 64
Results 73
Discussion 82
Literature Cited 87
CHAPTER 4: MANUSCRIPT II 94

Effect of weight gain, diet and exercise on insulin sensitivity in Thoroughbred geldings 94
Abstract 95
Introduction 97
Materials and methods 98
Results 107
Discussion 112
Literature Cited 120
GENERAL SUMMARY AND CONCLUSIONS 125

APPENDIX 128

LITERATURE CITED 134

Vitae 159

List of Tables

Literature Review

Table 1a. Percent fat, body condition score and relevant physiological information of horses and ponies, 1956 - 1979…… 8

Table 1b. Percent fat, body condition scores and relevant physiological information of horses and ponies, 1984 – 2004……..... 9

Manuscript I

Table 1. Nutrient analyses for commercial concentrate (CF) fed to Thoroughbred geldings for a three wk period at the start of the study, treatment concentrate (HS) fed from wk four to the end of weight gain (wk 41), and hay fed throughout the study…….…… 66

Table 2. Ingredient composition of treatment concentrate (HS) fed to Thoroughbred geldings from wk 4 to wk 41…… 68

Table 3. Least-squares means ± SEM for minimal model variables in Thoroughbred geldings derived from a frequently-sampled i.v. glucose tolerance test while consuming commercial concentrate (CFMM) or treatment concentrate (TXMM) at maintenance and throughout weight gain……..... 78

Manuscript II

Table 1. Nutrient analysis (DM basis) for commercial concentrate (CF), fed at maintenance to Thoroughbred geldings for a three wk period at the start of the study, the treatment concentrates (HS, high in starch and sugar and

HF, high in fat and fiber) fed from wk four to the end of weight gain (wk 41), and hay fed throughout the study…… 100

Table 2. Ingredient composition of treatment concentrates, formulated to be either high in starch and sugar (HS) or high in fat and fiber (HF) fed from wk four to the end of weight gain (wk 41)…….. 102

Table 3. Least-squares means ± SEM for minimal model variables during weight gain in Thoroughbred geldings fed a diet high in starch and sugar (HS, n = 9) or fat and fiber (HF, n = 6) after the third week of commercial concentrate feeding at maintenance (CFMM), after the fourth week of treatment concentrate feeding at maintenance (TXMM) and following 90.8 kg of BW gain (ENDMM)……..... 111

Table 4. Least-squares means ± SEM for minimal model variables in Thoroughbred geldings fed a diet high in starch and sugar (HS, n = 9) or fat and fiber (HF, n = 6) following the completion of 90.8 kg BW gain (ENDMM) and after 2 wk of minimal exercise (MINEX)…..……. 113

List of Figures

Literature Review

Figure 1. Release of insulin from the pancreatic β-cells……. 17

Figure 2. GLUT-4 translocation following insulin binding and activation of the signal transduction cascade……..……. 19

Figure 3. Insulin sensitivity and insulin effectiveness……..... 21

Figure 4. Insulin receptor……. 22

Figure 5. Glucose effectiveness…….……... 24

Figure 6. The minimal model of glucose and insulin dynamics following a frequently sampled i.v. glucose tolerance test…….. 52

Manuscript I

Figure 1. Changes in average BW and BCS of Thoroughbred geldings (n = 9) while fed commercial concentrate (wk 3) or treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41)……..……..… 75

Figure 2. Average digestible energy intake (Mcal/d; n = 8) and ADG (kg/d; n = 9) in Thoroughbred geldings while consuming commercial concentrate (wk 3) or treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41)……..……..…….…… 76

Figure 3. Average serum leptin levels (ng/mL) in Thoroughbred geldings (n = 9) while consuming commercial concentrate (wk 3) and treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41)……... 79

Figure 4. Average serum cortisol (nmol/L) in Thoroughbred geldings (n = 9) while consuming commercial concentrate (wk 3) or treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41)……..…..……... 80

Figure 5. Average plasma lipid hydroperoxide (LPO; uM/L, n = 9), a measure of oxidative stress-induced cell membrane lipid peroxidation damage, in Thoroughbred geldings while consuming commercial concentrate (wk 3) and treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41)……..… 81

Figure 6. Average whole-blood reduced (GSH; uM/L) and oxidized (GSSG; uM/L) glutathione, an indication of the ability of the glutathione peroxidase antioxidant system to combat oxidative stress, in Thoroughbred geldings (n = 9) while consuming commercial concentrate (wk 3) or treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41)……..……. 82

Manuscript II

Figure 1. Changes in average BW and BCS of Thoroughbred geldings (n = 15) while fed commercial concentrate (wk 3) or treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41)……..…….. 108

Figure 2. Average digestible energy intake (Mcal/d; n = 14) and ADG (kg/d; n = 15) in Thoroughbred geldings while consuming commercial concentrate (wk 3) or treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41)……..……..…….……. 110

Appendix

Figure I Raw plasma glucose and serum insulin responses of Thoroughbred geldings to the frequently sampled i.v. glucose tolerance test following the third week of commercial concentrate (CFMM)…… 128

Figure II Raw plasma glucose and serum insulin responses of Thoroughbred geldings to the frequently sampled i.v. glucose tolerance test following the fourth week of treatment concentrate (TXMM)…..……...……... 129

Figure III Raw plasma glucose responses of Thoroughbred geldings to the frequently sampled i.v. glucose tolerance test following each 22.7 kg gain,WG1, WG2, WG3 and WG4…….……... 130

Figure IV Raw serum insulin responses of Thoroughbred geldings to the frequently sampled i.v. glucose tolerance test following each 22.7 kg gain,WG1, WG2, WG3 and WG4……...……..…….. 131

Figure V Raw plasma glucose and serum insulin responses of Thoroughbred geldings to the frequently sampled i.v. glucose tolerance test following 90.8 kg BW gain (ENDMM)……...……. 132

Figure VI Raw plasma glucose and serum insulin responses of Thoroughbred geldings to the frequently sampled i.v. glucose tolerance test following 2 wk of minimal exercise (MINEX)…… 133

CHAPTER 1: INTRODUCTION AND OBJECTIVES

Introduction

Obesity in the horse and pony has been associated with several negative health conditions, including a decreased ability to thermoregulate in hot weather (Cymbaluk and Christison, 1990), reduced athletic ability (Webb et al., 1989), abnormal estrous cycling (Gentry et al., 2002; Fitzgerald et al., 2003), insulin resistance (IR; Hoffman et al., 2003) and laminitis (Treiber et al., 2006). Of the afore-mentioned issues, IR is the most insidious, appearing in otherwise healthy animals and slowly increasing the risk of metabolic diseases, such as laminitis, in susceptible individuals (Johnson et al., 2002). As the cornerstone of the human Metabolic Syndrome, IR has been linked to diabetes and cardiovascular disease and is a widely studied metabolic disorder with numerous contributing factors. In the equid, early observations of hormonal dysregulation during induced and naturally occurring laminitis led to key experiments which linked IR and laminitis, especially in obese ponies (Coffman and Colles, 1983). Insulin resistance in the horse and pony has been quantified using techniques developed in human medicine, adapted for use in veterinary medicine and equine research (Hoffman et al., 2003). An understanding of the etiology of IR as it relates to metabolic disease in humans will allow for parallels to be drawn to the horse, some of which have already been explored. The pages to follow are meant to both encompass current knowledge in equine nutrition as it relates to obesity in the horse and pony and to present original research findings based on related hypotheses.

Objectives

The objectives of this dissertation are to:

1. Summarize the current knowledge of obesity and IR in the horse in such a way that the link between IR and metabolic disease becomes apparent. Through a review of previous research in the horse and pony, the hypothesis of excessive adiposity as a contributing factor to laminitis will be established.

2. Provide background information on insulin sensitivity (SI) drawn from human and other research models to lend credence to this field as well as establish its general importance. The depth of information provided is meant to cover current knowledge of insulin secretion and action as well as highlight the many factors that interact in the complex etiology of the human Metabolic Syndrome and its applications to the horse.

3. Describe techniques used for the determination of glucose tolerance, ranging from simple, indirect estimates to sophisticated, complex protocols. Special emphasis will be place on the minimal model of glucose dynamics, as it is the method used in the original research presented herein.

4. Present original research findings related to body weight, diet and exercise effects on SI in the horse. Objectives of the research include

a. An evaluation of the effect of controlled weight gain on SI, the satiety hormone leptin and two markers of oxidative stress, lipid hydroperoxide (LPO) and glutathione in the mature Thoroughbred gelding.

b. An estimation of the contribution of dietary energy source to changes in SI that may occur prior to and following controlled weight gain utilizing two treatment concentrates, one high in starch and sugar and the other high in fat and fiber.

c. An appraisal of the impact of exercise on SI through the cessation of light, regular exercise as well as any contributing effects of dietary energy source.

CHAPTER 2: LITERATURE REVIEW

PART I: EQUINE BODY COMPOSITION

Equine obesity

Horses deposit s.c. fat in specific areas of the body, including the crest of the neck, on the withers, behind the shoulder, over the ribs, along the loin and around the tailhead (Henneke et al., 1983). Additionally, fat is deposited around the internal organs and viscera, with additional fat depots located in the perineum, supraorbital fossa, orbit of the eye, mediastinum, subepicardium, mesentery, omentum, around the kidney, in the epidural space and on the abdominal floor (Bianchi, 1989). Equine body fat has been measured using visual appraisal of the horse's body condition, ultrasound, total body water, cadaver dissection and others as discussed in a review by Kearns et al. (2002). The most common methods of estimating body fat composition in the horse are a standardized body condition score (BCS) system (Henneke et al., 1983; Carroll and Huntington, 1988) and the use of ultrasound (Westervelt et al. 1976; Kane et al., 1987).

The most widely used BCS system was developed by Henneke et al. (1983) and is a subjective measure of body fat accumulation determined via observation and palpation of fat deposition in specific areas on the horse (neck, withers, loin, tailhead, shoulder and ribs). The degree of body fat cover over the skeletal structure is used to create a numerical score that ranges from 1 to 9, with half scores possible. The Henneke BCS system was developed to study the effects of BCS on mare reproductive efficiency and was patterned after a similar system used in cattle (Henneke et al., 1983). Although it has

been recommended for use in all types of equids, including both light horse and draft breeds (Potter et al., 1987), as well as ponies (Freestone et al., 1992), the original system was developed in pre-parturient and lactating Quarter Horse mares (Henneke et al., 1983). While the Henneke BCS system is very useful for tracking body fat changes in an individual animal, its limitations are recognized when differences in gender, age, breed, and conformation among horses are considered. Additionally, users of a BCS system must be properly trained on the usage of the system in order to attain proper repeatability, reproducibility and predictability (Burkholder, 2002), which may limit the ability of a BCS to be compared across studies. Using the Henneke BCS system, it is generally recommended that horses be maintained at a BCS of 4.5-6, depending on the physiological status of the horse.

The use of the term 'obesity' has only recently begun to be used in published equine literature (Hoffman et al., 2003), with ambiguous terms such as 'fat' or 'well-conditioned' pervasive in the past literature. Such terms have always relied heavily on a visual component, as the 'fat' ponies or horses sometimes are found to weigh less than the 'normal' individuals (Jeffcott et al., 1986). It has generally been accepted, however, that any horse or pony with a BCS above 7 is most definitely 'fat' and may even be 'obese.' In the Henneke system, a BCS of 7 is defined as 'fleshy' with a noticeable filling of fat between the ribs, along the withers, behind the shoulder and along the neck. A BCS of 8 is considered 'fat,' with a visible crease down the back, the neck and shoulder are flush with the body due to fat filling the area behind the shoulder, the ribs can only be felt with difficulty and fat is beginning to be deposited between the thighs (gaskins; Henneke et al., 1983). The distinction between BCS 8 and BCS 9 ('extremely

fat') lies in the bulging of the fat in the six areas, as well as fat accumulation between the inner thighs to the point where the gaskins rub together.

While a true definition of equine obesity has not been presented in the literature, obesity in the human 'refers specifically to having an abnormally high proportion of body fat' and is measured using the body mass index (BMI, weight (kg)/height (m^2); NIH, 1988). Because an equation similar to the BMI does not yet exist in the horse, the designation of 'obese' in the horse is only ambiguously defined using the Henneke BCS system and the subsequently re-defined BCS of 7, 8 and 9 by Hoffman et al. (2003). Until a more objective system for estimating the degree of fatness in horses is developed, the use of the term 'obese' may be misleading, in that the current Henneke BCS system allows for a significant amount of individual interpretation for each score, resulting in a very broad definition of the term.

Characterization of equine body composition

The use of B-mode ultrasound provides a repeatable, objective measurement of equine body fat percentage and has been validated by carcass dissection (Westervelt et al., 1976; Kane et al., 1987). Ultrasonic measurements of adipose tissue depth on the rump and near the tailhead have been used to estimate body fat composition, with increasing fat depth correlated with increasing BCS (Freestone et al., 1992; Freestone et al., 1992b; Powell et al., 2002; Webb et al., 1989; Gentry, 2001). Variation in the estimation of body fat using ultrasound can occur due to variation in scan site location, which may be due to user error or animal conformation differences. Fat depth is greatest at the tailhead and

decreases with rostral progression (Westervelt et al., 1976, Kane et al., 1987). The scan site locations and prediction equations developed by Kane et al. (1987) are the most widely used in the literature.

Body composition studies on horses using carcass dissection, total body water methods and ultrasound have shown that body fat percentages range from 1.12 to 34.6 % in horses of various breed, physiological state and BCS (Table 1a, b). Of note, mature mares seem to carry the most body fat as compared to their younger counterparts and males (Fitzgerald and McManus, 2000; Lehnhard et al., 2004) and horses developed for speed or endurance or both, such as Thoroughbreds and Arabians, seem to be more lean than horses developed for power, such as Percherons (Julian, 1956), or even the hardy pony breeds (Gunn, 1986).

Adipose tissue is now characterized as a dynamic endocrine organ, producing hormones and inflammatory mediators that are believed to be contributing factors to many human metabolic diseases, including atherosclerosis, hypertension, IR and type II diabetes (Vettor et al., 2005). A distinction must be made, however, between s.c. and visceral adipose tissue, which contribute differently to the pathogenesis of disease (Wajchenberg, 2000; Kahn, 2003). Visceral fat is composed of omental, mesenteric and retroperitoneal fat masses surrounding the intestines and kidney and can only be accurately measured in the live individual using computed tomography or magnetic resonance imagery (Wajchenberg, 2000). Such measurements have not been used in the horse to date and the available carcass data does not differentiate between adipose tissue collection sites when determining body fat percentage.

Table 1a. Percent fat, body condition score and relevant physiological information of horses and ponies, 1956 - 1979

Study	Breed (n)	Sex[d]	Age	Physiological state (n)	BCS	Body weight (kg)	Body fat (%)
Julian et al., 1956[a]	Percheron (4)	M	Mature	-	-	865.75	24.5
	Thoroughbred (2)	M, G	Mature	-	-	472	14.1
	Quarterhorse (2)	M	Mature	-	-	386	7.55
	Arabian (1)	S	Mature	-	-	346	8.6
	Am. Saddlebred (1)	M	Mature	-	-	557	24.7
Robb et al., 1972[b]	Shetland pony (11)	S, G, M	8mo-18y	-	-	-	7 – 19
Westervelt et al., 1976[b,c]	unspecified horse (8)	-	-	-	-	336-559	15.88
	unspecified pony (15)	-	-	Ad libitum fed (8)	-	150.6	6.40
	Shetland pony (11)	-	-	Non-exercised (6)	-	167.8	15.03
		-	-	Exercised (5)	-	139.9	8.96
Webb & Weaver, 1979[b]	unspecified pony (12) Thoroughbred (5)	S, G, M	1.5 - 14	diseased (11) healthy (6)			5.06
Kane et al., 1987[b,c]	unspecified horse (6)	-	1 – 26	-	-	281-474	10.1-24

[a]TBW = total body water
[b]CD = carcass dissection
[c]US = ultrasound
[d]Sex, M = mare, S = stallion, G = gelding

Table 1b. Percent fat, body condition score and relevant physiological information of horses and ponies, 1984 - 2004

Study	Breed (n)	Sex[d]	Age	Physiological state (n)	BCS	BW (kg)	Body fat (%)
Henneke et al., 1984[c]	Quarterhorse (32)	M	-	Pre-foaling (32)	6.1	522.8	13.8
				Post-foaling (16)	7.6	532.5	16.25
				Post-foaling (16)	3.6	490	11.4
Gunn, 1986[b]	Thoroughbred (21)	-		-		0.6-490	1.12
	Other (16)					3-535	2.11
Powell et al., 1989[c]	Quarterhorse (12)	M	-	65d gestation	6.2	554.4	10.6
		M	-	215d gestation	6.9	619.2	14.2
Fitzgerald and McManus, 2000[c,e]	Thoroughbred cross (23)	M	>10	Non-pregnant	-	470 - 540	12 - 15.5
			2-5	Non-pregnant	-	419 - 459	7 - 11
Kearns et al., 2002[c]	Standardbred (14)	M, S	3.1 – 3.5	Elite racehorses	-	432 - 444	7.4 - 9.9
Powell et al., 2002[c]	Various breeds (12)	M	16-20	Lean	4.4	522	9.15
				Obese	8.8	594	31.3
Lehnhard et al., 2004[c]	Quarter Horse (31)	M	4-8	Young	-	512.7	20.5
		M	>20	Old, skinny (5)	-	507.8	14.2
				Old, fat (3)	-	563.1	26.6

[a]TBW = total body water
[b]CD = carcass dissection
[c]US = ultrasound
[d]Sex, M = mare, S = stallion, G = gelding
[e]estimated from authors' graph

Subcutaneous adipose tissue is found under the skin and distributed throughout the body, quantified in humans using techniques such as waist-to-hip ratio and skinfold thickness (Wajchenberg, 2000) and body condition scoring in horses (Henneke et al., 1983). In humans, both s.c. and visceral fat are metabolically active, producing both hormones and inflammatory mediators that are strong contributors to the onset of metabolic disease. However, it is the relative proportion of visceral fat which determines a person's risk for development of abnormal glucose and insulin regulation and ultimately type II diabetes (Wajchenberg, 2000; Kahn, 2003). Indeed was found that humans with greater intra-abdominal fat were more insulin resistant than those with increased s.c. fat, even if one group were more lean than the other (Kahn, 2003). As with humans and other mammals, it is possible that individual variations in visceral and s.c. fat deposition may partially explain the differences in susceptibility to metabolic disease between and within equine breeds. The metabolically active factors secreted from adipose tissue are numerous and a full discussion is beyond the scope of this review (see Wajchenberg, 2000). At this time in the horse, the factors of interest in the development of IR include leptin, adiponectin, oxidative stress and cytokines such as tumor necrosis factor-α (TNF-α), all of which will be discussed in future sections.

Weight manipulation in the adult horse

A standardized protocol for manipulating horse BW for research purposes does not exist in the literature. Because of this, many studies sample groups of horses at different BW and BCS and represent the data as a continuum of BW and BCS in order to correlate

the variable of interest to weight or adiposity. In the studies which have manipulated BW to attain a certain BCS, the resulting groups of horses are usually polarized into high or low BCS groups, depending on the objective of the study. Unfortunately, the procedures used to encourage either weight loss or weight gain in past studies have usually been omitted. In addition, those studies which specifically report the techniques used to manipulate BW often utilize mixed breeds and sexes, omitting the breed type but specifying only 'light-breed,' draft or pony in many cases. However, anecdotal evidence exists for differences in body type, metabolism and feed efficiency within the light-horse breeds which can have a profound effect on the success of a weight manipulation protocol. For example, Thoroughbred horses are traditionally known for having difficulty in maintaining BW and exhibit a well-defined bone structure visible under a typically lean physique. It is likely that this breed will respond faster to a weight loss protocol than a weight gain protocol and may require a more concentrated source of calories to encourage weight gain as compared to a Quarter Horse or Arabian, known for a more rapid weight gain response.

Weight loss. Few studies have directly examined the effect of caloric intake on weight loss or weight gain. Powell et al., (2002) fed timothy hay to six mares (16 – 20 years old) of 'various breeds' at 50% of their maintenance DE requirement (11 Mcal DE/d). The resulting weight loss was 1.6 kg/d and the mares dropped one BCS every 16 d. A slightly slower weight loss strategy was used by Gentry et al. (2002), who limit-fed 'light horse' mares on Alicia bermudagrass and winter ryegrass (2 h/d) until four body condition

scores were lost (BCS 7 to 3). The resulting weight loss was 1.1 kg/d and one BCS was lost every 23 d.

Weight gain. Within the new National Research Council Nutrient Requirements of the Horse (NRC, 2007), the DE recommendations for maintenance have been adjusted and expanded to account for draft horses, ponies and those horses with greater or lesser requirements. Similarly, it should be expected that the DE required for gain in the adult horse should vary with breed and type and that the recommendations made by Lawrence (2000) may not be adequate for all horses. Indeed, it is likely that the DE requirement for weight gain in the Thoroughbred would be different than that of the draft horse, as might the rapidity at which weight is gained and the ease of maintaining the achieved BW.

Heusner et al. (1993) fed horses of mixed sex (breed not given) 44 Mcal/d, corresponding to approximately 27 Mcal/d above maintenance for the 500 kg horse. Using this strategy, the horses gained 1 kg per day and increased their BCS by one unit every 20 d. A slightly lesser intake was found by Martin-Rosset and Vermorel (1991), who calculated 18 Mcal above maintenance was required per kg of gain. In draft horses, a gain of 1 kg/d required 16 – 21 Mcal above maintenance (Potter et al., 1987). The results of these three studies were used to outline recommendations for weight gain in the adult horse within the NRC (1989; 2007). However, research in Thoroughbreds (Quinn et al., 2007) found that only 0.35 kg/d BW gain could be achieved when horses were offered up to 20 Mcal/d above their daily DE requirements, taking 80 d for one BCS gain. Additionally, it was calculated that and intake of 40 Mcal/kg above maintenance would

have been required to achieve one kg of gain per day, an intake which was not achieved by any horse.

In addition to having a slower rate of gain, the weight gain associated with a one-unit increase in BCS in the Thoroughbred does not appear to be the same as the studies cited by the NRC. One unit of BCS is reported to correspond to 20 – 25 kg of BW in horses of mixed breed and sex (Heusner et al., 1993; Powell et al., 2002; Gentry et al., 2002) but has been found to be approximately 32 kg in the Thoroughbred (Quinn et al., 2007). A likely explanation for the breed-specific difference may be the body conformation of the Thoroughbred as compared to other light-horse breeds, such as the Quarter Horse. It may be that more fat or muscle or both is required to fill out the six BCS areas to the level defined by the Henneke BCS system, and in fact Suagee (unpublished data, 2007) found that the Henneke system did not accurately describe fat accretion in the neck, withers and loin in Thoroughbred geldings.

Obesity in the Thoroughbred has been reported in the literature (Hoffman et al., 2003), and while not as common as obesity in the pony, Morgan, Spanish Mustang or European Warmblood (Johnson, 2002), it may be similarly effective in inducing a state of metabolic dysfunction. Within the pony, IR and elevated blood triglycerides are risk factors for metabolic disease that have been implicated in the pathogenesis of pasture-associated laminitis (Treiber et al., 2006) and may similarly be involved in the obese Thoroughbred.

PART II: INSULIN ACTION AND CAUSES OF RESISTANCE

Before describing the complex interplay between hormones and inflammatory factors which may be involved in the pathogenesis of IR, the term must be adequately defined. Insulin resistance is defined as the impaired response of insulin-sensitive cells in the peripheral tissue, whereby the normal level of insulin secretion in response to glucose is not enough to encourage adequate glucose disposal into the peripheral tissues (Wilcox, 2005). Insulin resistance in peripheral tissues can be partitioned into two components, 'insulin sensitivity (SI)' and 'insulin effectiveness' (Kahn, 1979; Kronfeld et al., 2005), which are used interchangeably in the literature.

Insulin sensitivity refers to the cellular actions following insulin arrival at the target tissue, most prominently the binding of insulin to its receptor and initiation of the intra-cellular signal transduction cascade. Glucose transport and metabolism within the cell are affected by insulin effectiveness and IR occurs when there is a reduction in SI, insulin effectiveness or both. As both problems are usually occurring simultaneously, SI has become the preferred all-encompassing term to describe insulin's effect within the peripheral tissues, with a reduction in SI leading to IR. Indeed, known causes of IR in the peripheral tissue include impairments in receptor function and the subsequent signal transduction cascade and reduced intra-cellular insulin-dependent glucose transporter activity (Wilcox, 2005; Yang et al., 1989).

Insulin action

Release. The protein hormone insulin is released from the β-cells of the Islets of Langerhans within the pancreas (Figure 1), in response to blood glucose, and to a lesser extent fat and protein. Glucose is taken up into the β-cells by the glucose transporter-2 (GLUT-2), one of a series of transport proteins involved in glucose metabolism in various tissue types (Ren et al., 2007). Once inside the β-cell, glucose is metabolized, culminating in ATP production, the closure of sodium-potassium-ATPase channels and the generation of a membrane-depolarizing charge. Following membrane depolarization, an influx of extra-cellular calcium leads to exocytosis of insulin secretory granules (Ren et al., 2007). Two distinct pools of secretory granules are found within the β-cells and are each responsible for the characteristic insulin secretory response, which occurs in two phases. A small pool of secretory granules remains primed for quick release and is responsible for the first phase of the insulin response. The second pool is made up of reserved secretory granules and is released more slowly, creating the second phase of the insulin response.

The first-phase insulin response occurs during the first ten minutes following glucose administration and the second phase occurs as the first phase diminishes and may or may not be visible during blood testing (Wilcox, 2005). During meal feeding, this first response occurs before glucose is detected within the bloodstream and is driven by the central nervous system (Storlien et al., 2004). This response, known as the cephalic phase, is believed to occur in order to initiate rapid inhibition of hepatic glucose output and fatty acid release, preparing the peripheral tissues for anabolic metabolite uptake

(Storlien et al., 2004). Such a phenomenon is assumed to occur in the horse, as the insulin response to glucose administration or feed is not dissimilar to other animal models (Giraudet et al., 1994). Following intravenous glucose administration, horses fed a diet of ad libitum hay demonstrated a rapid increase in insulin, reaching its highest value within 5 minutes, followed by a plateau that lasted for the duration of the 30 min study (Giraudet et al., 1994). While glucose values began to decline almost immediately, baseline was not reached within 30 min. Garcia and Beech (1986) observed a return to baseline of 60 min for glucose and 180 min for insulin following intravenous glucose dosing.

Arrival and action. Insulin is released into the portal vein and, after passing through the liver, makes its way into the interstitial space by crossing the capillary-endothelial barrier (Bergman, 1997). The interstitial fluid is in intimate contact with cells of the target tissue (adipose, liver, muscle) and it is from this location that insulin has its action. Impaired movement of insulin from the pancreas to the target tissue (impaired trans-capillary movement) was at one time believed to be the primary defect underlying IR (Yang et al., 1989). However, lymph insulin, known to be reflective of the insulin concentration within the interstitial space (Bergman et al., 1990), circulates in proportion to body size, with obese individuals having greater lymph insulin than lean individuals (Castillo et al., 1994). Additionally, insulin actually arrives at the interstitial space faster under IR conditions, perhaps as a compensatory mechanism (Bergman et al., 1990).

Following binding of insulin to its receptor, the insulin-receptor substrate-1 (IRS-1) is activated, initiating the signal transduction cascade, culminating in the movement of

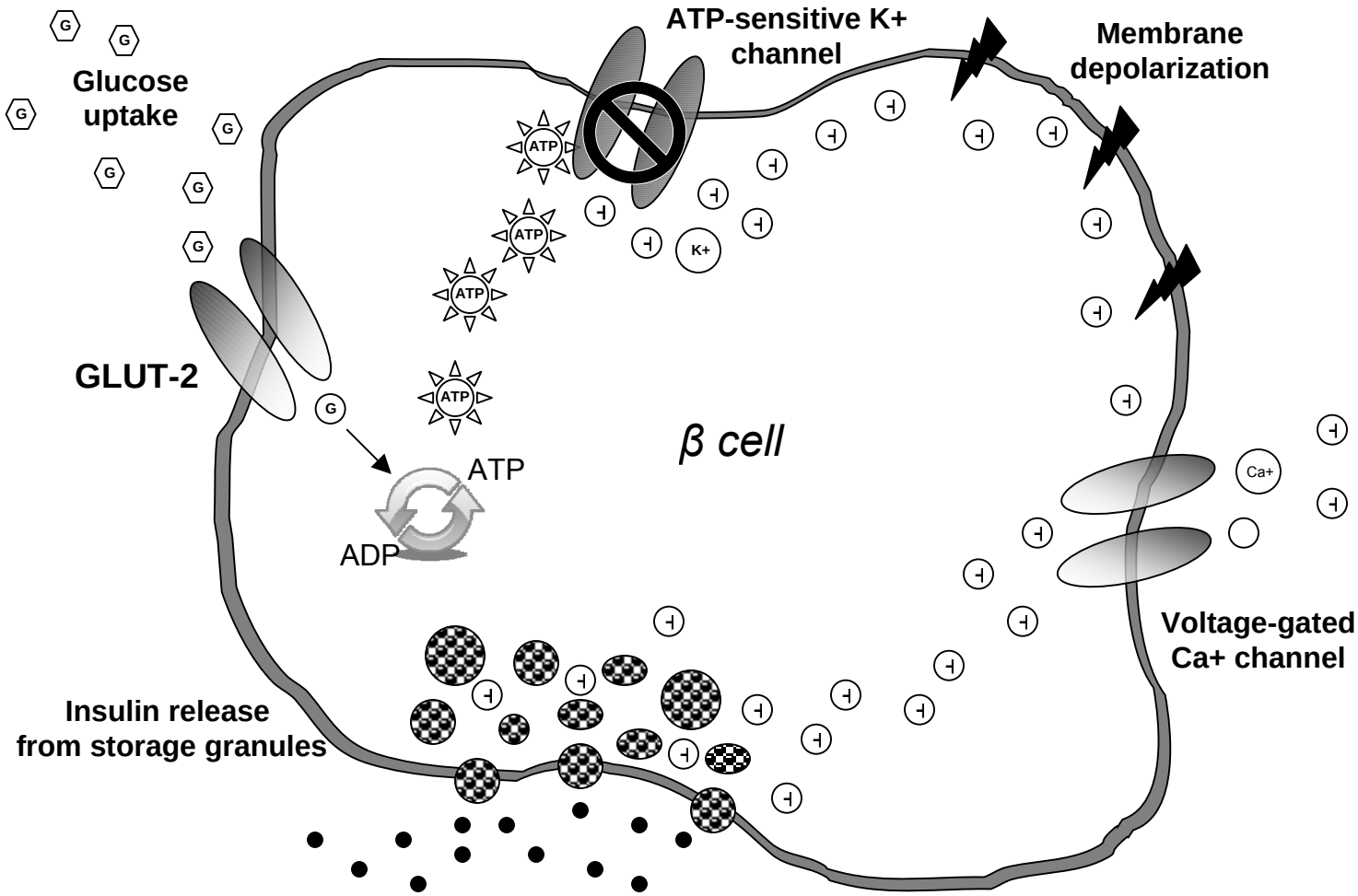


Figure 1. Release of insulin from the pancreatic β-cells. *Adapted from www.betacell.org*.

GLUT-4, the insulin-sensitive glucose transporter, to the cell surface (Figure 2; Chang et al., 2004). The level of whole-body insulin-stimulated glucose uptake is highly correlated with GLUT-4 activity (Koranyi et al., 1991) and equine tissues have been found to express GLUT-4 (Filho et al., 2006).

During fasting and at basal glucose levels, euglycemia is maintained via the actions of GLUT-1, the primary glucose transporter involved in glucose transport into the brain and central nervous system, as well as basal glucose uptake in peripheral tissues (Wilcox, 2005). The actions of GLUT-1 are controlled only by glucose and work to enhance glucose utilization within those tissues as well as suppress unnecessary endogenous hepatic production (Ader, 1997). Indeed, in diabetic individuals with insufficient SI, glucose-stimulated glucose disposal is the primary vector for glucose uptake into the brain, CNS and peripheral tissues (Ader, 1997). Additionally, over-expression of GLUT-1 in cell culture results in enhanced glucose uptake under hyperglycemic conditions (Heilig et al., 1995). The effects of glucose at euglycemia are almost hormone-like and have been termed 'glucose effectiveness (Sg).' While almost 70% of Sg is devoted to glucose uptake, the remainder is committed to the suppression of hepatic glucose production (Ader et al., 1997). Although Sg is the primary vector for glucose disposal at euglycemia, it is only responsible for approximately 80% of the total uptake during that time due to the reduced yet still present actions of insulin (Kahn et al., 1994). The action of Sg is not limited to euglycemia, however, in that following a meal or glucose infusion, the lull between glucose arrival at the pancreas and insulin arrival at the peripheral tissue is filled by Sg (Kahn et al., 1994).

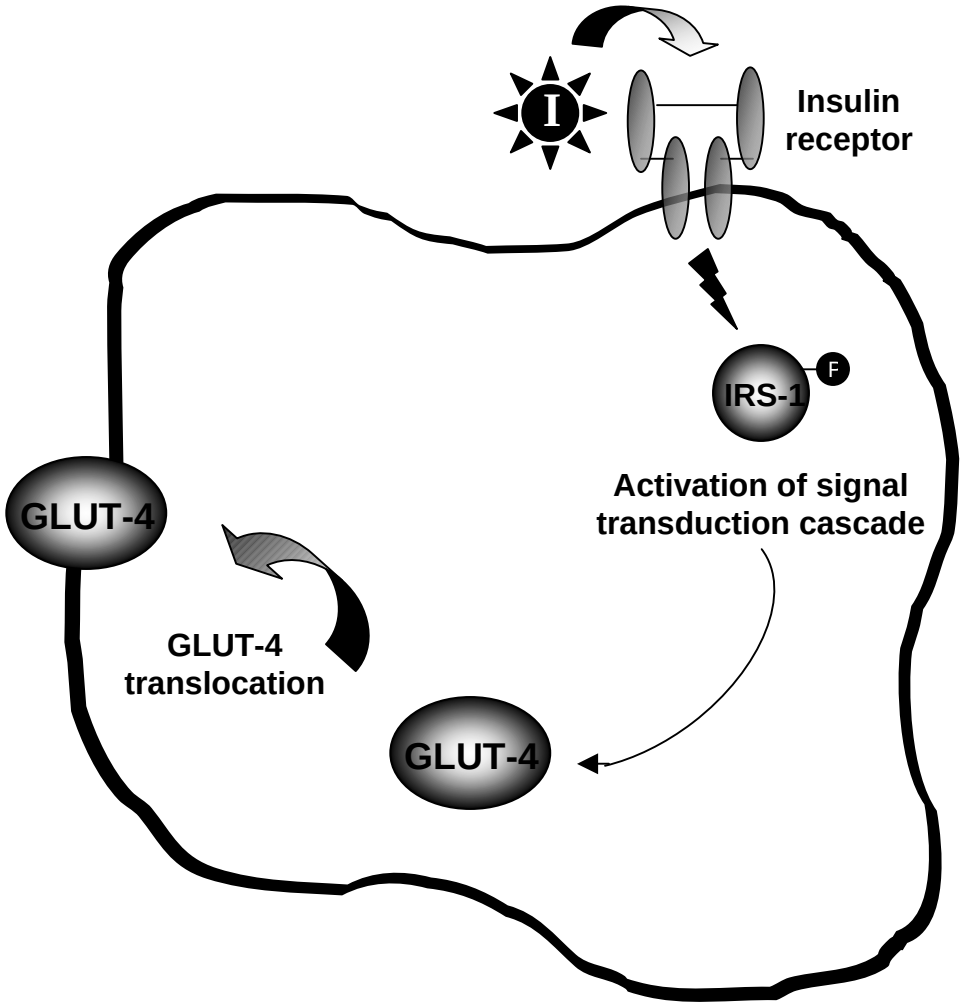


Figure 2. GLUT-4 translocation following insulin binding and activation of the signal transduction cascade.

Insulin resistance

The ultimate source of insulin resistance may be due to a reduction in the cellular response to an otherwise adequate concentration of insulin or a need for increased insulin secretion to stimulate an adequate cellular response (Kahn, 1978; Kronfeld et al., 2005b). When presented graphically (Figure 3), a reduction in SI requires that a greater concentration of insulin be released to achieve the maximal biological response. A change in insulin effectiveness occurs when the biological response to a normal concentration of hormone is reduced.

Impaired insulin sensitivity. Insulin sensitivity refers specifically to the ability of the insulin receptor to stimulate glucose transport into the cell (Kronfeld et al., 2005). The insulin receptor is a dimer, consisting of two α subunits and two β subunits, the latter of which spans the plasma membrane (Figure 4). Normal insulin binding at the extra-cellular α subunit initiates a series of biochemical reactions, including auto-phosphorylation of the β subunit, followed by the activation of intrinsic tyrosine kinase activity and the activation of the IRS-1 (Youngren, 2007). The IRS-1 then initiates the rest of the signal transduction cascade, culminating in GLUT-1 translocation to the cell surface. When SI is reduced, the ability of the receptor to auto-phosphorylate following insulin binding is reduced, as is the ensuing tyrosine kinase phosphorylation (Youngren, 2007). Without the ability to activate the IRS-1, activation of the downstream signal transduction cascade is short- circuited, resulting in a lack of GLUT-4 translocation

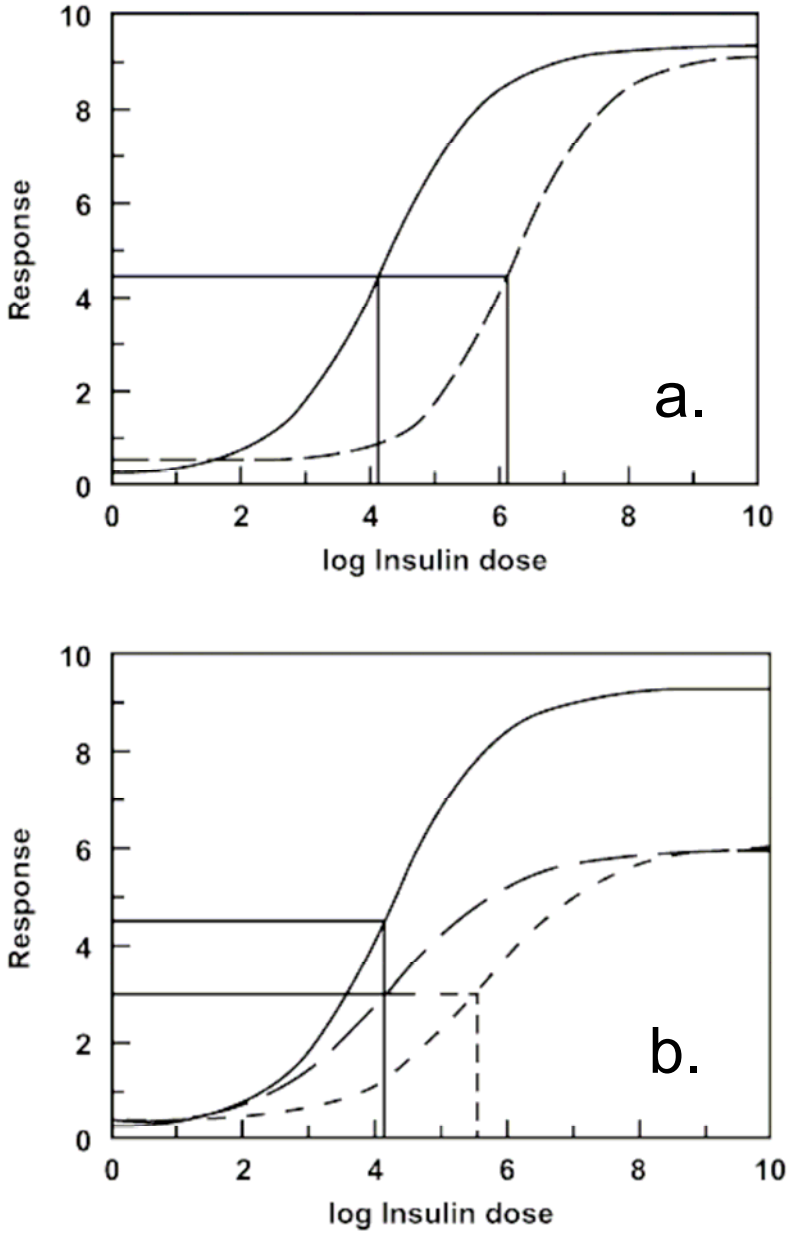


Figure 3. Insulin sensitivity and insulin effectiveness. Decreased SI (a) resulting from a shift in the curve to the right, increasing the amount of hormone released to encourage a half-maximal response (K_m). Decreased insulin effectiveness (b) resulting from a decrease in the upper asymptote and reduced maximal physiological response (V_{max}). From Kronfeld et al., 2005b.

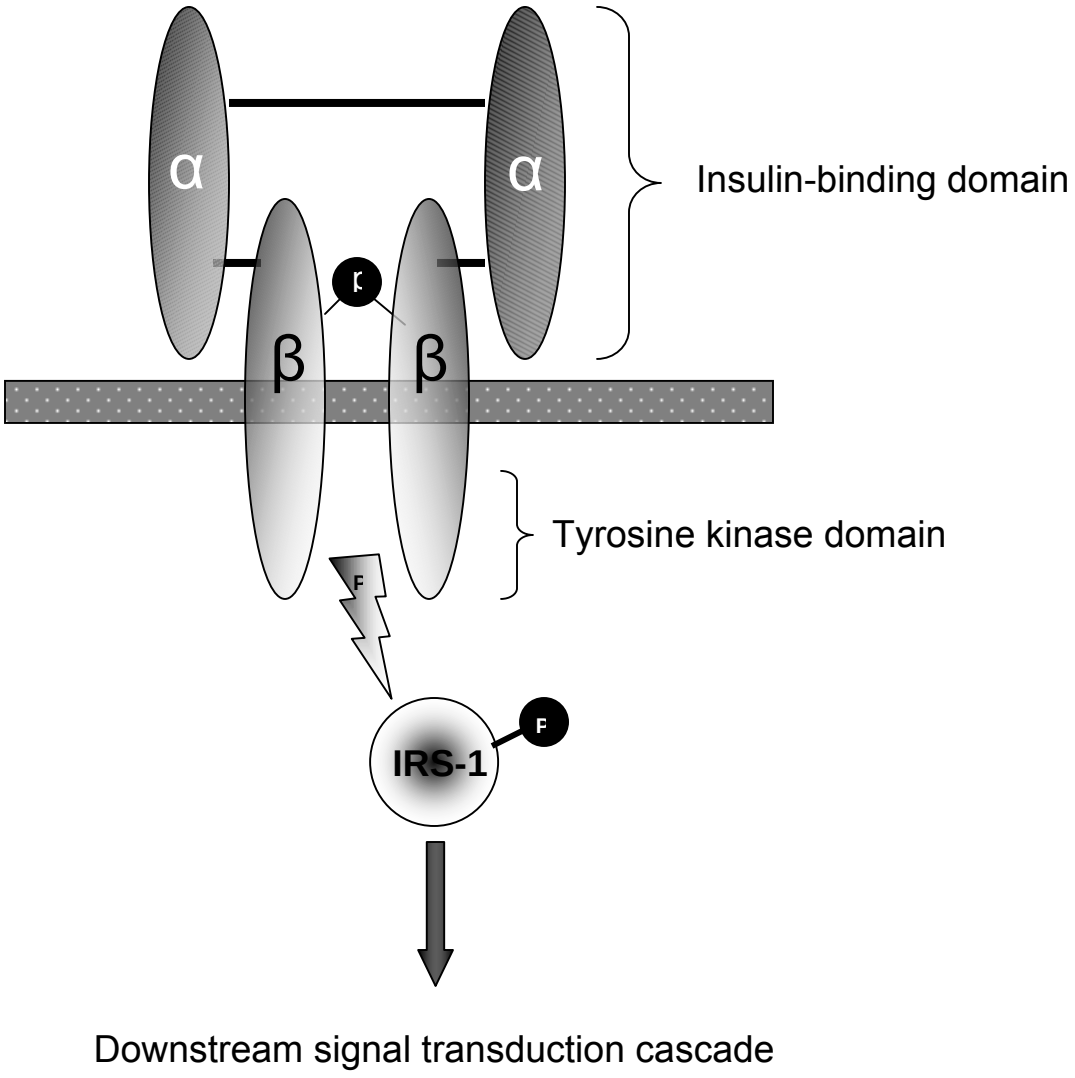


Figure 4. Insulin receptor. Following insulin binding at the α-subunit of the insulin receptor, autophosphorylation of the β-subunits and activation of intrinsic tyrosine kinase activity leads to the phosphorylation and subsequent activation of the IRS-1 and initiation of the downstream signal transduction cascade. Adapted from Schwartz et al., 2000.

to the cell surface and unsuccessful glucose uptake (Wilcox, 2005; Petersen and Schulman, 2006).

Impaired insulin effectiveness. The effectiveness of insulin ultimately depends on the completion of glycogen formation, resulting from successful GLUT-4 glucose uptake and activation of the cellular metabolic processes such as hexokinase, required for the conversion of glucose-6-phosphate to UDP-glucose (Figure 5; Petersen and Schulman, 2006). Excessive FFA and hyperinsulinemia are two important causes of impaired insulin effectiveness (Ye, 2007) and have been implicated in the inhibition of both GLUT-4 and hexokinase activity in human obesity and diabetes (Petersen and Schulman, 2006). Free fatty acids are also associated with a rise in protein kinase C (PKC), which phosphorylates the IRS-1 at a serine residue, resulting in inhibition of its actions (Yu et al., 2002).

The role of the B-cell. The full pathogenesis of IR is not entirely related to functional impairments in the peripheral tissues. Once IR has been diagnosed, it is usually found that there is some level of β-cell dysfunction already occurring, although it is usually unclear as to whether the IR was caused by the dysfunction or the dysfunction caused the IR (Kahn, 2003). For example, the pancreas can compensate for IR by increasing the sensitivity of the β-cells to glucose (Bergman, 1997). In turn, increased β-cell sensitivity leads to increased insulin secretion, eventually resulting in hyperinsulinemia, which in itself can cause IR (Polonsky et al., 1988; Marangou et al., 1986).

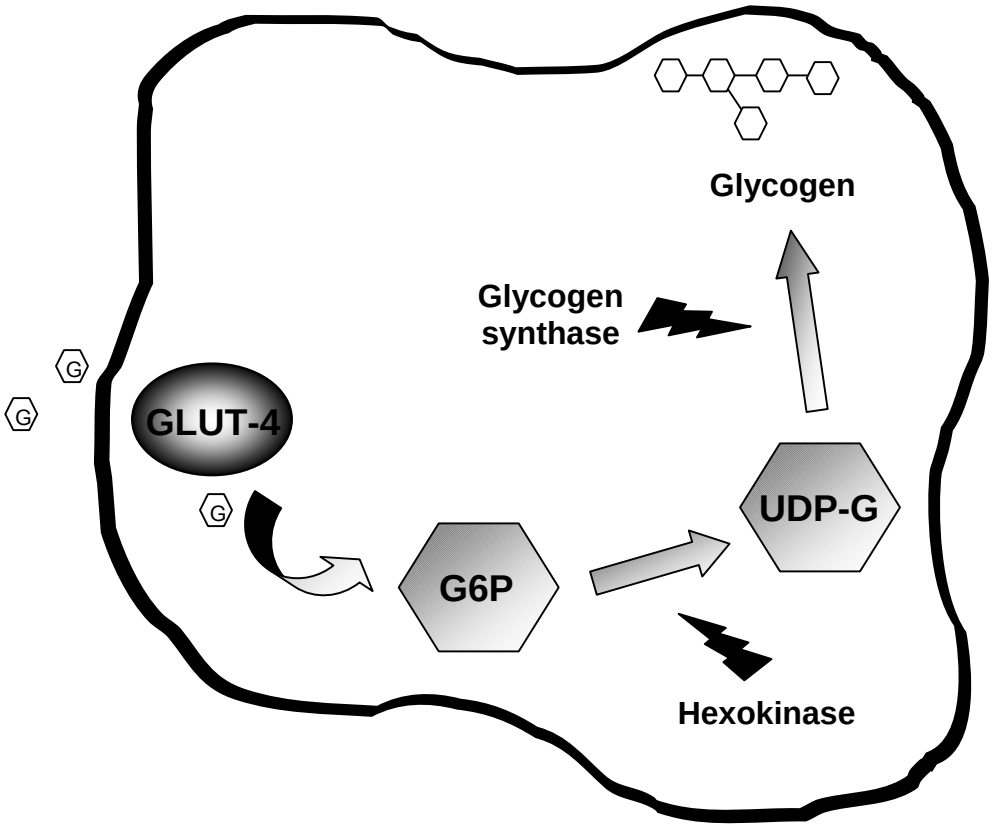


Figure 5. Glucose effectiveness. Glucose metabolism within the peripheral tissue. Adapted from Petersen and Schulman, 2006.

The ability of the β-cell to adjust the level of insulin secretion to the degree of SI is an important concept underlying the ultimate diagnosis of IR. The relationship between SI and insulin secretion in response to glucose is hyperbolic (Flanagan et al., 2000) and is described as the product of the two, for any given degree of glucose tolerance (Kahn, 2003). Because of this relationship, a change in SI is balanced by a comparative, reciprocal change in β-cell function (Kahn et al., 2001). Such a compensatory mechanism leads to the achievement and maintenance of euglycemia, at least for a period of time. Compensation is facilitated by both hyperinsulinemia and increased glucose-stimulated glucose disposal via enhanced GLUT-1 activity (Flanagan et al., 2000) however the exact mechanism by which the β-cell senses a need for increased insulin secretion is yet unknown (Ahren and Pacini, 2005). Candidate theories include hyperglycemia and high circulating FFA levels, as both stimulate insulin secretion.

While the compensatory mechanisms are functioning, an individual can maintain his resting blood glucose within the healthy range, only showing evidence of abnormal glucose dynamics when subjected to a clinical test, such as an oral or intravenous glucose tolerance test (OGTT, IVGTT; Expert Committee, 2003). During an OGTT or IVGTT, evidence of impaired glucose tolerance is exhibited by over-secretion of insulin and slower glucose disposal in response to a glucose bolus. The underlying cause of the impairment, such as abnormal β-cell function, can be identified using additional tests, such as a euglycemic-hyperinsulinemic clamp (EHC) or frequently-sampled IVGTT (FSIGT), which examine β-cell function, Sg and SI (Araujo-Vilar et al., 1998; Kahn et al., 1994).

The role of the central nervous system. While insulin is primarily considered an anabolic hormone within the peripheral tissue, the actions of insulin within the brain are counter-regulatory. Insulin is a long-term signal of energy balance, circulating in proportion to the level of body fat (Schwartz et al., 2000). In the normal individual, as adiposity increases, the concomitant increase in insulin leads to activation of neural pathways involved in the control of food intake, ultimately resulting in a decrease in intake and weight loss (Schwartz et al., 2000). These neural pathways are also regulated by the cytokine hormone leptin, an additional long-term indicator of energy balance. Both insulin and leptin cross the blood-brain barrier and, in times of excessive adiposity, suppress the activity of two hypothalamic neuropeptides, neuropeptide Y (NPY) and agouti-related protein (AgRP), and activate a third, α-Melanocyte stimulating hormone (α-MSH), resulting in a decrease in food intake (Schwartz et al., 2000). Lower levels of NPY have recently been found in the hypothalamus of overweight and obese horses (Buff et al., 2007).

Leptin is produced in by the brain, heart, placenta and s.c. adipocytes and is released in proportion to the level of body fat in many species, including horses (Buff et al., 2002; Gentry et al., 2002; Cartmill et al., 2003; Kearns et al., 2005; Frank et al., 2006). In addition to its effects within the hypothalamus, leptin stimulates glucose metabolism in the peripheral tissue by enhancing cellular uptake and increasing glucose and lipid oxidation, thus reducing the amount of glucose and lipid that can be stored as fat (Arch, 2005). Leptin has also been implicated in IR. The binding of leptin to receptors on the β-cell results in suppression of insulin mRNA expression, interference with secretory

granule exocytosis and ultimately IR through leptin-stimulated fatty acid oxidation (Ceddia, 2005; Zhao, 2006).

During obesity, however, the high circulating leptin levels are not associated with a reduction in food intake or BW, a condition which has been termed 'leptin resistance (Halaas et al., 1997).' While the ultimate cause of leptin resistance is not yet fully understood, a likely mechanism within the hypothalamus is the activation of negative feedback systems in response to excessive leptin. Such systems include impaired transport of leptin across the blood-brain barrier, alterations in post-receptor signal transduction within the hypothalamus and an increase in the negative regulators of leptin signaling (e.g. suppressor of cytokine signaling-3, SOCS3; Ceddia, 2005). Similar mechanisms have been proposed for insulin as well, as reduced insulin transport into the CNS was associated with high fat feeding in dogs (Kaiyala et al., 2000). In the horse, recent evaluation of hypothalamic leptin receptor expression has shown an increase in leptin receptor concentration with obesity, hypothesized to be a compensatory mechanism to counteract reduced sensitivity with increased receptor number (Buff et al., 2007).

Within the peripheral tissue, resistance to leptin, hypothesized to be caused by increased SOCS3 activity, can lead to hyperinsulinemia (Zhao et al., 2006). Hyperinsulinemia may contribute to leptin resistance as both rats and cultured rat adipocytes show an increase in leptin secretion or mRNA expression following insulin injection (Saladin et al., 1995; Mueller et al., 1998). Activity of SOCS3 has also been implicated in insulin receptor dysfunction in the peripheral tissues (Ukei et al., 2004). It

stands to reason that chronically elevated insulin would lead to chronically elevated leptin, and vice versa, with neither hormone able to carry out is appropriate action.

Leptin resistance has been observed in the obese horse and the causative mechanisms, while unknown, are likely to be similar to those previously described. Cartmill et al. (2003) divided horses of BCS 7.5-8.6 into two distinct groups, those with 'high' circulating leptin levels (14.1 ng/mL) and those with 'low' circulating leptin levels (2.8 ng/mL). The combination of high BCS and high leptin levels were taken to indicate leptin resistance. Additionally, the horses in the high leptin group had a greater insulin response to glucose infusion, indicating some level of IR was occurring in these animals (Cartmill et al., 2003). An additional requirement for the diagnosis of leptin resistance may also be an increased number of leptin receptors in the hypothalamus (Buff et al., 2007).

Adiponectin. The insulin-sensitizing hormone adiponectin is secreted from visceral fat tissue in proportion to the level of SI in an individual (Zhao et al., 2006). As SI is reduced in the obese, it is not surprising that adiponectin levels are negatively correlated to s.c. fat levels in many species including horses (Vettor, et al., 2005; Kearns et al., 2005). Adiponectin enhances SI and is believed to have a protective effect on the β-cells (Zhao et al., 2006). In addition to its ability to improve SI, adiponectin is negatively correlated with tumor-necrosis factor-α (TNF-α), an inflammatory cytokine involved in the progression of IR (Meier and Gressner, 1994).

TNF-α. Obesity in human and experimental animal models, such as rodents, is regarded as a state of chronic, low-grade inflammation (Elenkov, 2005). Cytokines, mediators of the immune system, are involved in the immune response against invading pathogens, such as during tissue trauma, sepsis, or a bacterial infection. However, some level of cytokine production occurs in healthy individuals, originating from adipose tissue. As adiposity increases, so does the production of cytokines (Elenkov, 2005). In response to a high fat diet, TNF-α has been shown to induce SI by interfering with insulin signaling via serine phosphorylation of the insulin receptor and IRS-1 within the hypothalamus and peripheral tissue (Souza et al., 2005; Schulman, 2000). Additionally, TNF-α reduces tyrosine phosphorylation of the insulin receptor, reduces GLUT-4 mRNA expression and instigates lipolysis by stimulating hormone-sensitive lipase and reducing lipoprotein lipase in the peripheral tissues (Coppack, 2001). In the horse, TNF-α is positively correlated to percent body fat and negatively correlated to SI, linking adiposity with IR and inflammation in the equid (Vick et al., 2007).

Oxidative stress. The mitochondrial electron transport chain (ETC) is required for ATP generation and relies on the transfer of electrons from a donor (NADH or $FADH_2$) to an end recipient (O_2) through a series of oxidation-reduction reactions. Leakage of the electrons from the ETC results in premature interaction of the electrons with O_2 and instead of forming H_2O, $O_2^{-}\cdot$ (superoxide) is formed (Clarkson and Thompson, 2001). Under normal conditions, the enzyme superoxide-dismutase (SOD) converts the superoxide to hydrogen peroxide (H_2O_2), which is further converted to H_2O and singlet oxygen by catalase and glutathione peroxidase (GPx; Droge, 2002). Overproduction of

superoxide can overwhelm these regulatory enzymes and H_2O_2 will be instead converted into the hydroxyl radical, a highly reactive molecule (Droge, 2002). In small amounts these reactive oxygen species (ROS) can be used for cellular processes such as signal transduction and smooth muscle relaxation but in large amounts can be toxic. Excessive ROS can damage DNA and lipid membranes, reducing the fluidity of the cellular membranes and inactivating membrane-bound receptors and enzymes (Clarkson and Thompson, 2001). Within cell membranes, the polyunsaturated fatty acids are prime targets for ROS and can themselves be converted into lipid hydroperoxides (LPO), which initiate further damage to the membrane as well as DNA.

Increased oxidative stress occurs in obesity and type II diabetes in humans. Excessive glucose uptake and metabolism increases the activity of the ETC, resulting in an increase in the production of superoxide and further ROS which can damage the functionality of the β-cell (Droge, 2002). Additionally, H_2O_2 can enhance insulin receptor autophosphorylation and at very high concentrations can actually stimulate receptor autophosphorylation and initiate the signal transduction cascade in the absence of insulin (Droge, 2002). However, prolonged exposure to high or medium H_2O_2 levels can actually decrease insulin receptor signaling (Droge, 2002).

In horses, increased oxidative stress is most often associated with exercise (Mills et al., 1996; de Moffarts et al., 2005) and treatment with antioxidants can offset the detrimental effects (de Moffarts et al., 2005; Williams et al., 2002). Antioxidants are compounds that interact with the ROS and render them neutral, for example Vitamin E, which interacts with superoxide to form the relatively innocuous tocopherol radical, further metabolized to the non-toxic reduced tocopherol (Clarkson and Thompson, 2001).

Evidence exists for increased oxidative stress in equine laminitis, as non-exercised, chronically laminitic ponies were shown to have increased urinary TBARS, a marker of lipid peroxidation damage, as compared to non-laminitic ponies (Neville et al., 2004). While tissue ischemia and reperfusion are associated with a massive increase in the production of ROS (Droge, 2002), the oxidative stress found in chronic laminitis was hypothesized to result from underlying IR (Neville et al., 2004). While this hypothesis remains untested, horses with equine Cushing's disease, a disease associated with obesity and a high risk of laminitis, had minimally increased oxidative stress levels and resting hyperglycemia (Keen et al., 2004). However, it is most likely that increased oxidative stress in the obese horse is not causative of laminitis but rather increases the risk of the disease following an appropriate stimulus. Indeed, Treiber and colleagues (2007) found no difference in oxidative stress markers in previously laminitic ponies as compared to non-laminitic ponies.

Equine insulin resistance

Insulin resistance in the equid was first discussed by Argenzio and Hintz (1971), who reported that reduced glucose tolerance in the fasted pony was not due to insulin insufficiency but rather resistance to insulin's action. While fasting and feed restriction are both known to induce IR in the horse and pony (Powell et al., 2000; Sticker et al., 1995), glucose intolerance has since been associated with developmental orthopedic disease (Ralston, 1996), diets high in starch and sugar (Williams et al., 2001; Hoffman et al., 2003), excessive adiposity (Hoffman et al., 2003) and laminitis (Coffman and Colles,

1983). Differences in susceptibility to IR exist within the equine breeds, however, with ponies generally exhibiting a greater degree of glucose intolerance than horses (Jeffcott et al., 1986).

The progression from normal glucose tolerance to IR in the fat pony seems to mimic that found in the obese human. Freestone and colleagues (1992) divided obese ponies with abnormal responses to an OGTT into three groups based on their pancreatic insulin response. The first group exhibited hyperinsulinemia and euglycemia, hypothesized by the authors to indicate pancreatic compensation for tissue insensitivity to insulin. The second group was both hyperglycemic and hyperinsulinemic, possibly signifying that excessive insulin secretion was no longer able to maintain euglycemia. The final group was hyperglycemic but with normal insulin levels, suggesting that pancreatic exhaustion may have been occurring. While the boundaries used to determine the divisions between each group were somewhat arbitrary, pancreatic compensation for IR has since been observed using direct measures of the insulin response to glucose in relation to SI in both laminitic ponies (Treiber et al., 2005b) and horses of BCS $\geq$ 7 (Hoffman et al., 2003). In addition, horses with excessive adiposity and evidence of pancreatic compensation for IR were found to rely more heavily on glucose-stimulated glucose uptake than lean horses, most likely to offset the decreased proficiency of insulin-stimulated glucose uptake (Hoffman et al., 2003).

Insulin resistance in the equine has been postulated to act as a contributor to the onset of laminitis, especially in susceptible individuals exposed to dietary trigger factors, such as excessive pasture fructans or grain overload (Johnson et al., 2002; Treiber et al., 2006). In the pony, laminitis is associated with reduced SI (Coffman and Colles, 1983; Jeffcott

et al., 1986; Treiber et al., 2005b) and obese-laminitic ponies have been shown to be even less sensitive to insulin than obese non-laminitic ponies (Jeffcott et al. 1986; Treiber et al., 2005b). Insulin resistance and obesity have been recognized as significant risk factors for laminitis in the pony (Treiber et al., 2006) but as yet remain tentative in the horse. Meta-analyses of laminitis cases from several university veterinary clinics found that obesity was associated with a greater risk of laminitis in the horse (Alford et al., 2001) and parallels have been drawn from the pony to include IR as an additional laminitic risk factor in the horse.

Insulin resistance is not believed to be a primary cause of laminitis in the equid. Instead, IR and associated conditions, such as oxidative stress and inflammation, are believed to increase the risk of laminitis through insidious damage to hoof structures, increasing the probability or severity of a laminitic episode. Both the laminae and the vasculature of the hoof are currently thought to be the primary strategic points where weakness is exploited.

Because glucose is not adequately taken up into the tissue during IR, a certain level of glucose deprivation may occur. In the hoof, glucose deprivation leads to weakening of the laminae, shown *in vitro* by French and Pollitt (2004). Hoof lamellar tissue incubated in media that lacked glucose had a weaker resistance to mechanical tension than did tissue incubated in glucose-rich media. Further weakening of the laminae may be due to upregulation of the matrix metalloproteinases (MMPs), enzymes responsible for tissue remodeling through degradation of the basement membrane of collagenous tissues (Mungall et al., 1998). When laminitis is induced experimentally, two MMPs are upregulated, MMP-2 and MMP-9 (Johnson et al., 1998). Insulin is a regulator of MMP-9

activity and is unable to suppress its activity in human obesity-associated IR (Dandona et al., 2003). In human studies, insulin has been shown to suppress the activity of MMP-9, indicating that the IR associated with obesity may be linked to the upregulation of the MMPs (Dandona et al., 2003).

In addition to its primary involvement with glucose metabolism, insulin, in conjunction with nitric oxide, also acts as a vasodilator. In humans, IR is associated with endothelial dysfunction and arteriosclerosis (Wilcox, 2005). During laminitis, tissue ischemia caused by vasoconstriction leads to tissue necrosis and coffin bone separation. It follows then that insulin's inability to improve vascular function during IR may be contributory to the progression of laminitis. Insulin administration reduces arterial blood pressure in laminitic ponies (Coffman and Colles, 1983) as does glyceryl trinitrate, a nitric oxide precursor (Hinckley et al., 1996).

Increased production of TNF-α may also contribute to the risk of laminitis, both insidiously through inhibition of nitric oxide in the obese horse (Lyon et al., 2003) and directly through the ability of the inflammatory immune response to cause IR (Fitzgerald, 2004). Additionally, heightened TNF-α production in human obesity increases the activity of 11-β-hydroxysteroid dehydrogenase-1 (11-β-HSD-1), an enzyme responsible for converting inactive cortisone to active cortisol in peripheral tissues (Hochberg et al., 2004). Glucocorticoid excess is associated with both IR and laminitis in horses affected with equine Cushing's disease and increased 11-β-HSD-1 activity has been observed in equine skin and lamellar tissue following the induction of laminitis (Johnson et al., 2004).

Dietary impacts on insulin sensitivity

Insulin sensitivity is impacted not just by obesity but by dietary factors as well, sometimes in the absence of excessive adiposity. Chronic hyperinsulinemia results after prolonged exposure to diets high in non-structural carbohydrates, leading to β-cell over-responsiveness and eventual pancreatic exhaustion (Kopp, 2003). In addition, insulin promotes FFA uptake and persistent high FFA levels are toxic to the pancreas, decreasing insulin secretion, increasing β-cell apoptosis, inhibiting insulin gene expression and interfering with IRS-1 activation (Grill and Qvigstad, 2000; Wilcox, 2005; Zhao et al., 2006).

Although excessive FFA metabolism is associated with IR, fatty acid intake at ≤ 30% of the total diet is not detrimental to SI or the ability of glucose to affect its own uptake in humans (Lovejoy et al., 2002). In horses, this may be the reason why few reports of IR associated with high fat diets exist in the literature. Dietary fat consumption was associated with impaired SI in two equine studies (Schmidt et al., 2001; Frank et al., 2005), but subsequent research into high fat diets has not supported these findings. High fat feeding was associated with impaired glucose tolerance in eight Shetland pony geldings fed a diet containing 20% of DE as fat from soybean oil (Schmidt et al., 2001). No appreciable weight was gained by the ponies and the authors did not hypothesize about the nature of the glucose intolerance. Frank et al. (2005) found reduced SI in mixed-breed Quarter Horse-type mares fed a commercial sweet feed top-dressed with 250 mL of oil (corn or rice bran). However, the authors attributed the reduction in SI to the sweet feed, hypothesizing that the level of fat was not high enough to affect the glycemic response.

Diets high in non-structural carbohydrates (NSC; starches and sugars) are associated with post-prandial glucose and insulin responses that are greater than those of forages alone or fat-supplemented rations (Stull and Rodiek, 1987; Pagan et al., 1999; Williams et al., 2001; Ropp et al., 2003). While long-term consumption of diets high in NSC leads to dietary adaptation and an improved ability of the horse to tolerate such diets (Hoffman et al., 2003b), overall SI is reduced when compared to diets where the NSC has been replaced by fat and fermentable fiber (Treiber et al., 2005; Hoffman et al., 2003). In addition to a reduction in SI, weanling horses fed a diet high in NSC had increased β-cell compensation, indicating that they were able to compensate for the reduced SI through increased insulin secretion (Treiber et al., 2005).

Onset of insulin resistance

A common observation among horse owners is that not all obese horses and ponies become laminitic. As with human IR (Alford et al., 1998), there appears to be a significant genetic component to the development of IR and laminitis, with ponies being highly susceptible (Treiber et al., 2006). However, obesity is a known factor associated with laminitis in horses (Alford et al., 2001) and it is possible that a genetic component exists within the horse as well.

The inability to predict both the susceptible individual and the time-course of obesity-associated laminitis in horses is difficult, if not impossible. In the pony, more accurate estimations of risk are available and sufficient evidence has been presented to this point. In the horse however, such evidence is lacking. It has been shown in other species that SI

decreases with increasing body weight, with dogs overfed energy by twice the recommended level showing at 44% increase in body weight with a concomitant 45% decrease in SI (Gayet et al., 2004). Rocchini et al. (1997) performed weekly HECs on dogs gaining weight on high fat diets and found that SI decreased as soon as one week following diet initiation and reached a nadir by two weeks. As the dogs fed fat in this study were gaining weight, as compared to the control dogs which did not gain weight, it is unclear as to whether the true source of the reduced SI was due to diet or BW. However, Rocchini and colleagues (1987) indicated that previous published work shows reduced glucose tolerance in dogs due to high fat diets alone. In cats, previous IR in lean individuals was associated with an increased risk of development of diabetes following weight gain to obesity (44% BW increase, 50% SI decrease; Appleton et al., 2001).

As not every obese horse will become IR and laminitic, not every obese human will become IR and diabetic. A wide range of SI exists within the human population (Hollenbeck and Reaven, 1987) and would be expected to occur in the equine population as well. In humans, it is thought that the ability of an individual to compensate for the negative physiological effects of obesity will ultimately determine whether or not glucose tolerance can be maintained (Reaven, 1988). So too in the horse, the ability to adapt to diets high in NSC or high levels of adiposity may be dependent on the genetic background of the animal.

PART III: EQUINE DIABETES AND METABOLIC SYNDROME

Equine diabetes

The IR associated with obesity and laminitis in the horse may not be the same as Type II diabetes in humans, dogs and cats. True type II diabetes is defined as chronic fasting hyperglycemia, impaired insulin action, either through under-secretion or over-secretion, polyurea, polydypsia and weight loss, with long-term effects such as ketosis, retinopathy, neuropathy, etc. (Expert Committee, 2003). Fasting hyperinsulinemia is not universally observed, in many cases due to insufficient insulin secretion for the level of plasma glucose.

Stogdale (1985) defines diabetes in animals as fitting in to one of three categories: insulin-dependent (Type I), non insulin-dependant (Type II) and secondary (S), occurring in response to a primary disease. According to Stogdale, neither Type I nor Type II diabetes had been described in the horse through the mid 1980's. In fact, up to 1985, the 24 reports of equine 'diabetes' in the literature were all attributable to pituitary tumor or pancreatitis (Stogdale, 1985).

Specific reports in the attainable literature attribute clinically abnormal glucose or insulin levels in horses to diabetes (Baker and Ritchie, 1974; Ross et al., 1983; Stogdale, 1985; McCoy, 1986, Ruoff et al., 1986, Muylle et al., 1986 and Johnson et al., 2005). Before a definitive diagnosis was (in most cases) confirmed through necropsy, horses classified as suffering from non-insulin-dependent diabetes mellitus (Type II) showed

symptoms including but not necessarily encompassing, weight loss, polyurea, polydypsia, fasting hyperglycemia or hyperinsulinemia or both and hirsutism. Following necropsy, a pituitary tumor was found to be the culprit in two cases (Baker and Ritchie, 1974; Garcia and Beech, 1986) and pancreatic hyperplasia due to adenoma in another (Ross et al., 1983). Cancer may have been responsible for a fourth case, a mare with bilateral ovarian granulosa cell tumors, but the authors were unclear as to how this would contribute to IR in such a case (McCoy et al., 1986). In several cases, the cause of the symptoms was unknown, with one horse testing negative for pituitary, pancreatic and adreno-cortical tumors, and a second and third euthanized without necropsy (Ruoff et al., 1986; Muylle et al., 1986). In a fourth case, reduced numbers of β-cells were hypothesized as the primary cause of diabetes (Johnson et al., 2005). Although it is tempting to suggest a diagnosis of Type II diabetes, especially in the fourth case, concerns raised by Kronfeld et al., (2005) regarding standardized methodology and adequate experimental design prevent this from being realized. Johnson et al. (2005) attempted to quantify the IR observed in a domesticated Spanish Mustang through glucose and insulin tolerance tests, culminating in a diagnosis of Type II diabetes after noting histological β-cell abnormalities that could explain the aberrant insulin and glucose dynamics. However, the strong evidence and assertive claims put forth in the study were not backed up by sound experimental design (Kronfeld et al., 2005), bringing the conclusions into question. Studies by Ruolf and colleagues (1986) and Muylle et al. (1986) documented both fasting hyperinsulinemia and hyperglycemia and Muylle and colleagues observed impaired glucose tolerance following oral glucose and meal tolerance tests. However, neither

study confirmed the presence of insulin resistance to a degree necessary to diagnose Type II diabetes (Kronfeld et al., 2005).

The equine Metabolic Syndrome

Several terms exist to describe those equids which are obese, insulin resistant and at high risk for the development of laminitis. Such terms include equine syndrome X (Kronfeld and Harris, 2003), equine grain-associated disorders (EGAD; Kronfeld and Harris, 2003), the equine Metabolic Syndrome (Johnson et al., 2002), peripheral Cushing's syndrome (Johnson et al., 2002) and pre-laminitic metabolic syndrome (PLMS; Treiber et al., 2006). Each term, however, can be more specifically applied to a subset of horses within the general population and it may be that no one label is adequate to describe every case.

'Equine grain-associated disorders,' has been previously favored due to its encompassing definition, "a group of digestive disturbances commonly involving rapid fermentation and a set of metabolic disorders probably involving insulin resistance (Kronfeld and Harris, 2003)." However, this term is quite broad, encompassing colic, typhlitis, colitis, diarrhea, gastric ulcers, laminitis, exertional rhabdomyolysis, osteochondrosis, growth rate fluctuations, flexure deformities, hyperlipidemia, oxidative stress, aging, obesity and abortion (Kronfeld and Harris, 2003). Its attractiveness, however, is in that it provides for a simple management strategy, namely the avoidance of high starch and sugar feeds. Because simple caloric restriction due to the removal of high-starch grain-based concentrates from the horse's diet can improve both body weight

and IR, a diagnosis of EGAD may be appropriate for use by the general horse owner. However, the recent identification of risk factors specific to the onset of laminitis in the obese, IR population, the need for a specialization within the grain-associated disorders specifically related to obesity and IR is warranted.

Under the umbrella of EGAD, several labels have been defined to address horses specifically at risk for laminitis. The term 'peripheral Cushing's syndrome' has been popular but is misleading in that the comprehensive profile of these animals is in fact distinct from those afflicted by true Cushing's Disease (see McCue, 2002). The equine Syndrome X and equine Metabolic Syndromes were developed to mirror terms widespread in the human literature (see Reaven, 1988).

In humans, Syndrome X was defined as abnormal glucose tolerance and one or more of the risk factors for cardiovascular disease (CVD; e.g. hypertension; Reaven, 1988). This definition was later widened to include central obesity and renamed the Metabolic Syndrome, a definition championed by the World Health Organization (WHO, 1999). The WHO defines the human Metabolic Syndrome as an individual diagnosed with glucose intolerance, type II diabetes mellitus or insulin or both resistance coupled with at least two of the following risk factors: impaired glucose regulation, diabetes, insulin resistance as determined using the euglycemic-hyperinsulinemic clamp (HEC), hypertension, raised arterial blood pressure, hypertriglyceridemia, central obesity or microalbuminuria (WHO, 1999). The Metabolic Syndrome is used throughout the world as a powerful diagnostic tool for physicians to identify persons at risk for diabetes or CVD (Reaven, 2006).

In order for an equine to be labeled as having the equine Metabolic Syndrome, it is recommended that the animal must meet the qualifications set forth by the World Health Organization (WHO) for the human Metabolic Syndrome (Kronfeld, 2003; Frank et al., 2006). Treiber et al. (2006) lists key risk factors for the development of laminitis as IR with compensatory hyperinsulinemia, hypertriglyceridemia and obesity, as well as a genetic predisposition (e.g. pony breeds) and access to diets high in starch.

It is the goal of such diagnostic tools to identify individuals at risk for developing the ultimate disease through the presence of risk factors that predict with certainty the onset of said disease. However, it has become clear from human studies that certain individuals who present with various components of the Metabolic Syndrome do not fit the total criteria due to the absence of one or more symptoms (Reaven, 2006). The symptoms presented by these individuals may still be taken together to indicate a strong risk of developing CVD or diabetes but may not be appropriately labeled the Metabolic Syndrome. Because of this, Reaven (2006) suggests taking a step back from the ultimate diagnosis of Metabolic Syndrome and instead identify individuals with IR, a strong risk factor for the onset of symptoms of the Metabolic Syndrome. Diagnosing individuals with IR as opposed to Metabolic Syndrome allows for circumventing an absolute diagnosis of Metabolic Syndrome while still identifying individuals at risk for diabetes or CVD.

So too with equids, a desire to identify at-risk animals may require identification of both individuals fitting the specific criteria of the equine Metabolic Syndrome and those which can be diagnosed with IR but do not fit all of the required categories for true equine Metabolic Syndrome. Such a distinction can be made in the literature when

examining the Pre-laminitic Metabolic Syndrome (PLMS; Treiber et al., 2006), which lists key risk factors for the development of laminitis as IR with compensatory hyperinsulinemia, hypertriglyceridemia and obesity, as well as a genetic predisposition (e.g. pony breeds) and access to diets high in starch.

A need then arises to adequately deal with those animals not fitting all of the above criteria but which still are at risk for the development of laminitis. Such an example may be the obese light horse with IR. He cannot be diagnosed with PLMS but may still be at risk of developing laminitis, especially if he is offered a high-starch diet. The diagnosis of IR will aid the owner and veterinarian in the preventative measures that should be taken to adequately manage the health of the horse. Those horses not fitting the criteria of PLMS, namely light-horse breeds with frank obesity and IR, may be better identified as having the 'equine Insulin Resistance Syndrome' (eIRS; Kronfeld and Harris, 2003), a term that may improve the detection, diagnosis and treatment of afflicted horses. Guidelines are currently being developed for the care and management of ponies with PLMS and such guidelines may be applicable to the horse with eIRS.

PART IV: MODULATION OF INSULIN SENSITIVITY THROUGH INTERVENTION

Diet and exercise

While numerous approaches to improving the health of the horse with obesity, IR or laminitis have been recommended (Johnson, 2004b), limited research exists on the specificity of such treatments in the horse (Kronfeld et al., 2005). Many 'common sense' recommendations have been put forth, including weight loss through feed reduction and increased exercise, as have those based on current research into the effects of diet on SI and laminitis. However, research into dietary supplements and pharmaceuticals still remains in the initial stages and as such should be considered secondary to the common sense approaches.

Insulin sensitivity is improved in obese humans following weight loss (Ferranini et al., 2004). While improvements have also been seen through dietary restriction, exercise and pharmaceutical intervention, the exact mechanisms by which IR is reversed are mostly unknown. In the human, aerobic exercise improves SI through increasing GLUT4 expression and translocation, and improving IRS-I signaling (Henricksen, 2002). In the horse, dietary restriction and exercise with or without concurrent weight loss (Freestone et al., 1992b; Powell et al., 2002) have been shown to improve SI. Previously *ad libitum*-fed hyperinsulinemic ponies (BCS 7 – 9) placed on a controlled, maintenance feeding regimine lost 7 kg over 6 wks and showed improved glucose tolerance during an OGTT

(Freestone et al., 1992b). In obese mares (BCS 8 – 9), short-term, low-intensity exercise (30 min trot, HR 120-140 bpm) resulted in a 60% improvement in SI as measured by the HEC, when compared to obese sedentary mares, even without concurrent weight loss (Powell et al., 2002). Submaximal exercise (treadmill 5d/wk for 10min, HR ≤ 140 bpm) also improved SI in ponies measured by the OGTT (Freestone et al., 1992b).

While feed restriction, defined here as a reduction in the level of energy offered to the horse, will stimulate weight loss, care must be taken to avoid detrimental side effects. Significant dietary restriction (50% of maintenance DE) in light horse mares (BCS 6 -7) resulted in impaired glucose tolerance, with restricted mares having larger insulin and glucose responses to an IVGTT (Sticker et al., 1996). In ponies, rapid weight loss can induce hyperlipaemia, in which excessive triglyceride mobilization from adipose tissue results in fatty infiltration of the liver, a potentially life-threatening condition (Hughes et al., 2004).

The best recommendation for weight loss and improved SI in the horse is to alter the ingredients in the feed offered to the horse, concurrent with increased exercise. Diets high in fat and fiber have been previously shown to increase SI (Treiber et al., 2005; Hoffman et al., 2003). Adding to the positive effect of dietary fat, the addition of beet pulp can also assist in increasing SI as it is theorized to cause increased fermentative propionate-derived glucose and increased lipoprotein lipase activity (Hallebeek and Beynen, 2003). The simplest recommendation to owners of obese horses is to remove energy-dense grain-based concentrates from the horse's diet and replace it with forage, providing no less than 75% of the energy required for maintenance and at an amount no less than 1% of BW.

Pharmaceutical intervention

The use of pharmacological agents to reduce BW and improve SI in the horse is a relatively new area of research and should be considered secondary to diet and exercise. Although primary hypothyroidism is not a cause of obesity as discussed above, administration of levothyroxine sodium to euthyroid, normal horses resulted in weight loss and improved SI (Sommerdahl et al., 2005; Frank et al., 2005b). Improvements in SI were also observed in a horse treated with metformin, a drug used to treat human insulin resistance, in concert with glyburide, an anti-hyperinsulinemia compound (Johnson et al., 2005). The mare had been diagnosed with hyperglycemia during an IVGTT (>400 mg/dL for up to 12h), hypertriglyceridemia, polydipsia and glycosuria Following metformin and glyburide administration, glucose levels fell to within the reference range for normal horses (72-114 mg/dL) by 4h (Johnson et al., 2005). The mare was not obese, however (BCS 3) and histopathology indicated a reduced number of β-cells within the pancreatic islets (Johnson et al., 2005).

Conflicting results using metformin were reported by Vick et al., (2006) in mares treated with a low dose (3 g/d) of metformin. During a dose-response study, SI was increased in mares treated with 3 g/d of metformin but not in mares treated with 6 or 9 g/d. However, successive use of the 3 g/d dosage failed to improve SI when used in a subsequent experiment.

Clenbuterol is a β-2 adrenoceptor agonist which facilitates anabolic tissue repartitioning, specifically a reduction in adipose tissue via increased lipolysis and an increase in lean muscle mass (Kearns et al., 2001). Although used in therapeutic doses to

treat chronic obstructive pulmonary disease, administration of pharmacological levels of clenbuterol to Standardbred mares (BCS 5.4) decreased the ultrasound-derived percent body fat of the mares from approximately 21% to approximately 10%, but without a concurrent change in BCS (BCS 5.6; Kearns et al., 2001). Although not measured in this study, research in rats has shown improved SI following clenbuterol treatment (Pan et al., 2001), which may be an added benefit to treatment with this drug.

An additional β-adrenergic agonist, ractopamine hydrochloride, tended (P=0.09) to enhance BW reduction in treated mares on a restricted feeding regimen (75% of ad libitum intake, n=5; Buff et al., 2006). However, no impact on leptin or insulin was observed. Caution must be taken, however, when considering the use of such β-adrenergic agonists as therapeutic treatments for obesity. Mares treated with clenbuterol for an 8 week period were at an increased risk for aortic rupture compared to untreated mares (Sleeper et al., 2002), indicating that administration of such drugs at pharmacuetical doses, which are much higher than therapeutic doses, may not be advisable.

Chromium tripicolinate shows promise as a dietary supplement, with research indicating improved glucose tolerance following treatment (Ott and Kivipelto, 1999). Administration of chromium tripicolinate to Thoroughbred (n=11) and Quarter Horse (n=13) yearlings of mixed sex resulted in a faster glucose clearance rate during an IVGTT, with no difference in body fat between treatment groups (Ott and Kivipelto, 1999). Chromium is believed to act at the level of the insulin receptor to aid in proper cellular signaling via increasing the activity of several components of the signal transduction cascade, including IRS-1 (Wang et al., 2006).

PART V: MODERN TECHNIQUES FOR THE QUANTIFICATION OF INSULIN SENSITIVITY

Simple techniques

Numerous methods of quantifying SI exist in the literature, ranging from simple fasting blood samples to highly sophisticated and complex methodologies. Each have been pioneered through human medicine and validated for use in the horse, with some techniques faring better than others for diagnosing IR in equids. The simplest strategy for assessing glucose and insulin dynamics consists of taking a single fasting blood sample and measuring serum insulin and plasma glucose. Although these diagnostic tools are attractive in their simplicity, caution must be taken due to inconsistent results in the literature as well as a lack of specificity as to where the resistance lies (Kronfeld et al., 2005b).

Recently, equations have been published allowing simple fasting glucose and insulin levels to more accurately reflect the SI status of the horse (Treiber et al., 2005c). Derived from the more complicated methods used to measure SI (the FSIGT, described below), these non-specific 'proxies' have been shown to accurately estimate both SI and the acute insulin response to glucose, indicative of the level of initial responsiveness of the β-cell to glucose. Through these tests, horses which are compensating for IR through increased β-cell insulin production can be detected, lending increased diagnostic power to veterinarians. Although not as accurate as direct measures of SI, these proxies were

found to be as accurate and comparable to proxies used in human diagnostics, including the HOMA-IR and QUICKI (Treiber et al., 2005c).

Oral and intravenous glucose tolerance tests

The increased complexity of the OGTT and IVGTT lend themselves to better approximation of the degree of IR, although as with the simple indices, the ultimate location of the defect, be it at the β-cell or peripheral tissue, cannot be determined (Kronfled, 2005). An additional weakness of the OGTT is that an accurate assessment of glucose tolerance may not be possible, due to absorptive differences of the equine gastrointestinal tract (Kronfeld, 2005). This same factor most likely hinders results gained from meal glycemic response tests, where instead of a glucose bolus, horses are fed a standard meal of oats or concentrate and the glucose response is measured. The use of a meal glycemic response test is best used to estimate the relative changes in blood glucose in response to various feeds, similar to the human glycemic index, and is more appropriately used by horse owners when deciding which feed is best for their horse.

The IVGTT is considered a more appropriate test of glucose removal from the bloodstream due to the bypassing of the gastro-intestinal tract. When the IVGTT is coupled with administration of insulin, the response of both endogenous insulin to the glucose bolus, read from the part of the curve prior to insulin administration, and exogenous insulin further facilitating glucose removal can be estimated (Kronfeld, 2005). The combined glucose and insulin test (CGIT) combines the IVGTT and an insulin tolerance test into one clinically relevant procedure (Eiler et al., 2005). However, this is

also non-specific test because it only shows the presence or absence of resistance and gives no quantitative values for SI (Frank et al., 2006).

Modern methods: the euglycemic-hyperinsulinemic clamp

Moving into more sophisticated techniques, the euglycemic-hyperinsulinemic clamp (HEC) directly estimates SI under hyperinsulinemic steady-state conditions (Defronzo et al., 1979). The rate of infusion of glucose under hyperinsulinemic conditions is directly proportional to SI. Information on insulin secretion and β-cell function can be derived from an adjustment in the protocol to attain hyperglycemia, while measuring changes in endogenous insulin secretion.

Long considered the 'gold standard' for measuring SI in humans and other experimental models, the HEC is both labor-intensive and complex, with almost constant glucose monitoring and flow adjustment required for adequate assessment of IR. An additional drawback of the HEC is that it does not reflect *in vivo* changes to glucose and insulin following bolus administration, as it is a steady-state approximation of glucose and insulin dynamics (Bergman, 1997). In order to evaluate glucose and insulin dynamics in a non-steady state system, a modification of the IVGTT can be used, wherein numerous blood samples are taken following insulin bolus (up to 35) followed by mathematical modeling of the glucose and insulin responses. The frequently-sampled intravenous glucose tolerance test (FSIGT), with SI derived directly from the minimal

model of glucose dynamics, was proposed in 1979 as an alternative to the HEC (Bergman et al., 1979).

Modern methods: the minimal model of glucose dynamics

The minimal model of glucose (and insulin) dynamics describes the changes in plasma insulin related to the changes in plasma glucose, superimposed on an understanding of typical glucose responses to insulin. Insulin and glucose concentrations over a 180-240 minute sampling protocol are entered into computer software (MinMod Millenium, Boston et al., 2003), and are integrated to produce estimates of SI and other important parameters. The intent of the original minimal model protocol was to mathematically describe the complex dynamics of glucose and insulin in the bloodstream from a simple clinical test, the FSIGT (Bergman, 1997). Using the multiple sampling protocol, four phases of glucose dynamics were discerned: the initial mixing of glucose in the distribution space (plasma), glucose uptake prior to insulin effects, accelerated glucose uptake via insulin action and the return to baseline (Bergman, 1997). A primary assumption of the minimal model is insulin's action from a 'remote compartment,' the interstitial space, instead of serum or plasma. Additionally, the model accounts for the effect of glucose on its own utilization, Sg, and the suppression of hepatic glucose output by both glucose and insulin (Figure 6; Bergman et al., 1979; Bergman, 1997).

Using knowledge of glucose kinetics, the minimal model can estimate both SI and β-cell function, previously only measurable under the purview of the HEC, as well as glucose-stimulated glucose disposal (Sg) and the acute insulin response to glucose AIRg), representative of the pancreatic insulin response (Best et al., 1990). The minimal

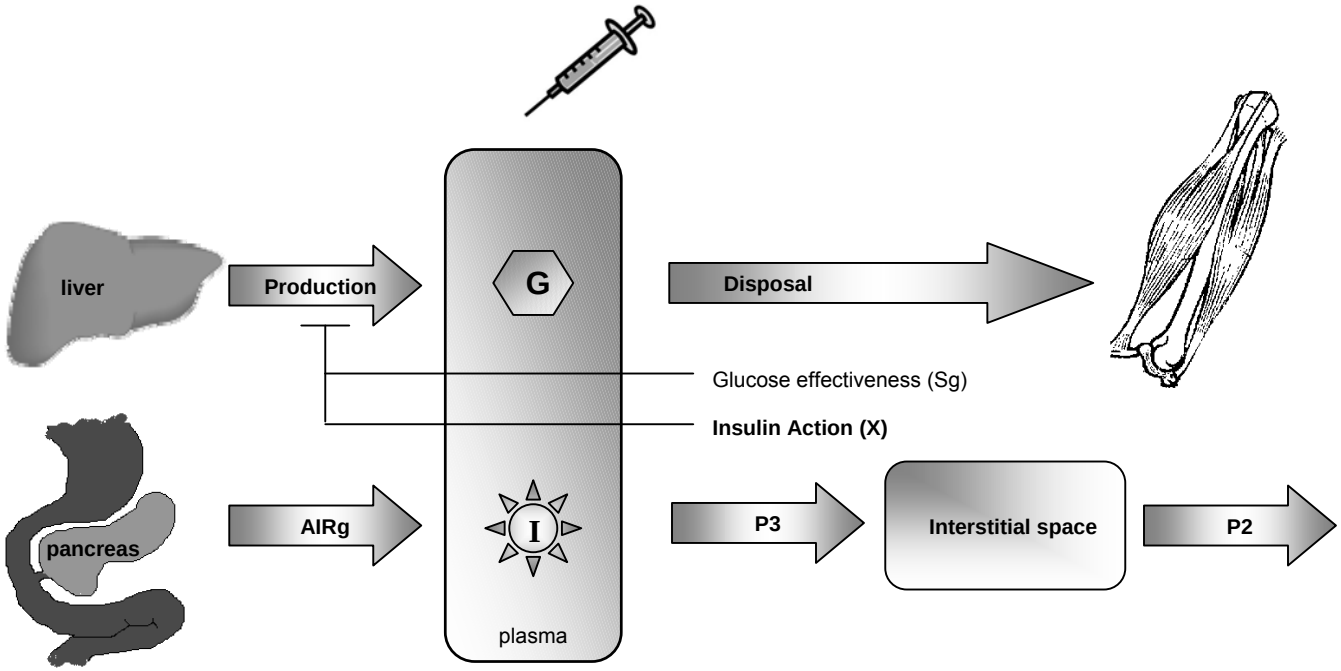


Figure 6. Minimal model of glucose dynamics following a frequently-sampled i.v. glucose tolerance test (FSIGT). Plasma glucose (G) enters the bloodstream from the liver or infusion and is taken to the peripheral tissues under the influence of insulin (I) or glucose (Sg). The AIRg represents the initial endogenous insulin response to plasma glucose during the first ten min of the test, P3 represents the efficiency of trans-endothelial transport, P2 represents the rate of insulin loss from the interstitial space and SI is calculated as P3/P2. Adapted from Bergman, 1997 and Hoffman et al., 2003.

model was compared to the HEC several times, with each independent study finding a significant, high correlation between SI measured by the two methods ($r = 0.85$, $P < 0.002$; Beard et al., 1986), ($r = 0.89$, $P < 0.001$, Bergman et al., 1987) indicating that the minimal model was an acceptable substitute for the HEC. In fact, when SI data from both methods are expressed in identical units, the two methods were found to be equivalent (Bergman et al., 1987).

Since its inception, the minimal model has seen several variations. Originally designed with only a glucose bolus, difficulty in estimating SI in advanced type II diabetes required the addition of tolbutamide, an insulin secretagogue, or exogenous insulin, at a time point beyond the initial insulin response (Beard et al., 1986; Saad et al., 1997). Additionally, somatostatin injections prior to glucose administration have been used to suppress the endogenous insulin response (Yang et al., 1987) and labeled glucose has been infused (Ader et al., 1997), both in attempts to quantify Sg.

The original FSIGT required numerous samples (at least 30) taken over 180 minutes following the glucose bolus. Research on different sampling strategies found that up to 240 minutes was acceptable (Finegood, 1997) and that more samples were necessary if the study had a small number of experimental units (Steil et al., 1994). The recommended glucose dosage (300 mg/kg, Finegood, 1997) was chosen due to good repeatability and correlations between studies and has also been used successfully in horses (Hoffman et al., 2003; Treiber et al., 2005; 2005c). Concerns have arisen as to this dose exceeding the renal threshold, the point whereby the kidneys begin to remove excess glucose, which would lead to improper estimation of glucose disappearance, primarily through overestimation of Sg (Ward et al., 1990; Finegood, 1997). However,

glucose levels exceeding the renal threshold would only be apparent during the first few minutes following glucose administration, and these points are excluded from analysis in the MinMod software as they only represent mixing of glucose in the plasma (Boston et al., 2003).

The insulin dose is recommended to be low enough to prevent receptor saturation, but high enough to elicit a response that can be fit by the computer software (Prigeon et al., 1996). As the amount of insulin administered has been shown to affect the estimate of SI (Welch et al., 1990), an attempt should be made to standardize the dosage. In horses, the insulin doses have been 1.5 mU/kg (Treiber et al., 2005), 20 mU/kg (Treiber et al., 2005c; Quinn et al., 2007) and 30 mU/kg (Hoffman et al., 2003). Administration of both glucose and insulin is done on a kg BW basis, which has been shown to be appropriate in human studies (Finegood, 1997).

Defining the equations. The following descriptions have been independently drawn from Bergman et al. (1979), Bergman (1997), Boston et al. (2003) and Treiber (K.H. Treiber, Virginia Polytechnic and State University, Blacksburg, VA, personal communication). The estimation of SI from the minimal model relies on the accurate description of changes in both glucose and insulin in response to their respective bolus and obtaining 'best fits' of the model to the data (Boston et al., 2003). Through the use of non-linear differential equations, changes in insulin and glucose in the serum and plasma can be used to estimate numerous parameters, via two separate yet intimate equations.

The minimal model equations were developed to provide the simplest description of the change in glucose in relation to the change in insulin, beginning in the absence of insulin and increasing in complexity as factors influencing glucose loss are added. Each equation has two components, 'input' and 'loss,' which are modified by the influencing factor (insulin). In a steady state, 'input' will equal 'loss' and during the non-steady state FSIGT, the increase in 'input' above basal is countered by increasing 'loss.'

Equation α $\Delta G = (\text{input}) - (\text{loss})$

Equation (1). When the contribution of insulin is insignificant, such as during advanced type II diabetes, suppression by somatostatin or during the lull between glucose dosing and endogenous insulin secretion, glucose loss from the circulation occurs by insulin-independent mechanisms (Sg). At basal, a steady state is achieved through the 'input' of hepatic glycolysis and the 'loss' through Sg. Assuming an insignificant contribution of insulin, the loss of glucose from the circulation (mg/dL per minute) is expressed as

Equation 1a $G'(t) = (Sg*Gb) - (Sg*G(t))$

Following the glucose bolus, the 'input' is increased over basal and after glucose detection by the pancreas, the 'loss' is accelerated by insulin secretion (X). While Sg is constantly functioning, its relative contribution to glucose loss will decrease as insulin levels increase. Equation 1 in its final form is:

Equation 1. $G'(t) = Sg * Gb - [Sg + X(t)]G(t)$

(Bergman, 1997)

Equation (2). The second equation is fundamentally similar to Equation 1, in that the change in insulin action within the interstitial space is also impacted by 'input' and 'loss' factors. The insulin input, I(t), is affected by the efficiency of trans-endothelial transport of insulin (P3), indicative of how rapidly insulin responds to glucose stimulation. The ability of insulin action, X(t), to dispose of glucose is dependent on the rate of insulin loss from the interstitial space (P2).

Equation 2. $X'(t) = [P3 * I(t) - Ib] - [P2*X(t)]$

(Bergman, 1997)

From equation 2, SI is derived:

Equation 3. $SI = P3/P2$

(Bergman, 1997)

By studying equation 2, it becomes apparent how SI can be manipulated based on the response of insulin to the glucose bolus. As an example, if insulin action is very high and insulin is working as it should, the first half of the equation will become increasingly large (very efficient insulin delivery, high concentration). In addition, clearance will be rapid and the concentration of insulin will fall rapidly, making the second half of the

equation increasingly small. As a result, the change in insulin action over time will increase, as will SI.

It is possible to quantify the response of the β-cell to glucose through the minimal model equations. As previously described, the pancreas has the ability to compensate for reduced tissue sensitivity, and thus an individual with low SI may show normal glucose tolerance due to an increase in insulin secretion. Insulin secretion is estimated through the response of the pancreas during the first ten minutes of the FSIGT, the acute insulin response to glucose (AIRg), which ultimately is a reflection of post-hepatic insulin concentration.

Equation 4. $AIRg = \int_0^{10} [I(t) - Ib]dt$

(Bergman, 1997)

The degree of compensation by the β-cell, the disposition index (DI), is quantified as the product of AIRg and SI and reflects β-cell insulin secretion, corrected for the level of SI. While a standardized reference range of DI has not yet been established in humans or horses, the increase or decrease in DI relative to treatment groups and the respective changes in SI and AIRg can be used for comparative purposes.

Equation 5. $DI = AIRg * SI$

(Bergman, 1997)

Limitations to the minimal model. A key limitation to the minimal model is the estimation of Sg, which is assumed to indicate the rate of glucose uptake independent of glucose. However, Sg is not truly independent of the actions of insulin as it is measured at basal insulin not zero insulin (Kahn et al., 1994). Even at basal levels, insulin is still impacting glucose uptake and hepatic output. Estimation of true Sg without insulin contribution is derived from subtracting out the effect of basal insulin and thus estimating the glucose effectiveness at zero insulin (GEZI; Kahn et al., 1994). Because of this, estimates of the rate of insulin-independent glucose uptake have been shown to overestimate the true value of Sg.

Aside from the confounding effects of insulin, assuming a constant rate of insulin-independent glucose uptake may not be entirely correct. The minimal model assumes that glucose is taken up into a single compartment, the easily accessible pool of plasma, liver, skeletal tissue and adipose tissue (Caumo et al., 1996). However, evidence has been shown for the existence of two pools into which glucose moves (Caumo et al., 1996). In the minimal model, the rate of removal of glucose from the plasma by insulin-independent mechanisms is assumed to occur uniformly at the rate identified as the variable '*Sg*.' However, '*Sg*' has been found only to adequately represent glucose effectiveness during the initial part of the curve, when glucose is moving into the first pool. Once the insulin effect has waned and glucose has returned to near basal, the estimate of Sg is no longer accurate and in fact overestimates the speed of glucose-stimulated glucose uptake into the second, more slowly equilibrating pool (Caumo et al., 1996).

All is not lost however, in that Sg is found to no longer be overestimated during advanced IR, such as type II diabetes (Finegood and Tzur, 1996). As the initial insulin response to glucose diminishes with disease progression, its contribution to Sg becomes less influential and the accuracy of Sg increases (Finegood and Tzur, 1996).

The use of the minimal model the horse was validated by Hoffman and colleagues in 2003 and has since been used to understand the interplay between body condition, diet, genetics and laminitis in several studies. Adapting a technique that has been validated and utilized in human diagnostics for almost 30 years to equine research increases both the accuracy and precision of data collected on IR, as well as improving the quality of equine research in general.

CHAPTER 3: MANUSCRIPT I

EFFECT OF WEIGHT GAIN ON GLUCOSE AND INSULIN DYNAMICS, LEPTIN AND MEASURES OF OXIDATIVE STRESS IN THOROUGHBRED GELDINGS

Effects of weight gain on glucose and insulin dynamics, leptin and measures of oxidative stress in Thoroughbred geldings[1].

R.W. Quinn[2*] A.O. Burk[*], T.G. Hartsock[*], N.C. Whitley[**], D.L. Keisler[***], K.H. Treiber[†] and R.C. Boston[‡]

[*]*University of Maryland, College Park, MD 20742,* [**]*University of Maryland Eastern Shore, Princess Anne, MD 21853,* [***]*University of Missouri, Columbia, MO 65211,* [†]*Virginia Polytechnic and State University, Blacksburg, VA 24061;* [‡]*University of Pennsylvania, Kennett Square, PA, 19348*

[1]This research was supported in part by the University Maryland College of Agriculture and Natural Resources Agriculture Experiment Station Competitive Grants Program. The authors would like to thank the staff at the Central Maryland Research and Education Center - Clarksville Dairy and Equine Units, specifically J. Schreiber, T. Shellem, J. Suagee and K. Swyers, as well as D. Cazac from the University of Maryland Eastern Shore.

[2]Correspondence: Animal Sciences Center, College Park, MD 20742 (phone: 301-405-8337; fax: 301-405-7960; email: rwquinn@cmh.edu)

ABSTRACT

Altered glucose and insulin dynamics, including reduced insulin sensitivity (SI) have been observed in overweight horses and may contribute to metabolic disease. The objective of this study was to evaluate SI, leptin, lipid hydroperoxide and glutathione in horses during controlled weight gain to determine the point along the continuum of BW or BCS gain where the risk of metabolic disease may be increased. Nine mature Thoroughbred geldings (BW 522 ± 15 kg; BCS 4.4 ± 0.1) were fed hay and a commercial concentrate twice daily at maintenance for three wk followed by a six wk acclimation to the high starch treatment concentrate. After the sixth wk, an additional 20 Mcal/d above maintenance was offered, resulting in an ADG of 0.35 kg/d over 41 wk (total gain 90.8 kg; final BW 615 ± 15 kg, BCS 6.8 ± 0.1). To assess SI, frequently-sampled i.v. glucose tolerance tests were completed prior to treatment initiation (wk 3), after four wk of treatment concentrate feeding (wk 7) and with each 22.7 kg BW gain. The minimal model of glucose and insulin dynamics was used to estimate SI, glucose effectiveness and the acute insulin response to glucose. Leptin, lipid hydroperoxide and glutathione concentrations were measured monthly. By wk 7, SI had decreased ($P = 0.04$) from initial values. At the end of weight gain, SI and Sg remained unchanged ($P = 0.88$; $P = 0.84$) and the acute insulin response to glucose increased ($P = 0.001$) compared to wk 7. Lipid hydroperoxide and glutathione were unaffected by weight gain ($P > 0.05$). Leptin increased ($P < 0.001$) but was not correlated to SI ($P = 0.31$). Because the BCS and BW gain in this study were not associated with a reduction in SI, increase in oxidative stress

or leptin resistance, the BCS and BW gain where the risk of metabolic disease is increased likely lies above BCS 7.0.

Key Words: equine, insulin sensitivity, obesity, laminitis

INTRODUCTION

Changes in tissue insulin sensitivity (**SI**) can result in insulin resistance (**IR**), where the normal level of insulin required to facilitate glucose uptake is no longer adequate (Wilcox, 2005). In the equine, IR is associated with obesity (Hoffman et al., 2003), laminitis (Treiber et al., 2005) and leptin resistance (Cartmill et al., 2003). While horses are more insulin sensitive than ponies (Jeffcott et al., 1986), the prevalence of obese horses acquiring IR and laminitis is increasing (Johnson, 2002) and it is not known whether IR in the horse occurs progressively with increasing adiposity or develops following prolonged obesity.

The onset of IR in the horse may be concurrent with resistance to leptin, a hormone secreted from adipose tissue in proportion to fat levels that acts on neural pathways to promote reduced energy intake (Arch, 2005). Because elevated leptin in a group of obese horses was associated with an exaggerated insulin response to a glucose challenge (Cartmill et al., 2003), changes in SI with weight gain may be mirrored by changes in leptin. In addition, increasing adiposity may increase the risk of cellular damage from oxidative stress due to the accumulation of reactive oxygen species (ROS), end products of intracellular metabolism that interfere with cell signaling and membrane integrity

(Vincent and Taylor, 2006). In obese humans, oxidative stress is associated with impaired endothelial function and IR (Vincent and Taylor, 2006) and as such may contribute to the pathogenesis of laminitis in horses (Neville et al., 2004).

It was the goal of this study to assess the impact of controlled BW gain on SI, leptin and two markers of oxidative stress, lipid hydroperoxide (**LPO**) and reduced (**GSH**) and oxidized (**GSSG**) glutathione, in order to identify the limit of BW gain associated with an increased risk of metabolic disease and its relation to an achieved level of adiposity, quantified as BCS.

MATERIALS AND METHODS

This experiment was run in conjunction with another experiment assessing the impacts of weight gain, diet and exercise on insulin sensitivity in the horse. The experiments were carried out at the University of Maryland Equine Research Unit (Clarksville, MD) and the experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Maryland (Protocol # R - 05 - 16).

Animals. Nine Thoroughbred geldings (10.3 ± 3.4 yr) with an initial BW and BCS of 516 ± 13 kg and 4.4 ± 0.1, respectively were housed in individual box stalls (3.6 m^2) with rubber mat flooring and pelleted wood fiber bedding (Woody Pet, Surrey, BC, Canada). All horses received routine dental care, vaccinations and deworming with a broad-spectrum dewormer prior to the start of the study. Horses were exercised twice daily at

0900 and 1600 for 35 min at a speed of 1.2 m/s using an automated 6-horse exerciser (Priefert, Mt. Pleasant, TX).

***Diets*.** Prior to the start of the study, horses were maintained in the study facility and fed only mixed grass-alfalfa hay for a four wk period. At the start of the study, horses were given three wk to acclimate to a diet of mixed grass-alfalfa hay and a texturized commercially available concentrate (**CF**; Legends 12, Southern States Cooperative, Richmond, VA; Table 1) at a level meeting their maintenance requirement for DE and providing vitamins and minerals at a level meeting or exceeding the minimum requirements for horses (NRC, 1989).

Throughout the study, all horses had ad libitum access to water and iodized salt. Hay was fed twice daily at 0800 and 1900 in floor-mounted wooden hay boxes and orts collected and weighed prior to the morning feeding to estimate daily hay intake. Due to the length of the study, three separate lots of hay were fed (Table 1) and three to five random core samples of hay taken from each lot were submitted to a commercial forage testing lab for nutrient analysis (Dairy One, Ithaca, NY). Concentrate was offered in individual canvas nose bags (Derby Originals, North Canton, OH) and refusals weighed following each feeding to estimate concentrate intake, with horses given 45 min during which to consume the concentrate. Grab samples of concentrate (n = 8) were submitted to a commecial laboratory for nutrient analysis (Dairy One, Ithaca, NY). All diets were offered at a ratio of 60% hay to 40% concentrate. Upon inititation of weight gain (wk 9), the 60:40 hay-to-concentrate ratio resulted in hay being fed ad libitum. All dietary transitions were made over a 7d period.

Table 1. Nutrient analyses [1] (DM basis) for commercial concentrate (CF [2]) fed to Thoroughbred geldings for a three wk period at the start of the study, treatment concentrate (HS) fed from wk four to the end of weight gain (wk 41), and hay fed throughout the study. Data are presented as means ± SEM

	Concentrate		Hay (wk fed)		
Item, %	CF	HS	Lot 1 (pre-study to wk 25)	Lot 2 (wk 26 to 29)	Lot 3 (wk 30 to 41)
ADF	10.0 ± 3	8.04 ± 1.3	31.40 ± 0.84 [b]	39.00 ± 2.21 [c]	35.05 ± 1.11 [bc]
NDF	20.0 ± 2	17 ± 1.3	51.37 ± 2.27	63.00 ± 6.00	50.75 ± 3.00
NSC [3]	53.0 ± 0.1	56.7 ± 1.4	17.47 ± 2.04	13.10 ± 5.39	10.38 ± 2.69
Fat	8.2 ± 0.4	4.3 ± 0.1	3.43 ± 0.14	2.40 ± 0.37	2.83 ± 0.19
CP	14.4 ± 0.3	10.0 ± 0.6	14.35 ± 0.54	11.00 ± 1.44	17.5 ± 0.72 [b]
Ca	1.1 ± 0.02	0.73 ± 0.10	0.92 ± 0.07	0.69 ± 0.19	0.86 ± 0.09
P	0.61 ± 0.04	0.38 ± 0.03 [a]	0.26 ± 0.03	0.17 ± 0.07	0.31 ± 0.04
DE, Mcal/kg	3.4 ± 0.04	3.6 ± 0.07	2.34 ± 0.04	1.98 ± 0.10 [b]	2.31 ± 0.05

[1] Dairy One, Ithaca, NY
[2] Legends 12, Southern States Cooperative, Richmond, VA
[3] NSC = Non-structural carbohydrates calculated as starch plus sugar (as water-soluble carbohydrates)
[a] Means within rows are significantly different at $P < 0.05$ for concentrate
[b,c] Means within rows are significantly different at $P < 0.05$ for hay

At wk 4, the horses were transitioned onto the treatment concentrate (**HS**, Table 2) fed to meet their maintenance DE requirement (NRC, 1989). After six wk, the amount of DE offered was increased to 20 Mcal above maintenance per day to encourage weight gain. The DE offered was recalculated weekly following the assesment of weight gain to account for the increasing maintenance requirement. While hay lot two was being fed, the rate of weight gain began to decline and so a third concentrate feeding was added (at 1300), which offered an additional 7 Mcal DE per day. Refusals of hay and concentrate were recorded daily and a seven d period at the end of every 4 wk period was averaged to estimate intakes of feed and DE. Data from one horse was not used for intake calculations due to excesive urination on refused hay. Weight gain was terminated at wk 41 when the average initial BW had increased by 90.8 kg.

Assessment of weight gain: Horses were weighed every two wk on a livestock scale (Preifert, Mt. Pleasant, TX) beginning at wk 1. BCS was assessed at wk 3, wk 7, wk 9 and every four wk thereafter by two independent researchers using the Henneke BCS system (Henneke et al., 1983). Each researcher assigned a numerical score (range 1 to 9 with half-scores possible) to the six different body areas which were then averaged to calculate the BCS. The final BCS was calculated by averaging the BCS scores from both researchers.

At the end of weight gain, rump fat thickness was measured via ultrasound and the equation of Kane et al. (1987) was used to calculate percent body fat (% fat = 2.47 + 5.47 (rump fat_{cm})). Fat depth was measured 6 cm lateral from the midline at the center of

Table 2. Ingredient composition of treatment concentrate (HS) fed to Thoroughbred geldings from wk 4 to wk 41. Concentrate was fed to meet the animals' maintenance DE requirement from wk 4 to wk 9, followed by an increase to 20 Mcal above maintenance from wk 9 to wk 41

Ingredient, %	HS
Corn	56.0
Oats	12.5
Distillers grains	7.0
Soybean meal	5.0
Beet pulp	5.0
Molasses	5.0
Mixed hay	5.0
Limestone	0.5
Vitamin E	0.5
Vitamin D	0.2
Vitamin, mineral and amino acid premix[1]	2.3
Salt and trace mineral premix[2]	1.0

[1] Grass-plus Mineral, Buckeye Nutrition, Dalton, OH; Guaranteed analysis: CP, 8%; lysine, 2.5 %; methionine, 1.3%; Ca (minimum) 19%; Ca (maximum) 22%; P, 6%; K, 2%; Mg, 1.5%; Mn, 900 ppm; Fe, 1150 ppm; Cu, 550 ppm; Zn, 1300 ppm; Co, 15 ppm; I, 15 ppm; Se, 9 ppm; vitamin A, 176,000 IU/kg; vitamin D-3, 17,600 IU/kg; vitamin E, 1,760 IU/kg.

[2] Harvest salt, Buckeye Nutrition, Dalton, OH; Guaranteed analysis: NaCl (minimum), 93%; NaCl (maximum), 96%; Mn, 500 ppm; Cu, 2500 ppm; Zn, 5000 ppm; Co, 150 ppm; I, 40 ppm; Se, 40 ppm.

the pelvic bone using b-mode ultrasound with a 15.24 cm linear transducer and frequency of 3.5 Hz (Aloka 500, Tokyo, Japan).

***Blood sampling*:** Twenty milliliters of blood was collected by jugular venipuncture every 4 wk over the course of the study for the determination of serum leptin, serum cortisol, plasma LPO and whole-blood GSH and GSSG. Samples were taken prior to the initiation of weight gain (wk 9), after the fourth wk of weight gain (wk 13) and every 4 wk thereafter for 41 wk. For the collection of serum, 10 mL of whole blood was placed into an additive-free evacuated glass collection tube (Vacutainer, Becton Dickenson, Franklin Lakes, NJ), allowed to clot for 2 h at room temperature and centrifuged at 4°C for 15 min at 2000 x g. For plasma collection, 10 mL of whole blood was placed into an evacuated glass collection tube containing EDTA as an anticoagulant (Vacutainer, Becton Dickenson, Franklin Lakes, NJ). Prior to centrifugation, 100 uL of whole blood was placed into a 1.5 mL microtube (Fisher Scientific, Pittsburgh, PA), to which 10 uL of 1-methyl-2-vinyl-pyridium trifluoromethane sulfonate (M2VP) was added for later determination of GSH concentrations. The remaining blood from the EDTA tubes was immediately centrifuged at 4°C for 15 min at 2000 x g. Serum and plasma used for the determination of hormone and metabolite concentrations were transferred into 1.5 mL microtubes and stored at -20°C until analysis. Plasma and whole blood used to determine the concentrations of LPO, GSH and GSSG were stored at -80°C until analysis.

FSIGT: Horses were subjected to frequently-sampled intravenous glucose tolerance tests (**FSIGT**) previously validated in the horse (Hoffman et al., 2003). The first FSIGT

occurred at wk 3, while the horses were consuming the CF concentrate at maintenance (**CFMM**) and the second at wk 7, while the horses were consuming the HS concentrate at maintenance (**TXMM**). Subsequent FSIGTs occurred with each 22.7 ± 4.5 kg BW gain (**WG1, WG2, WG3** and **WG4**). Following each weekly estimation of BW, total gain above maintenance BW was calculated. After a 22.7 ± 4.5 kg gain was observed, horses were scheduled for an FSIGT the following week, with 5 d elapsing between weighing and FSIGT. No more than four horses were scheduled for an FSIGT on any given day. By wk 41, all horses had completed FSIGTs CFMM through WG3, with only five of the nine completing WG4.

The night before each FSIGT, horses were asceptically fitted with an indwelling 14 guage jugular catheter with attached 5.5 mL total volume extension set, kept patent with 12 mL of heparinized saline (0.75 mL heparin/1000 mL isotonic saline). At 0600 on the day of the FSIGT, horses were fed their morning ration of hay only and had access to hay and water during the entire FSIGT. No concentrate was fed to any horse on the morning of an FSIGT. Provision of hay during the FSIGT (Hoffman et al., 2003; Treiber et al., 2005b) is thought to reduce the effect of fasting on glucose dynamics (Forhead and Dobson, 1997), as well as maintain quiet, calm behavior in all horses housed in the barn.

The morning of the FSIGT, horse BW was measured in order to accurately determine the level of glucose and insulin to be administered. Glucose (50% dextrose solution, AmTech Group, Inc., St. Joseph, MO) was administered as an i.v. bolus of 300 mg/kg BW. Insulin (HumulinR, Eli Lilly and Co., Indianapolis, IA) was administered as an i.v. bolus of 20 mIU/kg BW. Prior to glucose administration, three blood samples were collected (0730, 0745 and 0759) for determination of basal glucose and insulin levels.

Intravenous glucose was administered over a 2 - 3 minute period beginning at 0800. Thirty-five venous blood samples were taken at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 210 and 240 minutes after glucose was administered. Insulin dosing occurred 20 min after the glucose was administered. For each sample, 20 mL of whole blood was immediately transferred into one of two glass specimen tubes containing either sodium fluoride/potassium oxalate (Vacutainer, Becton Dickenson, Franklin Lakes, NJ) or no additive (Fisher Scientific, Pittsburgh, PA). Blood from the sodium fluoride/potassium oxalate tubes were immediately centrifuged at 4° C for 15 min at 2000 x g while blood from the additive-free tubes was allowed to clot for 2 h at room temperature before being centrifuged at 4° C for 15 min at 2000 x g. Serum and plasma were transferred to microtubes and stored at -20° C until analysis.

Minimal Model Analysis: Raw glucose and insulin data were modeled by MinMod Millenium (Ver. 6.02, 2001) using equations previously validated in the horse (Hoffman et al., 2003). Variables estimated include SI, insulin-independent glucose uptake (glucose effectiveness; **Sg**), the acute insulin response to glucose (**AIRg**) and the disposition index (**DI**; Bergman, 1997). Data from two individual horse FSIGTs were excluded from the analysis due to poor parameter resolution (f_sd $\geq$ 0.5; 3%).

Analysis of Blood Hormones: Serum insulin and cortisol were measured in duplicate using RIA techniques (Coat-A-Count Insulin and Coat-A-Count Cortisol, Diagnostics Products Corporation, Los Angeles, CA), previously validated for use in the horse

(Freestone et al., 1991). Serum leptin was measured using a double-antibody RIA procedure utilizing rabbit and anti-ovine antibodies, previously validated for use in the horse (Buff et al., 2002). Plasma glucose was measured in triplicate by colorimetric assay (Glucose C-2, Wako Chemicals, Richmond, VA), wherein samples were read at 505 nm on an absorbance microplate reader (Tecan Sunrise, Phenix Research Products, Hayward, CA). The intraassay CV of was 3% for insulin, 3% for cortisol and 5% for glucose. The interassay CV for insulin was 16%.

The levels of reduced and oxidized glutathione were determined in triplicate using a commercially available kit (Bioxytech, GSH/GSSG-412, OXIS Research, Portland, OR). Levels of GSSG were measured through an enzymatic reaction of GSH with dithiobis-2-nitrobenzoic acid, resulting in the conversion of 2-GSH to GSSG. The rate of color change, read at 412 nm every 15 sec over a 3-min period in an absorbance microplate reader, was proportional to the change in concentration of GSSG. Increased accuracy is achieved by the addition of M2VP to the whole blood prior to freezing, which scavenges any GSH already present and prevents back-conversion to GSSG. Data were not available for wk 3, wk 25 and wk 33 as M2VP was mistakenly not added to the whole blood before freezing.

For the determination of LPO in plasma, the oxidiation of Fe II to Fe III by LPO was quantified via its complexing to xylenol orange (Kinnunen et al., 2005). The absorption of the sample and concurrent blank were measured at 560 nm in an absorbance microplate reader and the net absorbance calculated as the difference in absorbance between the sample and the blank. The net absorbance was divided by the molar

extinction coefficient of LPO (0.0521) to determine the final concentration. The intraassay CV of triplicate samples was 6% for GSSG, 5% for GSH and 4% for LPO.

Statistical Analysis. All statistical analyses were performed using SAS statistical software (v. 9.1, Cary, N.C.). Assumptions of normality and homogeneity of variances were evaluated by examination of the residual plots and found to be met for all variables except SI, leptin, basal glucose and LPO. Variables not meeting assumptions were adjusted by base-10 logarithmic transformation prior to analysis. All variables were analyzed by repeated measures mixed model ANOVA using orthoganal contrasts for least-squares mean comparisons and to test for linear and quadratic changes over time. The values for SI, Airg, Sg and basal insulin at CFMM were used as a covariate and for all ANOVA analyses, the appropriate correlation matrix for repeated measures was chosen based on the smallest Akaike's Information Criteria. Fixed model effects included day, treatment and the treatment by day interaction and the random effect was horse (within treatment). Relationships between variables were explored by partial correlation analysis adjusted for horse (within horse) using the GLM procedure of SAS. Data are presented as least squared means ± SEM and Tukeys' HSD test was used for least-squares mean comparisons when appropriate. Although the data for log-normal variables are presented as least-squares means ± SEM, significant differences reflected in the superscripts refer to the test of significance run on the log-transformed data.

RESULTS

Average BW and BCS are presented in Figure 1. At wk 3, average BW was 522 ± 15 kg (range 466 to 576 kg) and BCS was 4.4 ± 0.1 (range 3.8 to 5.0). Horses maintained BW and BCS through the start of weight gain (BW, 519 ± 15 kg, range 473 to 590 kg, $P = 0.57$; BCS, 4.2 ± 0.1, range 4.0 to 4.6, $P = 0.77$). Following the initiation of weight gain, average BW and BCS increased ($P < 0.0001$) to wk 41. Final average BW was 615 ± 15 kg (range 539 to 684 kg) and BCS was 6.8 ± 0.1 (range 6.3 – 7.3). Regressions of BCS on BW and time (data not shown) indicated that an increase of one BCS required a gain of 32 kg ($r^2 = 0.36$, $P < 0.0001$) and required 80 d ($r^2 = 0.89$, $P < 0.0001$). Ultrasound-derived rump fat depth at the end of weight gain was 2.7 ± 0.2 cm (range 1.8 – 4.0 cm) and calculated percent body fat averaged 17 ± 1 % (range 12 – 24 %).

When fed at maintenance, all feed offered was consumed, indicating that the hay and both concentrates were acceptable. Despite small and occasional significant differences between weeks, a linear change between wk 9 and wk 41 for both DE intake and ADG was not detected (Figure 2; DE, $P = 0.29$; ADG, $P = 0.20$). The ADG was 0.35 kg/d.

The slight decline in DE intake and ADG at wk 29 coincided with the offering of the second lot of hay (hay # 2), which was chemically assessed to be lower in nutritional quality. The additional concentrate feeding added following the wk 33 sample did not result in a total DE intake greater than the previous month ($P = 0.21$) but concentrate intake was greater between wk 33 and 37 than during all previous weeks ($P < 0.001$, data not shown).

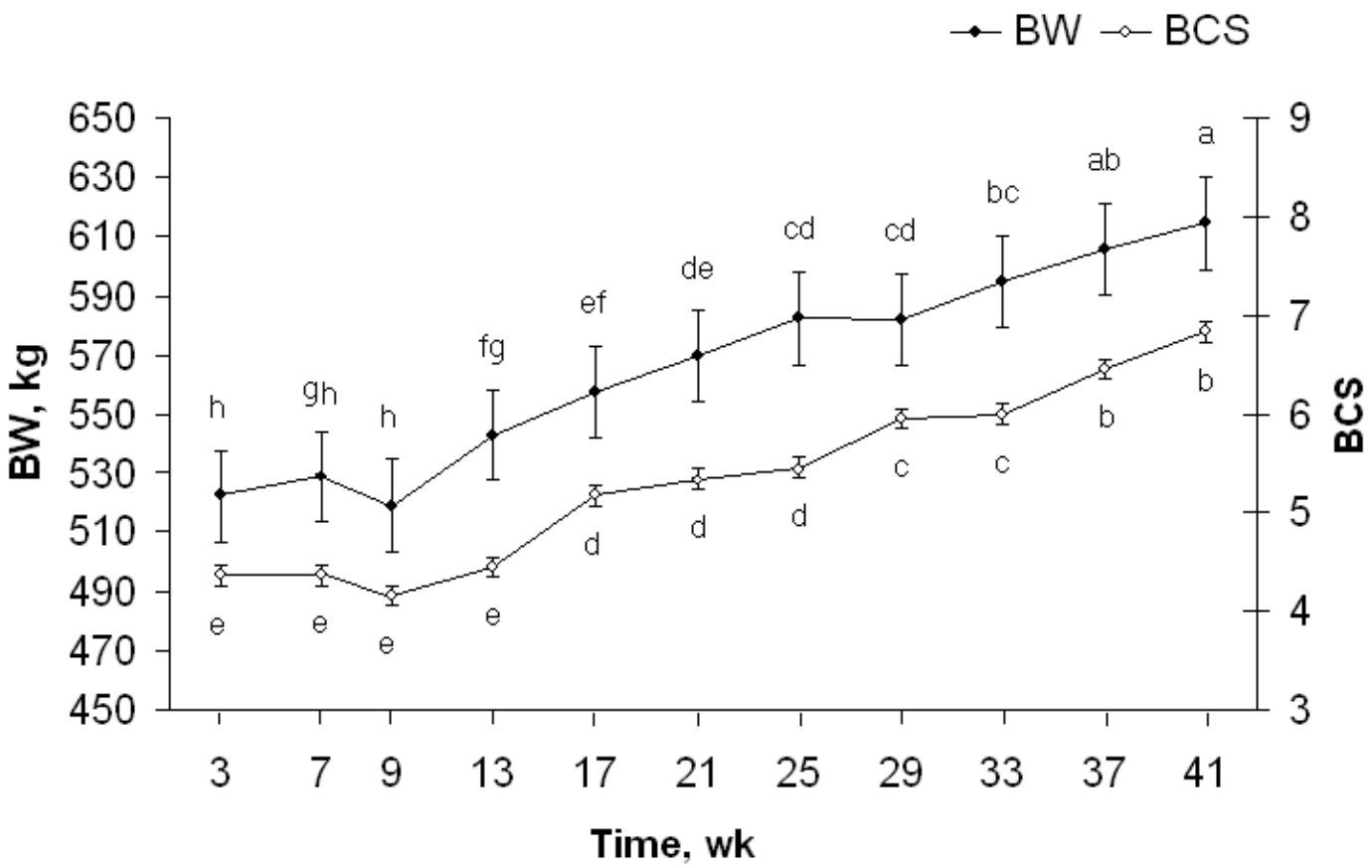


Figure 1. Changes in average BW and BCS of Thoroughbred geldings (n = 9) while fed commercial concentrate (wk 3) or treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41). Pooled SEM was 12 kg for BW and 0.1 for BCS. [abcdefgh] Least-squares means within each variable with uncommon superscripts differ ($P < 0.05$).

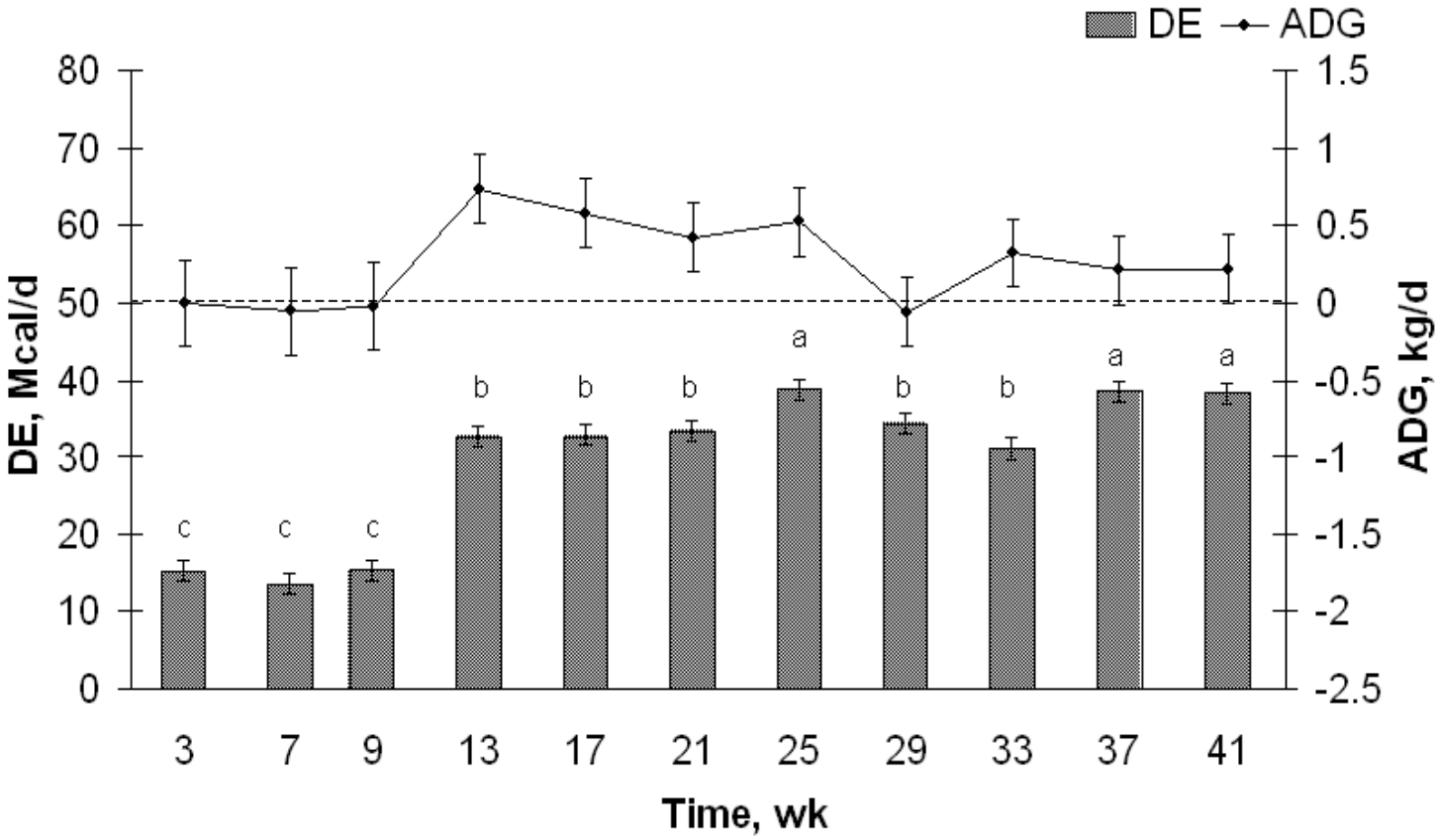


Figure 2. Average digestible energy intake (Mcal/d; n = 8) and ADG (kg/d; n = 9) in Thoroughbred geldings while fed commercial concentrate (wk 3) and treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41). Pooled SEM was 1.0 Mcal for DE and 0.2 kg for ADG. [abc] Least-squares means with uncommon superscripts differ ($P < 0.05$).

The target weight gain between each FSIGT was 22.7 ± 4.5 kg and actual average weight gain ranged from 17.3 – 24.0 kg. The increase in BCS between each FSIGT ranged from 0.1 – 0.5 scores. Data for all minimal model variables are presented in Table 3. Between CFMM and TXMM, Sg, AIRg, DI and basal glucose remained unchanged (Sg, $P = 0.10$; AIRg, $P = 0.49$; DI, $P = 0.07$; basal glucose, $P = 0.37$) while log-normal SI and basal insulin were decreased ($P = 0.04$; $P = 0.001$). No linear change in log-normal SI, Sg or basal glucose occurred between TXMM and WG4 (SI, $P = 0.88$; Sg, $P = 0.84$; Basal glucose, $P = 0.92$). A significant linear increase was found between TXMM and WG4 for AIRg ($P = 0.01$) and basal insulin ($P = 0.002$) while a quadratic effect was found for DI ($P = 002$). Neither BCS nor BW were correlated with SI (data not shown; BCS, r = - 0.132, $P = 0.40$; BW, r = - 0.12, $P = 0.45$) but were positively correlated with AIRg (data not shown; BCS, r = 0.32, $P = 0.04$; BW, r = 0.39, $P = 0.01$).

Log-normal serum leptin increased between wk 9 and wk 41 ($P < 0.001$; Figure 3) and was positively correlated (data not shown) to BCS (r = 0.67, $P < 0.001$) and BW (r = 0.59, $P < 0.001$) but not to SI (r = - 0.038; $P = 0.81$). No change was found between wk 9 and wk 41 for serum cortisol ($P = 0.65$; Figure 4) or log-normal plasma LPO ($P = 0.26$; Figure 5). Between wk 9 and wk 41, the concentration of whole blood GSH decreased ($P = 0.01$) while GSSG remained unchanged ($P = 0.65$; Figure 6).

Table 3. Least-squares means ± SEM for minimal model variables in Thoroughbred geldings derived from a frequently-sampled i.v. glucose tolerance test while fed commercial concentrate [1] (CFMM) or treatment concentrate (TXMM) at maintenance and throughout weight gain. Minimal model variables during weight gain were calculated after 22.7 (WG1), 45.4 (WG2), 68.1 (WG3) and 90.8 (WG4) kg of gain.

Variable [2]	CFMM (n = 9)	TXMM (n = 9)	WG1 (n = 8)	WG2 (n = 9)	WG3 (n = 7)	WG4 (n = 5)
SI, x 10^{-4} $(mU/L)^{-1}$ min^{-1}	5.37 ± 0.73 [a]	2.22 ± 0.73 [b]	3.28 ± 0.77 [ab]	3.69 ± 0.73 [ab]	3.87 ± 0.82 [ab]	2.66 ± 0.98 [ab]
Sg, x10^{-2} min^{-1}	2.21 ± 0.30 [a]	2.93 ± 0.30 [ab]	2.67 ± 0.32 [ab]	3.18 ± 0.30 [b]	2.44 ± 0.34 [ab]	2.28 ± 0.40 [ab]
AIRg, mU L^{-1} min	467 ± 55 [c]	523 ± 55 [bc]	396 ± 59 [c]	431 ± 55 [c]	640 ± 63 [ab]	794 ± 75 [a]
DI, x10[1]	192± 27 [ab]	121 ± 27 [bc]	78 ± 28 [c]	113 ± 27 [c]	203 ± 30 [a]	211 ± 36 [a]
Basal insulin, mU/L	14.16 ± 2.05 [bc]	6.19 ± 2.05 [d]	17.65 ± 2.17 [ab]	10.20 ± 2.05 [cd]	20.91 ± 2.17 [a]	21.35 ± 2.75 [a]
Basal glucose, mg/dL	86.1 ± 8.8	75.2 ± 8.8	80.9 ± 9.3	81.6 ± 8.8	84.6 ± 9.3	79.4 ± 11.8

[a – d] Means within rows with uncommon superscripts differ ($P < 0.05$).
[1] Legends 12, Southern States Cooperative, Richmond, VA.
[2] SI, insulin sensitivity; Sg, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index.

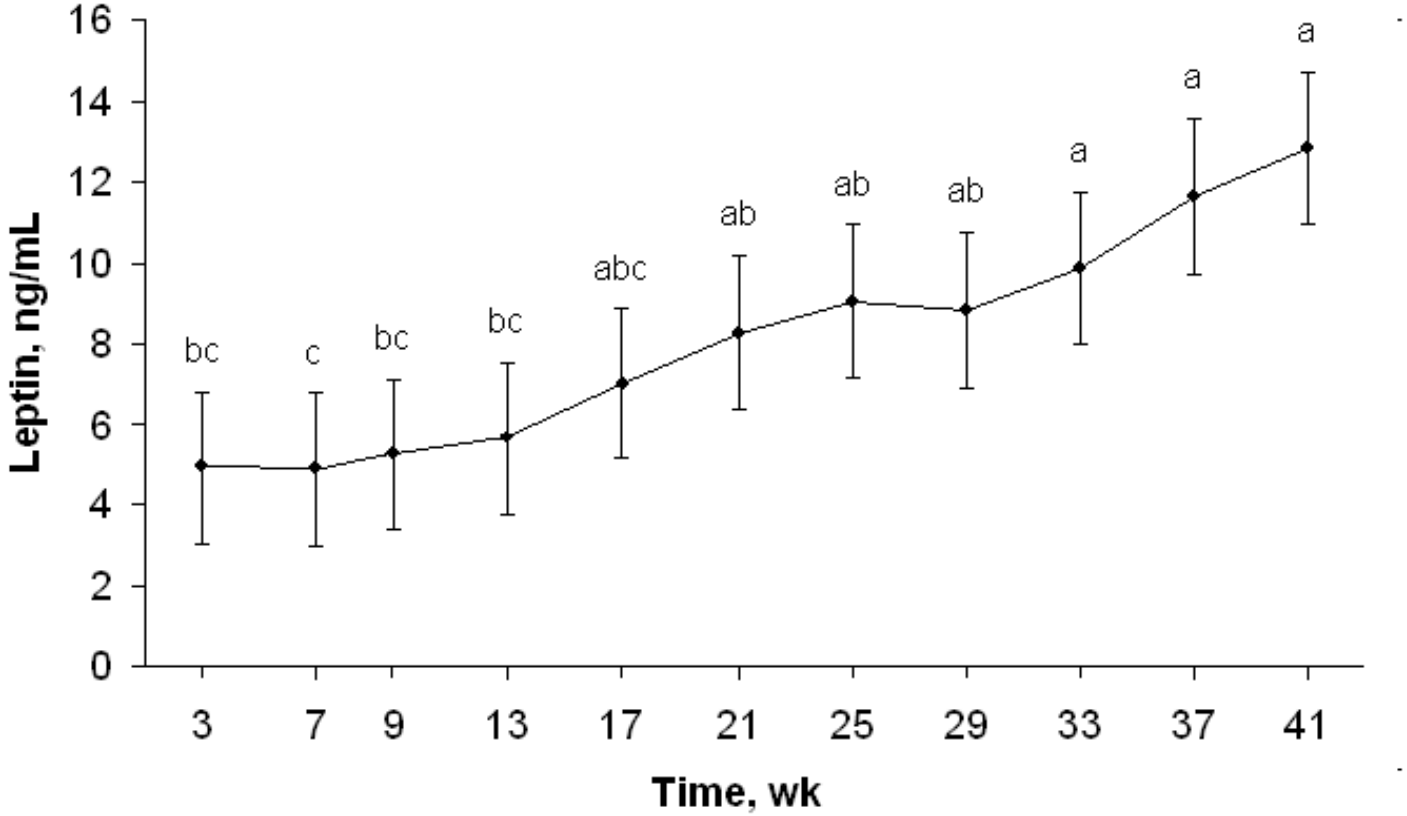


Figure 3. Average serum leptin levels (ng/mL) in Thoroughbred geldings (n = 9) while fed commercial concentrate (wk 3) or treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41). [abc] Least-squares means with uncommon superscripts differ ($P < 0.05$).

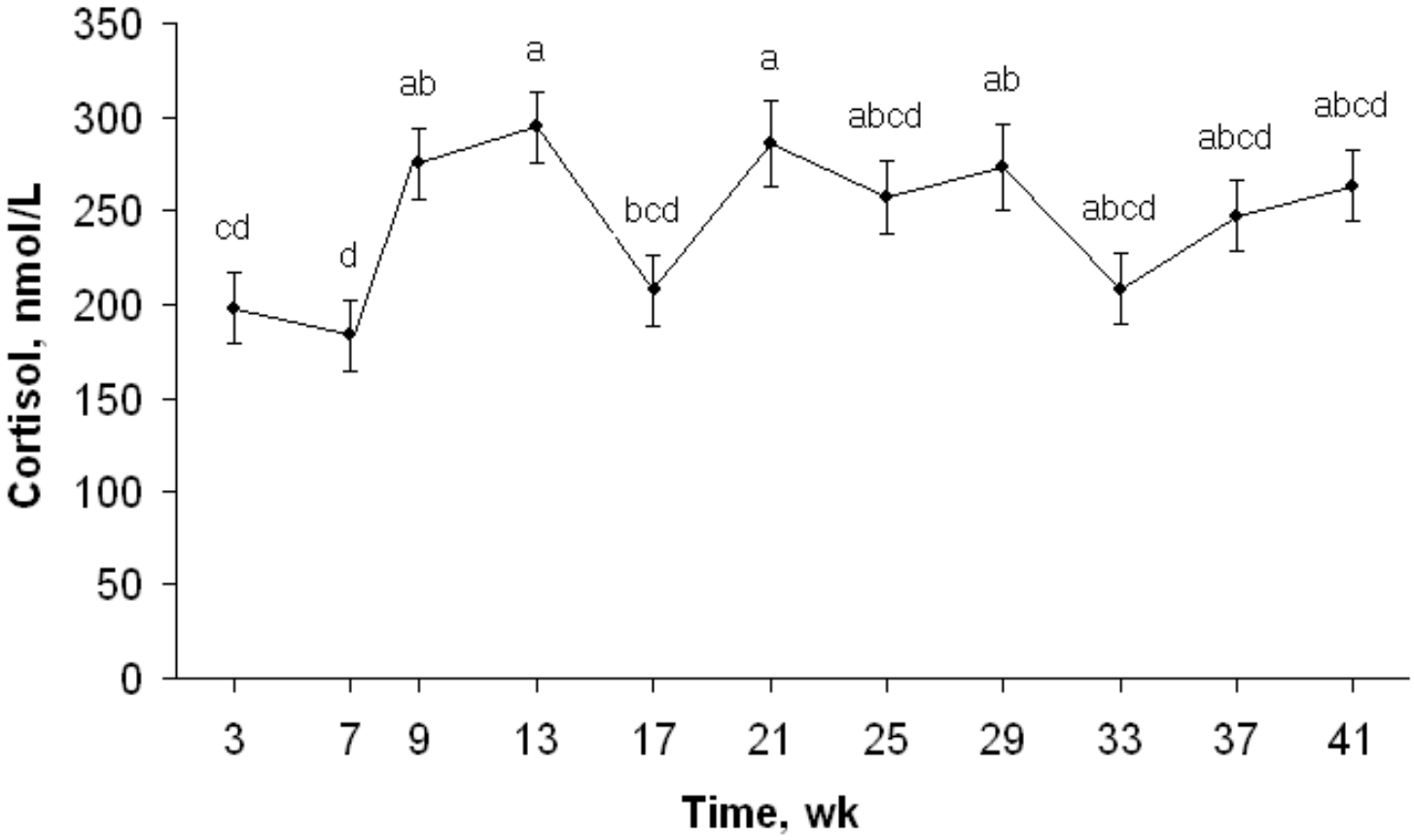


Figure 4. Average serum cortisol (nmol/L) in Thoroughbred geldings (n = 9) while fed commercial concentrate (wk 3) or treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41). Pooled SEM was 19.0 nmol/L. [abcd] Least-squares means with uncommon superscripts differ ($P < 0.05$).

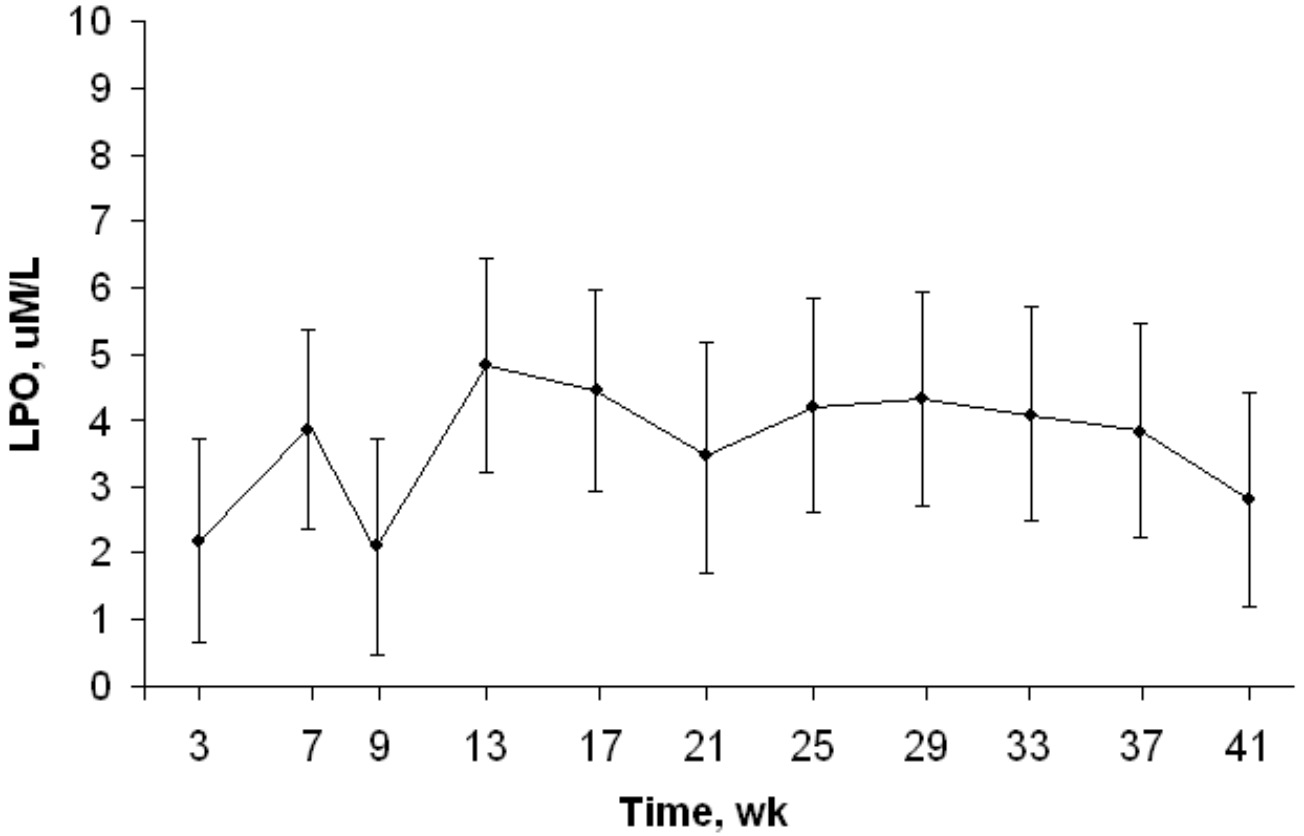


Figure 5. Average plasma lipid hydroperoxide (LPO; uM/L), a measure of oxidative stress-induced cell membrane lipid peroxidation damage, in Thoroughbred geldings (n = 9) while fed commercial concentrate (wk 3) and treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41). Data are presented as least-squares means ± SEM.

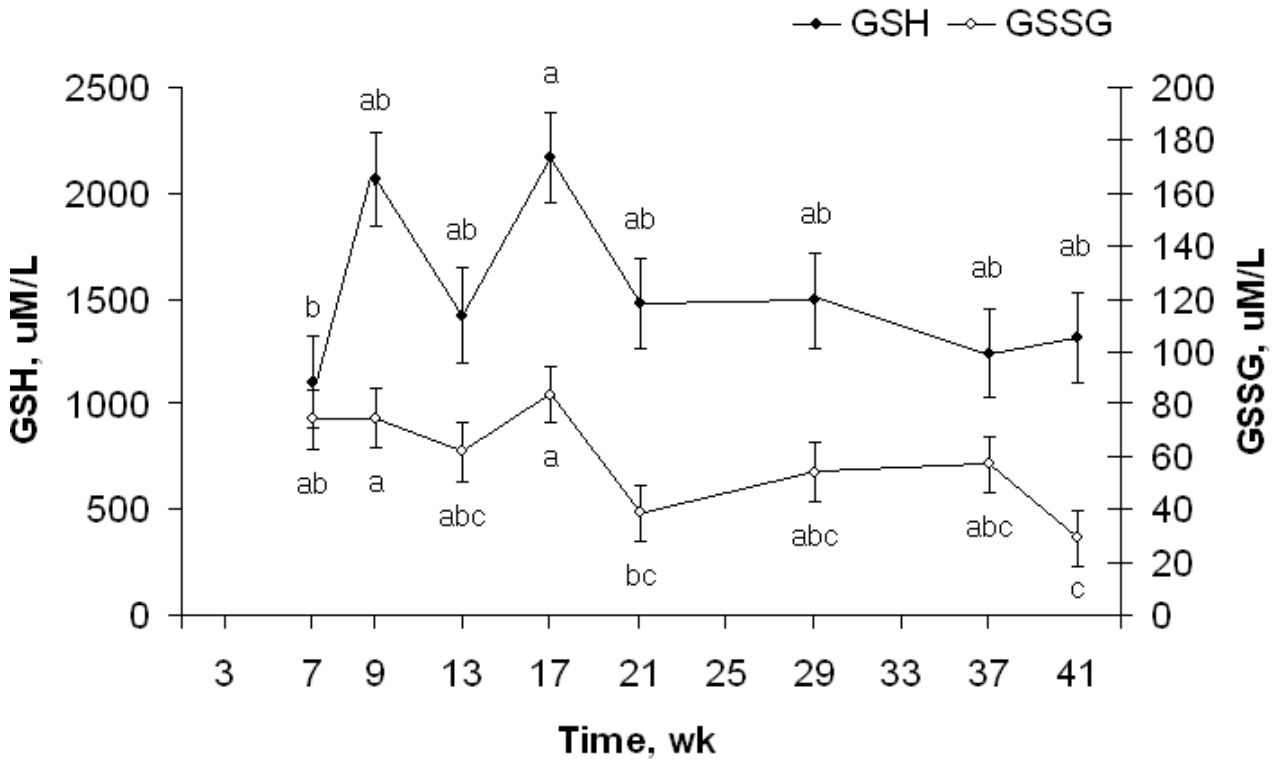


Figure 6. Average whole-blood reduced (GSH; uM/L) and oxidized (GSSG; uM/L) glutathione, an indication of the ability of the glutathione peroxidase antioxidant system to combat oxidative stress, in Thoroughbred geldings (n = 9) while fed commercial concentrate (wk 3) and treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41). Pooled SEM for GSH, 225.8 uM/L; GSSG, 7.7 uM/L. [abc] Least-squares means with uncommon superscripts within each variable differ ($P < 0.05$).

DISCUSSION

Glucose tolerance is altered in obese (Jeffcott et al., 1986; Freestone et al., 1992), laminitic (Coffman and Colles, 1983) and obese-laminitic (Jeffcott et al., 1986) ponies. Research using direct measures of SI indicates that IR is a significant risk factor for pasture-associated laminitis in obese ponies (Treiber et al., 2006). In the horse, SI is negatively correlated with BCS (Vick et al., 2007) and is lower in horses of high BCS (≥ 7) than low BCS (≤ 6; Powell et al., 2002; Hoffman et al., 2003). However, it is not known if SI deteriorates as BW is gained or if excessive adiposity must be maintained for a prolonged period of time before SI is affected.

During the weight gain portion of the current study, the horses gained an average of 0.35 kg/d and reached a maximum BCS ranging from 6.3 to 7.3. The slight decline in ADG in January was most likely due to the provision of lower quality hay (hay # 2). Because a more rapid gain was observed during the first month (1.08 kg/d), the horses may have experienced an initial period of compensatory gain, a phenomenon common in feedlot cattle and finishing pigs where the provision of ad libitum feed following a period of energy restriction results in an initial period of rapid lean tissue growth (Hornick et al., 2000). In addition, during the current study, 32 kg were gained per one unit increase in BCS, an amount greater than that previously determined for mixed light-breed horses (16 – 20 kg; Lawrence, 2000), possibly due to the leaner phenotype of the Thoroughbred.

The reduction in SI between CFMM and TXMM likely reflects the continuing adaptation to the starches and sugars in the concentrates (Hoffman et al., 2003b), as both the CF concentrate and treatment concentrate were very similar in nutrient composition.

Previous studies in horses have shown that adaptive changes in glucose and insulin dynamics begin after 27 – 29 d (Sticker et al., 1995; Powell et al., 2002) and it is likely that the SI at CFMM, being only 21 d after the start of concentrate feeding, was not reflective of full starch and sugar adaptation. Prior to the start of the study, horses had been maintained solely on mixed grass-alfalfa hay, which would be expected to be associated with a higher SI (Hoffman et al., 2003). Full adaptation to the starches and sugars in the concentrates, including an increase in digestive enzyme production (Flores et al., 1988) and pancreatic insulin secretion (Kopp, 2003) most likely occurred between CFMM and TXMM. Because the horses were able to maintain the SI seen at TXMM for the duration of weight gain, they were able to maintain an appropriate insulin secretion in response to the glucose challenge even while consuming a highly insulinogenic diet, which has been associated with IR in human obesity (Kopp, 2003).

At the end of weight gain, SI was higher in the current study than that of obese horses (BCS ≥ 7, SI = 0.37 ± 0.27 x 10^{-4} $(mU/L)^{-1}$ min^{-1}; Hoffman et al., 2003) or laminitic ponies (BCS 6 - 8; SI = 0.39 ± 0.07 x 10^{-4} $(mU/L)^{-1}$ min^{-1}; Treiber et al., 2005b), indicating that the level of weight and BCS achieved in the current study was not likely to increase the risk of metabolic dysfunction. While BCS has been correlated to SI (BCS 4 – 9; r = -0.57, $P < 0.001$; Vick et al., 2007), no correlation between SI and BCS was found in the current study using horses between BCS 4.3 ± 0.1 and BCS 6.8 ± 0.1. This may be due to the narrow range of BCS scores in the current study or the utilization of different breeds, ages and gender by Vick et al. (2007). Mares, such as those studied by Vick et al. (2007; 3 – 29 yr), especially mature mares, have been previously shown to have an increased proportion of s.c. body fat as compared to younger mares and geldings

(Fitzgerald and McManus, 2000; Lenhard et al., 2004) and as excessive adiposity is associated with reduced SI (Hoffman et al., 2003), this may explain the lack of congruency between studies.

The increases in AIRg and basal insulin between TXMM and WG4 may be due to increasing BW or in response to the treatment diet. Similar results were noted for rats fed high glycemic diets, which had higher insulin secretion during an IVGTT than did rats fed low glycemic diets but no difference in SI (Pawlak et al., 2001). The relationship between SI and AIRg is such that insulin secretion will be increased in order to prevent a decline in insulin action and ultimately maintain normal glucose tolerance (Araujo-Vilar et al., 1998). In the present study, the increasing AIRg without a concurrent decrease in SI, reflected in the increasing DI, may be an early indication of pancreatic compensation for either weight gain or diet which, without intervention, may eventually lead to reduced SI (Hoffman et al., 2003). Indeed, AIRg was reduced in obese horses with low SI (Hoffman et al., 2003), indicating that the insulin response was no longer adequate to maintain glucose tolerance. The effect of diet on AIRg in the horse is unclear, but higher AIRg was found in yearling horses fed a concentrate high in starch and sugar (Treiber et al., 2005b), although with a concomitant decrease in SI.

Resistance to the satiety hormone leptin has been implicated in IR in various experimental models (Ceddia, 2005). Leptin circulates in proportion to s.c. body fat levels in the horse (Buff et al., 2002) and has been shown in other models to promote a reduction in feed intake and increase in energy expenditure to compensate for excessive adiposity (Arch, 2005). Leptin resistance refers to the maintenance of high circulating leptin levels in the presence of obesity and hyperinsulinemia (Halaas, 1997). In the

current study, serum leptin levels increased by 60% between August and April, which was expected (Buff et al., 2002; Gentry et al., 2002; Kearns et al., 2005). However, changes in leptin were not mirrored by changes in SI, indicating that the horses were not leptin resistant as defined by Cartmill et al. (2003) in mares (BCS $\geq$ 7.5) fed native grass and ryegrass pasture.

Cortisol was measured both to track the stress levels of the horses, as they were confined to stalls for the majority of the day, and to rule out pituitary adenoma in any horse with abnormal glucose dynamics. Cortisol levels were slightly lower than those reported by Van der Kolk et al. (2001) for normal horses and did not change over the course of the study, indicating that the horses were not unduly stressed or afflicted with a pituitary tumor.

Oxidative stress is a risk factor for cardiovascular disease and type II diabetes in humans (Vincent and Taylor, 2006) and may be a risk factor underlying laminitis in horses (Neville et al., 2004). In humans, obesity-induced hyperglycemia is responsible for an increase in oxidative stress, resulting in endothelial dysfunction and poor vascular health (Vincent and Taylor, 2006). The existence of similar dysfunction in the horse may increase the risk of laminitis through damage to the vasculature within the hoof (Neville et al., 2004), however no evidence of oxidative stress was found in a recent study comparing previously laminitic ponies to non-laminitic ponies (Treiber et al., 2007).

In the current study, the ratio of GSH:GSSG was chosen as an indication of the activity of glutathione peroxidase, an enzyme responsible for ROS elimination, while LPO reflects the level of cellular membrane lipid peroxidation damage (Vincent and Taylor, 2006). Because neither index of oxidative stress changed appreciably with

weight gain in the present study, oxidative damage was likely obviated by the appropriate antioxidant systems. Concentrations of GSH and GSSG were similar to those previously reported in mature, healthy athletic horses (Art et al., 1999; Marlin et al., 2002; DeMoffarts et al., 2002) while LPO levels were lower than previously reported (Art et al., 1999b; Williams et al., 2002).

In conclusion, neither BW gain nor increased BCS affected SI within the limits of the current study, indicating that changes in adiposity between BCS 4.3 and 6.8 are not likely to be associated with an increased risk of metabolic disease in the Thoroughbred gelding. As many horses fluctuate between the BCS range of the current experiment from season to season, the results of this study indicate that such changes are not likely to impair SI, which has positive implications for horse owners. However, changes in SI might have been seen if weight gain had been continued such that horses reached a BCS > 7 (Hoffman et al., 2003; Vick et al., 2007), or if the horses had been assessed after maintaining BCS 7 for an extended period of time.

LITERATURE CITED

Arch, J.R.S. 2005. Central regulation of energy balance: inputs, outputs and leptin resistance. Proc. Nutr. Soc. 64:39-46.

Art, T., N. Kirschvink, N. Smith and P. Lekeux. 1999. Indices of oxidative stress in blood and pulmonary epithelium lining fluid in horses suffering from recurrent airway obstruction. Equine Vet. J. 31:397-401.

Art, T., N. Kirschvink, N. Smith, D. Votion and P. Lekeux. 1999b. Cardiorespiratory measurements and indices of oxidative stress in exercising COPD horses. Equine Vet. J. Suppl. 30:83-7.

Aurajo-Vilar, D., D.A. Garcia-Estevez and J. Cabezas-Cerrato. 1998. Both a reduced acute insulin response to glucose and lower glucose effectiveness are responsible for the worsening of intravenous glucose tolerance in healthy subjects independently of the degree of obesity. Metab. Clin. Exp.. 47:313-320

Bergman, R.N. 1997. The minimal model: yesterday, today and tomorrow. In: The minimal model approach and determinants of glucose tolerance. Ed: G.A. Bray and D.H. Ryan. Louisiana State University Press, Baton Rouge, LA**.**

Buff, P.R., A.C. Dodds, C.D. Morrison, N.C. Whitley, E.L. McFadin, J.A. Daniel, J. Djiane, and D.H. Keisler. 2002. Leptin in horses: Tissue localization and relationship between peripheral concentrations of leptin and body condition. J. Anim. Sci. 80:2942-2948.

Cartmill, J.A., D.L. Thompson, W.A. Storer, L.R. Gentry, and N.K. Huff. 2003. Endocrine responses in mares and geldings with high body condition scores grouped by high vs. low resting leptin concentrations. J. Anim. Sci. 81:2311-2321.

Ceddia, R.B. 2005. Direct metabolic regulation in skeletal muscle and fat tissue by leptin: implications for glucose and fatty acids homeostasis. Int. J. Obes. 29:1175-1183.

Coffman, J.R., and C.M. Colles. 1983. Insulin tolerance in laminitic ponies. Can. J. Comp. Med. 47:347-351.

De Moffarts, B., N. Kirschvink, T. Art, J. Pincemail and P. Lekeux. 2002. Effect of oral antioxidant status in trained Thoroughbred horses. Vet. J. 169:65-74.

Fitzgerald, B.P., and C.J. McManus. 2000. Photoperiodic versus metabolic signals as determinants of seasonal anaestrus in the mare. Biol. Reprod. 63:335-340.

Flores, C.A., P.M. Brannon, S.A. Bustamante, J. Bezerra, K.T. Butler, T. Goda and O. Koldovsky. 1988. Effect of diet on intestinal and pancreatic enzyme activities in the pig. Pediatr. Gastroenterol. Nutr. 7:914-21.

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

Freestone, J.F., K. Shoemaker, R. Bessin, and K.J. Wolfsheimer. 1992. Insulin and glucose response following oral glucose administration in well-conditioned ponies. Equine Vet. J. Suppl. 11:13-17.

Forhead, A.J. and H. Dobson, 1997. Plasma glucose and cortisol responses to exogenous insulin in fasted donkeys. Res. Vet. Sci. 62:265-269.

Gentry, L., D.L. Thompson, G.T. Gentry, K.A. Davis, R.A. Godke, and J.A. Cartmill. 2002. The relationship between body condition, leptin, and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. J. Anim. Sci. 80:2695-2703.

Halaas, J.L., C. Boozer, J. Blair-West, N. Fidahusein, D.A. Denton, and J.M. Freidman. 1997. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc. Natl. Acad. Sci. U.S.A. 94:8878-8883

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

Heusner, G. 1993. Ad libitum feeding of mature horses to achieve rapid weight gain. Page 86. In: Proc. 13th Equine Nutr. Physiol. Symp., Gainesville, FL.

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

Hoffman, R.M., D.S. Kronfeld, W.L. Cooper and P.A. Harris. 2003b. Glucose clearance in grazing mares is affected by diet, pregnancy and lactation. J. Anim. Sci. 81:1746-1771.

Hornick, J.L., C. Van Eenaeme, O. Gérard, I. Dufrasne and L. Istasse. 2000. Mechanisms of reduced and compensatory growth. Domest. Anim. Endocrinol. 19: 121–132.

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

Johnson, P.J. 2002. The equine metabolic syndrome Peripheral Cushing's syndrome. Vet. Clin. North Am. Equine Pract.. 18:271-293.

Kane, R.A., M. Fisher, D. Parrett, and L.M. Lawrence. 1987. Estimating fatness in horses. Pages 127-131. In: Proc. 10th Equine Nutr. Phsyiol. Symp., Ft. Collins, CO.

Kearns, C.F., K.H. McKeever, V. Roegner, S.M. Brady, and K. Malinowski. 2005. Adiponectin and leptin are related to fat mass in horses. Vet. J. Epub. ahead of print. www.elsevier.com/locate/tvjl.

Kinnunen, S., S. Hyyppa, A. Lehmuskero, N. Oksala, P. Maenpaa, O. Hannienen and M. Atalay. 2005. Oxygen radical absorbace capacity and exercise-induced oxidative stress in trotters. Eur. J. Appl. Physiol. 95:550-556.

Kopp, W. 2003. High-insulinogenic nutrition - an etiologic factor for obesity and the metabolic syndrome? Metab. Clin. Exp., Vol 52:40-844.

Lawrence, L.M. 2000. Advances and gaps in energy nutrition. In: Advances in Equine Nutrition II. J.D. Pagan and R.J. Geor, ed. Nottingham Univ. Press, p.227-236.

Lehnhard, R.A., K.H. McKeever, C.F. Kearns, and M.D. Beekley. 2004. Myosin heavy chain profiles and body composition are different in old versus young Standardbred mares. Vet. J. 167:59-66.

Marlin, D.J., K. Fenn, N. Smith, C.D. Deaton, C.A. Roberts, P.A. Harris, C. Dunster and F.J. Kelly. Changes in circulatory antioxidant status in horses during prolonged exercise. J. Nutr. 132:1622S-1627S.

Neville, R.F., T. Hollands, S.N. Collins and F.V. Keyte. 2004. Evaluation of urinary TBARS in normal and chronic laminitic ponies. Equine Vet. J. 36:292-294.

NRC. 1989. Pages 90 - 110 in Nutrient Requirements of the Horse. 6th rev. ed. Natl. Acad. Press, Washington, DC.

Pawlak, D.B., J.M. Bryson, G.S. Denyer and J.C. Brand-Miller. 2001. High glycemic index starch promotes hypersecretion of insulin and higher body fat in rats without affecting insulin sensitivity. J. Nutr. 131:99-104.

Powell, D., S. Reedy, D. Sessions, and B. Fitzgerald. 2002. Effect of short-term exercise training on insulin sensitivity in obese and lean mares. Equine Vet. J. 34:81-84.

Sticker, L.S., D.L. Thompson, L.D. Bunting, J.M. Fernandez and C.L. DePew. 1995. Dietary protein and(or) energy restriction in mares: plasma glucose, insulin, non-esterified fatty acid and urea nitrogen responses to feeding, glucose and epinephrine. J. Anim. Sci. 73:136-144.

Treiber, K.H., T.M. Hess and D.S. Kronfeld. 2005. Insulin resistance and compensation in laminitis-predisposed ponies characterized by the minimal model. Pferdeheilkunde. 21:91-92

Treiber, K.H., R.C. Boston, D.S. Kronfeld, W.B. Staniar, and P.A. Harris. 2005b. Insulin resistance and compensation in Thoroughbred weanlings adapted to high-glycemic meals. J. Anim. Sci. 83:2357-236.

Treiber, K.H., D.S. Kronfeld, T.M. Hess, B.M. Byrd, R.K. Splan and W.B. Staniar. 2006. Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. J. Am. Vet. Med. Assoc. 228:1538-1545.

Treiber, K.H. 2006. 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. Ph.D. Diss. Virginia Polytechnic and State University, Blacksburg, VA.

Treiber, K.H., R.A. Carter, L.A. Gay, C.A. Williams and R. Geor. 2007. Oxidative stress and inflammatory markers in laminitis-prone ponies. Pages 142-143. In: Proc. 20th Equine Sci. Soc., Hunt Valley, MD.

Van der Kolk, J.H., R.F. Nachreiner, H.C. Schott, K.R. Refsal and A.J. Zanella. 2001. Equine Vet. J. 33:211-213.

Vick, M.M., A.A. Adams, B.A. Murphy, D.R. Sessions, D.W. Horohov, R.F. Cook, B.J. Shelton and B.P. Fitzgerald. 2007. Relationship between inflammatory cytokines, obesity and insulin sensitivity in the horse. J. Anim. Sci. 85:1144-55.

Vincent, H.K. and A.G. Taylor. 2006. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. Int. J. Obes. 30:400-418.

Wilcox, G. 2005. Insulin and insulin resistance. Clin. Biochem. 26:19-39.

Williams C.A., R.M. Hoffman, D.S. Kronfeld, T.M. Hess, K.E. Saker and P.A. Harris. 2002. Lipoic acid as an antioxidant in mature thoroughbred geldings: a preliminary study. J. Nutr. 132:S1628-1631.

CHAPTER 4: MANUSCRIPT II

EFFECT OF WEIGHT GAIN, DIET AND EXERCISE ON INSULIN SENSITIVITY IN THOROUGHBRED GELDINGS

Effect of weight gain, diet and exercise on insulin sensitivity in Thoroughbred geldings[1]

R.W. Quinn[2*] **A.O. Burk**[*], **T.G. Hartsock**[*], **E.D. Petersen**[*], **N.C. Whitley**[**], **K.H. Treiber**[†] **and R.C. Boston**[‡]

[*]*University of Maryland, College Park, MD 20742,* [**] *University of Maryland Eastern Shore, Princess Anne, MD 21853,* [†]*Virginia Polytechnic and State University, Blacksburg, VA 24061;* [‡]*University of Pennsylvania, Kennett Square, PA, 19348*

[1]This research was supported in part by the University Maryland College of Agriculture and Natural Resources Agriculture Experiment Station Competitive Grants Program. The authors would like to thank the staff at the Central Maryland Research and Education Center - Clarksville Dairy and Equine Units, specifically J. Schreiber, T. Shellem, J. Suagee and K. Swyers, as well as D. Cazac from the University of Maryland Eastern Shore.

[2]Correspondence: Animal Sciences Center, College Park, MD 20742 (phone: 301-405-8337; fax: 301-405-7960; email: rwquinn@cmh.edu).

ABSTRACT

Insulin sensitivity (SI) in the horse is reduced in obesity, following the consumption of high starch and sugar concentrates and in the absence of forced exercise. Utilizing two

concentrates designed to differentially impact glucose and insulin dynamics, two experiments were carried out to assess the impact of controlled weight gain and exercise on SI so as to determine whether weight-gain associated changes in SI would be modulated by dietary energy source or exercise restriction. Fifteen mature Thoroughbred geldings (BW 519 ± 10 kg, BCS 4.3 ± 0.1) were fed hay and a commercial concentrate twice daily at maintenance for 3 wk followed by 6 wk acclimation to the treatment concentrates, one high in starch and sugar (HS; 57% non-structural carbohydrates, 4% fat; n = 9) and the other high in fat and fiber (HF, 20% non-structural carbohydrates, 17% fat; n = 6). After the sixth wk, an additional 20 Mcal/d above maintenance was offered, resulting in an ADG of 0.35 kg/d over 41 wk (total gain 90.8 kg; final BW 609 ± 12 kg, BCS 7 ± 0.1). To assess SI, frequently-sampled i.v. glucose tolerance tests were conducted prior to treatment initiation (wk 3), after the fourth wk of treatment concentrate feeding (wk 7), at the end of weight gain (wk 41) and following a 2 wk minimal exercise period. During the minimal exercise period, exercise was reduced from twice-daily walking in a motorized horse exerciser to box stall confinement except during twice-daily stall cleaning. The minimal model of glucose dynamics was used to estimate SI, glucose effectiveness and the acute insulin response to glucose (AIRg) from each glucose tolerance test. At wk 3, no significant differences existed between treatment groups for any variable. Within HF, SI and AIRg remained unchanged at all time points. Within HS, SI decreased at wk 7 ($P = 0.01$) and remained unchanged at wk 41. The SI in HS was lower than HF at wk 7 (P = 0.004) and wk 41 (P = 0.07). At wk 41, AIRg was higher in HS than HF (P = 0.01) and glucose effectiveness was reduced in both diets ($P <$ 0.05). Following the minimal exercise period, SI decreased in HF (P = 0.03) but was

unchanged in HS. Overall, dietary energy source may be more influential than weight gain on SI in the mature Thoroughbred gelding between BCS 4 and 7. The higher SI found in the horses consuming the high fat and fiber diet appears to be partially dependent on some level of physical activity.

Key Words: Equine obesity, insulin sensitivity, minimal model of glucose dynamics

INTRODUCTION

Insulin resistance (**IR**) in the equine is associated with breed (Jeffcott et al., 1986), obesity (Hoffman et al., 2003) and laminitis (Treiber et al., 2006). During IR, tissue sensitivity to insulin is reduced and the normal level of insulin required to promote glucose uptake is no longer adequate (Wilcox, 2005). Insulin sensitivity (**SI**) is also reduced following the consumption of high starch and sugar diets (Hoffman et al., 2003), which may be due to the increased glycemic response that occurs following ingestion (Stull and Rodiek, 1987). An increasing number of obese horses are acquiring IR and laminitis (Johnson, 2002) and it is not known whether SI deteriorates as adiposity increases or as long periods of obesity are sustained.

As dietary energy source impacts SI in both lean (Treiber et al., 2005) and obese (Hoffman et al., 2003) horses, the observation of IR in horses with pre-existing obesity may be due at least in part to diet. Substitution of soluble carbohydrates with fat and fermentable fiber may improve SI in the non-obese horse (Treiber et al., 2005; Hoffman et al., 2003) but it is unknown as to whether the improvement would be maintained as

adiposity increases. The goal of the first experiment was to evaluate SI at the onset (BCS 4; Henneke et al., 1983) and completion (BCS 7) of controlled weight gain utilizing two treatment concentrates, one high in starch and sugar and the other high in fat and fiber.

In addition to encouraging weight loss and thereby improving SI (Freestone et al., 1992), forced, sub-maximal exercise can improve SI independent of weight loss (Powell et al., 2002). In unfit horses used primarily as companion animals, however, the level of daily exercise may not be enough to overcome the negative effects of diet or weight gain. Therefore, the goal of the second experiment was to evaluate the effect of exercise restriction on SI.

MATERIALS AND METHODS

Two experiments were conducted to assess changes in SI following controlled weight gain on two experimental concentrates (Exp. 1) and cessation of light exercise (Exp. 2). The experiments were carried out at the University of Maryland Equine Research Unit (Clarksville, MD). The experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Maryland (Protocol # R – 05 – 16).

Animals. Fifteen Thoroughbred geldings (9.5 ± 2.9 yr) with an initial BW and BCS of 519 ± 10 kg and 4.3 ± 0.1, respectively were housed in individual box stalls (3.6 m^2) with rubber mat flooring and pelleted wood fiber bedding (Woody Pet, Surrey, BC, Canada). All horses received routine dental care, vaccinations and deworming with a broad-spectrum dewormer prior to the start of the study. Horses were exercised twice daily at

0900 and 1600 for 35 min at a speed of 1.2 m/s using an automated 6-horse exerciser (Priefert, Mt. Pleasant, TX) except during Exp. 2 when they were placed on a minimal exercise protocol. During the 14 d minimal exercise period, the horses received no exercise with the exception of being removed from their stalls twice daily during routine stall cleaning.

Diets. Prior to the start of the study, horses were maintained in the study facility and fed only mixed grass-alfalfa hay for a four wk period. At the start of the study, horses were given three wk to acclimate to a diet of mixed grass-alfalfa hay and a texturized commercially available concentrate (**CF**; Legends 12, Southern States Cooperative, Richmond, VA; Table 1) at a level meeting their maintenance requirement for DE and providing vitamins and minerals at a level meeting or exceeding the minimum requirements for horses (NRC, 1989).

Throughout the study, all horses had ad libitum access to water and iodized salt. The hay was fed twice daily at 0800 and 1900 in floor-mounted wooden hay boxes and orts collected and weighed prior to the morning feeding to estimate daily hay intake. Due to the length of the study, three separate lots of hay were fed (Table 1) and three to five random core samples of hay taken from each lot were submitted to a commercial forage testing lab for nutrient analysis (Dairy One, Ithaca, NY). Concentrate was offered in individual canvas nose bags (Derby Originals, North Canton, OH) and refusals weighed following each feeding to estimate concentrate intake, with horses given 45 min during which to consume the concentrate. All diets were offered at a ratio of 60% hay to 40% concentrate. Upon inititation of weight gain (wk 9), the 60:40 hay-to-concentrate ratio

Table 1. Nutrient analysis [1] (DM basis) for commercial concentrate [2] (CF), fed at maintenance to Thoroughbred geldings for a three wk period at the start of the study, treatment concentrates (HS, high in starch and sugar and HF, high in fat and fiber) fed from wk four to the end of weight gain (wk 41), and hay fed throughout the study. Data are presented as means ± SEM

	Concentrate			Hay (wk fed)		
Item	CF	HS	HF	Lot 1 (pre-study to wk 25)	Lot 2 (wk 26 to wk 29)	Lot 3 (wk 30 to wk 41)
P, %	0.61 ± 0.04	0.38 ± 0.03 [a]	0.29 ± 0.03 [a]	0.26 ± 0.03	0.17 ± 0.07	0.31 ± 0.04
Ca, %	1.1 ± 0.02 [ab]	0.73 ± 0.10 [a]	1.2 ± 0.10 [b]	0.92 ± 0.07	0.69 ± 0.19	0.86 ± 0.09
Fat, %	8.2 ± 0.4	4.3 ± 0.1	17.2 ± 1.3 [a]	3.43 ± 0.14	2.40 ± 0.37	2.83 ± 0.19
CP, %	14.4 ± 0.3 [a]	10.0 ± 0.6	10.1 ± 0.6	14.35 ± 0.54	11.00 ± 1.44	17.5 ± 0.72 [b]
ADF, %	10.0 ± 3	8.04 ± 1.3	21.7 ± 1.3 [a]	31.40 ± 0.84 [b]	39.00 ± 2.21 [c]	35.05 ± 1.11 [bc]
NDF, %	20.0 ± 2	17 ± 1.3	32.8 ± 1.3 [a]	51.37 ± 2.27	63.00 ± 6.00	50.75 ± 3.00
NSC [2], %	53.0 ± 0.1	56.7 ± 1.4	20.1 ± 1.4 [a]	17.47 ± 2.04	13.10 ± 5.39	10.38 ± 2.69
DE, Mcal/kg	3.4 ± 0.04 [1]	3.59 [3]	3.48 [3]	2.34 ± 0.04	1.98 ± 0.10 [b]	2.31 ± 0.05

[1] Dairy One, Ithaca, NY
[2] Legends 12, Southern States Cooperative, Richmond, VA
[3] NSC = Non-structural carbohydrates calculated as starch plus sugar (water-soluble carbohydrates)
[4] Calculation adjusted for fat and fiber contribution of ingredients (Harris and Kronfeld, 2003)
[a] Means within rows are significantly different at $P < 0.05$ for concentrate
[b,c] Means within rows are significantly different at $P < 0.05$ for hay

resulted in hay being essentially fed ad libitum. All dietary transitions were made over a 7d period. Grab samples of concentrate (n = 8) were submitted to a commercial laboratory (Dairy One, Ithaca, NY) for nutrient analysis.

At wk 4, the horses were transitioned onto one of two treatment concentrates (Table 1) offered at a level meeting their maintenance DE requirement (NRC, 1989). After six wk, the amount of DE offered was increased to 20 Mcal above maintenance per day to encourage weight gain. The DE offered was recalculated weekly following the assesment of weight gain to account for the increasing maintenance requirement. While hay lot two was being fed, the rate of weight gain began to decline and so a third concentrate feeding was added (at 1300), which offered an additional 7 Mcal DE. Refusals of hay and concentrate were recorded daily and a seven day period at the end of every month was averaged to estimate feed intake. Data from one horse was excluded from the estimation of intake due to excessive urination on the refused hay. Weight gain was terminated after 41 wk when the average initial BW had increased by 90.8 kg. Following the end of weight gain, horses continued on the same diets fed at maintenance through Exp. 2.

The two treatment concentrates to which the horses were randomly assigned consisted of a high starch and sugar concentrate (**HS**, n = 9) and high fat and fiber concentrate (**HF**, n = 6), formulated to meet or exceed the minimum requirements for DE, vitamins and minerals for horses at maintenance (Table 2; NRC, 1989). The diets were formulated to be isoenergetic (HF, 3.48 Mcal/kg; HS, 3.59 Mcal/kg) but the DE estimated by the commercial laboratory indicated a significant difference between the two diets (data not shown; $P < 0.05$). The current method of determining DE in equine

Table 2. Ingredient composition of treatment concentrates, formulated to be either high in starch and sugar (HS) or high in fat and fiber (HF) fed from wk four to the end of weight gain (wk 41)

Ingredient, %	HS	HF
Corn	56.0	4.0
Corn oil	0	14
Oats	12.5	4.0
Distillers grains	7.0	10.0
Soybean meal	5.0	10.0
Beet pulp	5.0	46.0
Molasses	5.0	3.0
Mixed hay	5.0	5.0
Limestone	0.5	0.0
Vitamin E	0.5	0.5
Vitamin D	0.2	0.1
Mineral premix [1]	2.3	2.3
Vitamin, mineral and amino acid premix [2]	1.0	1.0
Chelated mineral supplement [3]	0.0	0.1

[1] Grass-plus Mineral, Buckeye Nutrition, Dalton, OH; Guaranteed analysis: CP, 8%; lysine, 2.5 %; methionine, 1.3%; Ca (minimum) 19%; Ca (maximum) 22%; P, 6%; K, 2%; Mg, 1.5%; Mn, 900 ppm; Fe, 1150 ppm; Cu, 550 ppm; Zn, 1300 ppm; Co, 15 ppm; I, 15 ppm; Se, 9 ppm; vitamin A, 176,000 IU/kg; vitamin D-3, 17,600 IU/kg; vitamin E, 1,760 IU/kg.

[2] Harvest Salt, Buckeye Nutrition, Dalton, OH; Guaranteed analysis: NaCl (minimum), 93%; NaCl (maximum), 96%; Mn, 500 ppm; Cu, 2500 ppm; Zn, 5000 ppm; Co, 150 ppm; I, 40 ppm; Se, 40 ppm.

[3] Zinpro 4 - plex, Zinpro Corporation, Eden Prairie, MN; Typical analysis: Zn, 2.58%; Mn, 1.43%; Cu, 0.90%; Co, 0.18%; methionine, 8.21%; lysine, 3.80%; protein, 11.5%; fat, 1.5%; fiber, 22.0%; ash, 26.5%.

feeds is by using one of several equations (Harris, 1999) utilizing the chemical composition of the investigated feed. However, these equations have been found to underestimate the DE available to the horse fed a diet high in fat and fiber. This is because the calculated DE is thought to underestimate the energy contribution of added fat (e.g. corn oil, 9 kcal DE/g) and fermentable fiber (e.g. beet pulp, 2 kcal DE/g; Harris and Kronfeld, 2003). The DE for the HS and HF concentrates in the current study was calculated from the ingredient composition of the diets (Table 2) using the recommendations of Harris and Kronfeld (2003) while the DE calculated by the commercial laboratory was used for the CF concentrate due to the proprietary nature of the commercial feed ingredients.

Assessment of weight gain: Horses were weighed every two wk on a livestock scale (Preifert, Mt. Pleasant, TX) beginning at wk 1. BCS was assessed at wk 3, wk 7, wk 9 and every four wk thereafter by two independent researchers using the Henneke BCS system (Henneke et al., 1983). Each researcher assigned a numerical score (range 1 to 9 with half-scores possible) to the six different body areas which were then averaged to calculate the BCS. The final BCS was calculated by averaging the BCS scores from both researchers.

At the end of weight gain, rump fat thickness was measured via ultrasound and percent body fat calculated by the equation of Kane et al. (1987), % fat = 2.47 + 5.47 (rump fat_{cm}). Fat depth was measured 6 cm lateral from the midline at the center of the pelvic bone using b-mode ultrasound with a 15.24 cm linear transducer and frequency of 3.5 Hz (Aloka 500, Tokyo, Japan).

Minimal exercise period. Following completion of Exp. 1, a 14 d minimal exercise period was initiated for all horses (Exp. 2). Exercise was limited to removal of horses from stalls twice daily during routine stall cleaning. Horses were restrained using cross-ties and immediately returned to their stall following completion of cleaning. All other procedures were conducted as for Exp. 1.

FSIGT: Horses were subjected to frequently sampled intravenous glucose tolerance tests (**FSIGT**) previously validated in the horse (Hoffman et al., 2003). During Exp. 1, FSIGTs were performed following the third wk of CF feeding at maintenance (**CFMM**), following the fourth wk of treatment concentrate feeding at maintenance (**TXMM**) and when the horses had reached an average weight gain of 90.8 kg (wk 41; **ENDMM**). The third FSIGT from Exp. 1 (ENDMM) was used as the starting FSIGT in Exp. 2, with a final FSIGT carried out at the end of the 2 wk minimal exercise period (**MINEX**). No more than four horses were scheduled for an FSIGT on any given day.

The night before each FSIGT, horses were asceptically fitted with an indwelling 14 guage jugular catheter with attached 5.5 mL total volume extension set, kept patent with 12 mL of heparinized saline (0.75 mL heparin/1000 mL isotonic saline). At 0600 on the day of the FSIGT, horses were fed their morning ration of hay only and had access to hay and water during the entire FSIGT. No concentrate was provided to any horse during an FSIGT. Provision of hay during the FSIGT (Hoffman et al., 2003; Treiber et al., 2005) is thought to reduce the effect of fasting on glucose dynamics (Forhead and Dobson, 1997), as well as maintain quiet, calm behavior in all horses housed in the barn.

The morning of the FSIGT, horse BW was measured in order to accurately determine the level of glucose and insulin to be administered. Glucose (50% dextrose solution, AmTech Group, Inc., St. Joseph, MO) was administered as an i.v. bolus of 300 mg/kg BW. Insulin (HumulinR, Eli Lilly and Co., Indianapolis, IA) was administered as an i.v. bolus of 20 mIU/kg BW. Prior to glucose administration, three blood samples were taken (0730, 0745 and 0759) for determination of basal glucose and insulin levels. Intravenous glucose was administered over a 2 - 3 minute period beginning at 0800. Thirty-five venous blood samples were taken at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180, 210 and 240 minutes after glucose was administered. Insulin dosing occurred 20 min after the glucose was administered. For each sample, 20 mL of whole blood was immediately transferred into one of two glass specimen tubes containing either sodium fluoride/potassium oxalate (Vacutainer, Becton Dickenson, Franklin Lakes, NJ) or no additive (Fisher Scientific, Pittsburgh, PA). Blood from the sodium fluoride/potassium oxalate tubes were immediately centrifuged at 4° C for 15 min at 2000 x g while blood from the additive-free tubes was allowed to clot for 2 h at room temperature before being centrifuged at 4° C for 15 min at 2000 x g. Serum and plasma were transferred to 1.5 mL microtubes and stored at -20° C until analysis.

Analysis of Blood Hormones: Serum insulin was measured in duplicate using RIA techniques (Coat-A-Count Insulin, Diagnostics Products Corporation, Los Angeles, CA) previously validated for use in the horse (Freestone et al., 1991). The intraassay CV was 3% and the interassay CV was 16%. Plasma glucose was measured in triplicate by

colorimetric assay (Glucose C-2, Wako Chemicals, Richmond, VA), wherein samples were read at 505 nm on an absorbance microplate reader (Tecan Sunrise, Phenix Research Products, Hayward, CA). The intraassay CV of triplicate samples was 5%.

Minimal Model Analysis: Raw glucose and insulin data were modeled by MinMod Millenium (Ver. 6.02, 2001) using equations previously validated in the horse (Hoffman et al., 2003). Variables estimated included SI, insulin-independent glucose uptake (glucose effectiveness; **Sg**) and the acute insulin response to glucose (**AIRg**), which is a reflection of post-hepatic insulin concentration derived from the first ten minutes of the FSIGT. The disposition index (**DI**), calculated from the product of SI and AIRg, is a reflection of pancreatic β-cell function, corrected for the degree of IR (Bergman, 1997). Data from six individual horse FSIGTs were excluded from the analysis due to poor parameter resolution (f_sd ≥ 0.5, 6.7%).

Statistical Analysis. All statistical analyses were performed using SAS statistical software (v. 9.1, Cary, N.C.). Assumptions of normality and homogeneity of variances were evaluated by examination of the residual plot and found to be met for all variables except SI and basal glucose, which were adjusted by base-10 logarithmic transformation for analysis. All variables were analyzed by repeated measures mixed model ANOVA using orthoganal contrasts for least-squares mean comparisons and to test for linear changes over time. The values for SI, Airg, Sg and basal insulin at CFMM were used as a covariate and for all ANOVA analyses, the appropriate correlation matrix for repeated measures was chosen based on the smallest Akaike's Information Criteria and the fixed

model effects included day, treatment and treatment*day and the random effect was horse (within treatment). Data are presented as least-squares means ± SEM and Tukeys' HSD test was used for least-squares mean comparisons when appropriate. Although the data for log-normal variables are presented as least-squares means ± SEM, significant differences reflected in the superscripts refer to the test of significance run on the log-transformed means.

RESULTS

Experiment 1.

Average BW and BCS are presented in Figure 1. No treatment group differences were found for BW ($P = 0.93$) or BCS ($P = 0.08$) so data were pooled for analysis. Following the three wk adaptation to the CF concentrate, average BW was 524 ± 12 kg (range 466 to 596 kg) and BCS was 4.4 ± 0.1 (range 3.7 –to 5.1). Horses maintained BW and BCS through the start of weight gain (wk 9; BW, 519 ± 15 kg, range 473 – 590 kg, $P = 0.57$; BCS, 4.3 ± 0.1, range 4.0 – 4.6, $P = 0.77$). Following the initiation of weight gain, mean BW and BCS increased ($P < 0.0001$) to wk 41. Final average BW was 608 ± 12 kg (range 539 to 702 kg) and average BCS was 6.9 ± 0.1 (range 6.3 to 7.5). Regression of BCS on BW (data not shown) indicated that a one unit increase in BCS required a gain of 32 kg ($r^2 = 0.32$, $P < 0.0001$). Regression of BCS on time (data not shown) indicated that it required 80 d to gain one BCS ($r^2 = 0.89$, $P < 0.0001$). Ultrasound-derived rump

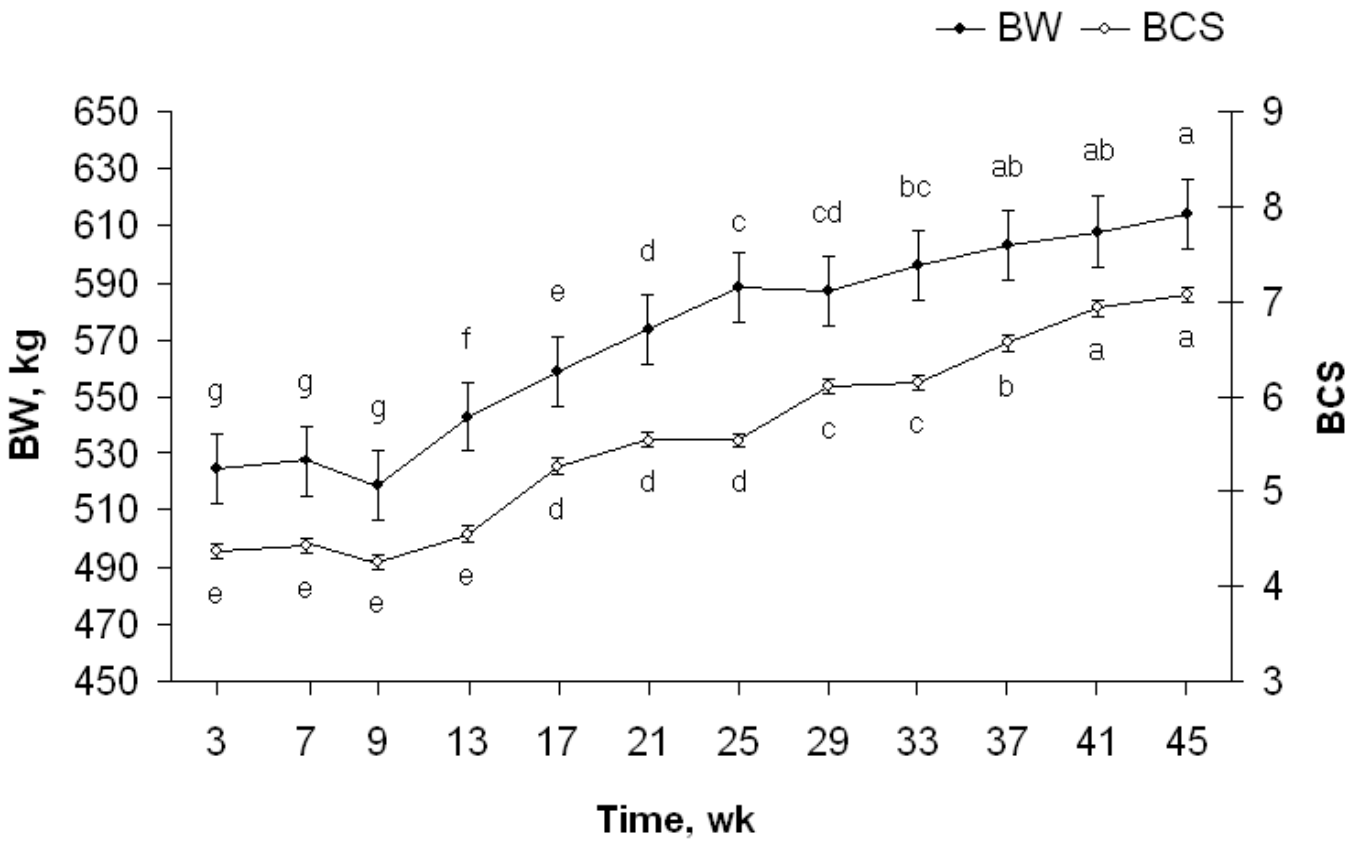


Figure 1. Changes in average BW and BCS of Thoroughbred geldings (n = 15) while fed commercial concentrate (wk 3) or treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 – 41). Pooled SEM was 15 kg for BW and 0.1 for BCS. [abcdefg] Means with uncommon superscripts within each variable differ ($P < 0.05$).

fat depth at the end of weight gain was 2.4 ± 0.2 cm (range 1.3 – 4.0 cm) and calculated percent body fat averaged 15 ± 1 % (range 10 – 24 %).

When fed at maintenance, the horses consumed all of feed offered, indicating that the hay and both concentrates were acceptable. Despite small and occasional significant differences between weeks, a linear change between wk 9 and wk 41 for both DE intake and ADG was not detected (Figure 2; DE, $P = 0.29$; ADG, $P = 0.20$). The ADG over the course of weight gain was 0.35 kg/d. The slight decline in DE intake and ADG at wk 29 coincided with the consumption of the second lot of hay (hay # 2), which was chemically assessed to be lower in nutritional quality. The additional concentrate feeding added following the wk 33 sample did not result in a total DE intake greater than the previous month ($P = 0.21$) but concentrate intake was greater between wk 33 and 37 than during all previous weeks ($P < 0.001$, data not shown).

Data for minimal model variables are presented in Table 3. At CFMM, no differences were found between treatment groups for any variable (SI, $P = 0.97$; Sg, $P = 0.71$; AIRg, $P = 0.78$; DI, $P = 0.28$; basal glucose, $P = 0.83$; basal insulin, $P = 0.98$). At TXMM, SI decreased in HS from the CFMM value ($P = 0.01$) to a level that was also lower than that of HF at TXMM ($P = 0.01$). The SI of HS remained numerically lower than the SI of HF at ENDMM. The Sg decreased between TXMM and ENDMM in both HS ($P = 0.03$) and HF ($P = 0.02$) and no treatment differences were found for Sg at either TXMM ($P = 0.43$) or ENDMM ($P = 0.96$). No treatment differences for AIRg were found at TXMM ($P = 0.16$) but AIRg was higher in HS than HF at ENDMM ($P = 0.01$). While log-normal basal glucose was not affected by time ($P = 0.10$) or treatment ($P = 0.44$), basal insulin was reduced at TXMM in both HS ($P = 0.003$) and HF ($P = 0.03$). No treatment

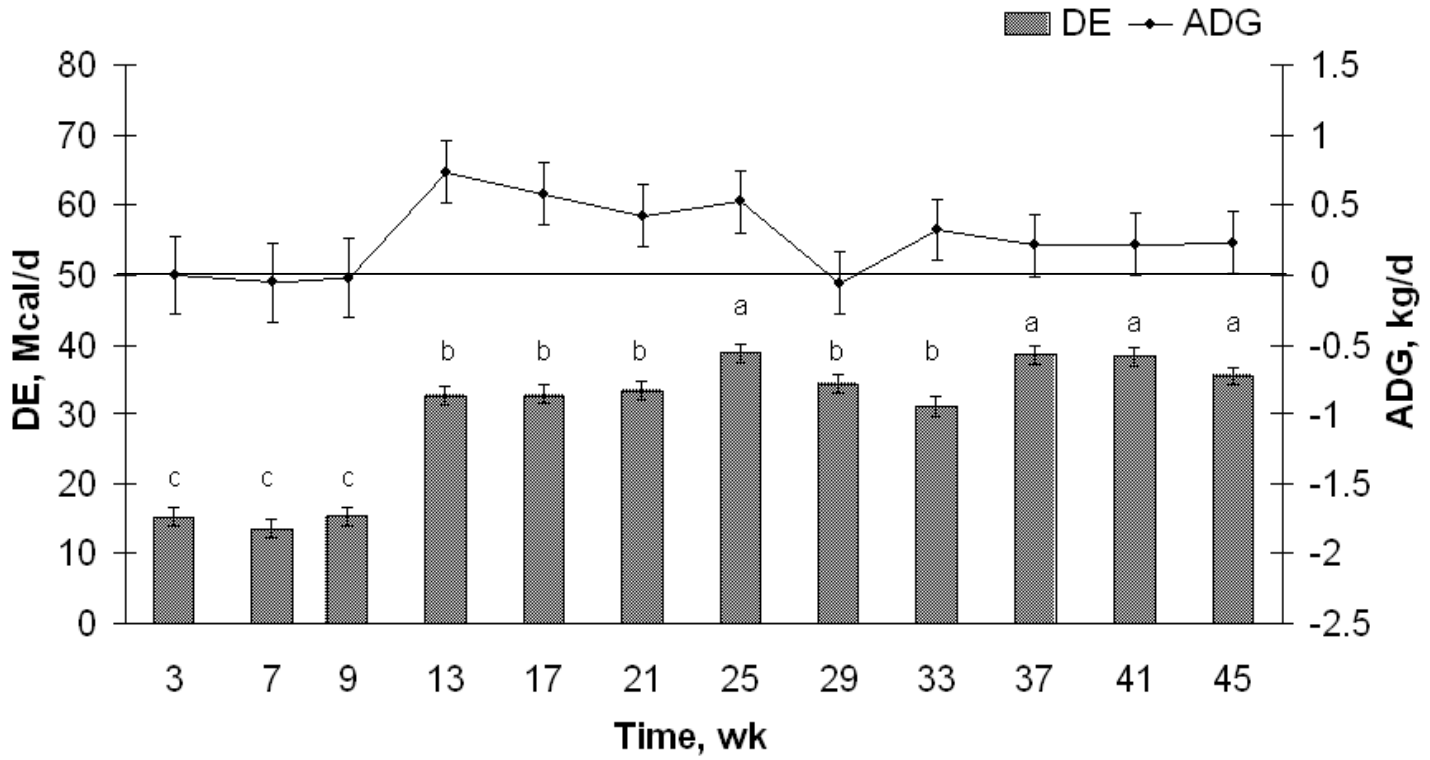


Figure 2. Digestible energy intake (Mcal/d; n = 14) and ADG (kg/d; n = 15) in Thoroughbred geldings while fed commercial concentrate (wk 3) and treatment concentrate (wk 9) at maintenance and throughout weight gain (wk 10 to 41). ADG was 0.35 kg/d. Pooled SEM was 1.0 Mcal for DE and 0.2 kg for ADG. [abc] Means with uncommon superscripts within each variable differ ($P < 0.05$).

Table 3. Least-squares means ± SEM for minimal model variables during weight gain in Thoroughbred geldings fed a diet high in starch and sugar (HS, n = 9) or fat and fiber (HF, n = 6) after the third week of commercial concentrate[1] feeding at maintenance (CFMM), after the fourth week of treatment concentrate feeding at maintenance (TXMM) and following 90.8 kg of BW gain (ENDMM)

	HS			HF		
Variable[2]	CFMM (n = 9)	TXMM (n = 9)	ENDMM (n = 9)	CFMM (n = 6)	TXMM (n = 4)	ENDMM (n = 6)
SI, $x10^{-4}$ $(mU/L)^{-1}$ min^{-1}	5.13 ± 0.74 [a]	2.09 ± 0.74 [b]	2.63 ± 0.74 [ab]	4.48 ± 0.70 [a]	5.07 ± 0.85 [a]	4.97 ± 0.70 [a]
Sg, $x10^{-2}$ min^{-1}	2.27 ± 0.30 [cd]	2.99 ± 0.30 [ab]	2.01 ± 0.30 [d]	2.47 ± 0.46 [abc]	3.50 ± 0.55 [ab]	2.04 ± 0.46 [cd]
AIRg, mU L^{-1} min	424 ± 57 [ab]	479 ± 57 [a]	549 ± 57 [a]	400 ± 64 [ab]	375 ± 78 [ab]	291 ± 64 [b]
DI, $x10^{1}$	192 ± 34	121 ± 34	152 ± 34	141 ± 32	153 ± 39	109 ± 32
Basal insulin, mUL	13.93 ± 1.41	5.96 ± 1.41 [a]	14.18 ± 1.41	13.92 ± 2.62	6.61 ± 3.12 [b]	14.02 ± 2.62
Basal glucose, mg/dL	86.1 ± 8.3	75.2 ± 8.3	85.0 ± 8.3	90.3 ± 9.8	68.9 ± 11.7	84.2 ± 9.8

[1] Legends 12, Southern States Cooperative, Richmond, VA.
[2] SI, insulin sensitivity; Sg, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index.
[a,b] Means within rows with uncommon superscripts differ ($P < 0.05$).

differences for basal insulin were found at TXMM (P = 0.64) or ENDMM (P = 0.96). The DI was not affected by time (P = 0.92) or treatment (P = 0.86).

Experiment 2.

Between the end of weight gain (wk 41) and the end of the minimal exercise period (wk 43), no treatment or time differences were found for BCS (P = 0.53; P = 0.79) but a significant time effect (P = 0.001) and treatment by time interaction (P = 0.01) were found within BW. Evaluation of the simple effect mean comparisons revealed a significant increase in BW within HF, from 602 ± 24 kg at wk 41 to 611 ± 24 kg at wk 43 (P = 0.002). Average BW at wk 41 within HS (616 ± 19kg) was not different from BW at wk 43 (617 ± 19 kg; P = 0.90). Data for minimal model variables are presented in Table 4. At MINEX, SI had decreased (P = 0.07) in HF but was unchanged in HS. No changes to Sg, AIRg, DI, basal insulin or log-normal basal glucose were found ($P \geq$ 0.10).

DISCUSSION

Experiment 1.

Obesity in the Thoroughbred has been reported in the literature (Hoffman et al., 2003), and while not as common as obesity in the pony, Morgan, Spanish Mustang or European Warmblood (Johnson, 2002), it may be similarly effective in inducing a state of metabolic dysfunction. The reduction in SI that accompanies increased adiposity in

Table 4. Least-squares means ± SEM for minimal model variables in Thoroughbred geldings fed a diet high in starch and sugar (HS, n = 9) or fat and fiber (HF, n = 6) following the completion of 90.8 kg BW gain (ENDMM) and after 2 wk of minimal exercise (MINEX)

Variable [1]	HS		HF	
	ENDMM (n = 9)	MINEX (n = 9)	ENDMM (n = 6)	MINEX (n = 6)
SI, $x10^{-4}$ $(mU/L)^{-1}$ min^{-1}	2.63± 0.74 [ab]	2.64 ± 0.73 [ab]	4.97 ± 0.70 [a]	2.99 ± 0.76 [b]
Sg, $x10^{-2}$ min^{-1}	2.01 ± 0.30	2.16 ± 0.30	2.04 ± 0.46	1.33 ± 0.50
AIRg, mU L^{-1} min	549 ± 57 [a]	562 ± 57 [a]	291 ± 64 [b]	353 ± 70 [b]
DI, $x10^{1}$	152 ± 34	133 ± 34	109 ± 32	72 ± 35
Basal insulin, mU/L	14.18 ± 1.41	17.36 ± 1.41	14.02 ± 2.62	16.71 ± 2.62
Basal glucose, mg/dL	85.0 ± 8.3 [ab]	79.7 ± 8.3 [a]	84.2 ± 9.8 [ab]	101.3 ± 9.8 [b]

[a,b] Means within rows with uncommon superscripts differ ($P < 0.05$).
[1] SI, insulin sensitivity; Sg, glucose effectiveness; AIRg, acute insulin response to glucose; DI, disposition index.

ponies is a significant risk factor for the development of pasture-associated laminitis (Treiber et al., 2006). In the horse, SI is negatively correlated with BCS (Vick et al., 2007) 2007) and is lower in horses of high BCS (≥7) than low BCS (≤ 6; Powell et 2002; Hoffman et al., 2003). However, the SI threshold at which the risk of metabolic disease increases is unknown and may be related to an achieved level of body fat or the length of sustained obesity or both.

Weight gain in the Thoroughbred, as measured in this study, appears to occur at a slower rate than that of other breeds and types of horse (Heusner et al., 1993; Pagan et al., 1987). Additionally, the Thoroughbred seems to require a greater BW gain per unit of BCS as compared to mixed light-breed horses (16 – 20 kg; Lawrence, 2000), as results of the current study indicate an average of 32 kg gain per unit of BCS. Such breed-specific differences suggest that recommendations for weight gain in the mature horse should be specialized beyond the basic generalization of 'light breed,' 'pony' or 'draft' and that research studying the effects of BW or BCS on physiological parameters utilize a single breed, gender and maturity of horse.

Diets high in starches and sugars are associated with changes in glucose and insulin dynamics, including post-prandial glucose and insulin responses that are greater than those from forages alone or fat-supplemented rations (Stull and Rodiek, 1987; Pagan et al., 1999; Williams et al., 2001; Ropp et al., 2003). Additionally, SI is reduced in horses fed high starch and sugar concentrates as compared to concentrates where the energy from non-structural carbohydrates is replaced by fat and fermentable fiber (Treiber et al., 2005; Hoffman et al., 2003). Such an effect was found in the present study, with SI in the HS group lower than HF at TXMM and ENDMM. However, SI in the current study

(BCS 4.3 – 7.0) was not reduced to levels previously found in obese horses (BCS ≥ 7, SI = $0.37 \pm 0.27 \times 10^{-4}$ $(mU/L)^{-1}$ min^{-1}; Hoffman et al., 2003) or laminitic ponies (BCS 6 - 8; SI = $0.39 \pm 0.07 \times 10^{-4}$ $(mU/L)^{-1}$ min^{-1}; Treiber et al., 2005b).

The HS concentrate was formulated to be very similar to the CF concentrate in the amount of starches and sugars provided and would therefore be expected to have the same effect on SI. It was assumed that adaptation to the starches and sugars would have occurred in both groups during the 3 wk prior to CFMM, but it is likely that a longer period of time was required (Sticker et al., 1995; Powell et al., 2002). The SI for HS at TXMM likely represents sufficient adaptation to the starches and sugars, which was not achieved in HF due to the dietary switch. Prior to the start of the study, the horses had been fed a diet consisting solely of mixed grass-alfalfa hay and the lack of change in SI in HF may indicate that the horses were able to maintain an SI hypothesized to be more similar to that of hay alone. Because both groups were able to maintain SI at the TXMM level for the duration of the study, they appear to have developed long-term tolerance to the diets (Hoffman et al., 2003b).

The lack of statistical significance between dietary treatments may be due to the higher variability and lower repeatability of the minimal model as compared to other experimental paradigms, such as the euglycemic-hyperinsulinemic clamp (Frank et al., 2005; Pratt et al., 2005). Additionally, although all horses were tattooed Thoroughbreds, differences in phenotype within the pool of study subjects may have contributed to the variation within treatment groups. However, the consistency of SI measured in HF and its tendency to remain higher than HS is a reflection of the physiological response to the ingredients of the HF concentrate. Both dietary corn oil and beet pulp have been shown

to dampen the glycemic response of feeds high in non-structural carbohydrates in the horse (Stull and Rodiek, 1987; Lindberg and Palmgren-Karlsson, 2001) as well as improve SI (Treiber et al., 2005).

The higher AIRg in HS at ENDMM may be in response to the lower SI and may be reflective of the ability of the horse to adapt to chronic feeding of diets high in starch and sugar (Treiber et al., 2005). Normal glucose tolerance can be maintained even with reduced SI through a compensatory increase in insulin secretion, reflected in AIRg (Araujo-Vilar et al., 1998). However, previous research using obese horses (BCS $\geq$ 7; Hoffman et al., 2003) found that AIRg was reduced in obesity, indicating that the compensatory insulin response may have no longer been adequate to maintain glucose tolerance (Hoffman et al., 2003).

Diet does not appear to influence Sg in the horse (Hoffman et al., 2003; Treiber et al., 2005). The reduction in Sg in both treatment groups between TXMM and ENDMM may reflect weight gain, although obese geldings (BCS $\geq$ 7; Hoffman et al., 2003) had a higher Sg than did moderately- and non-obese geldings (BCS 5 – 5.9 and BCS 6 – 6.9, respectively). However, Sg was found to be reduced in obese cats (Hoenig et al., 2006) but differentially increased or decreased in obese humans depending on their state of glucose tolerance (Taniguchi et al., 1994). In the current study, the reduction in Sg at ENDMM in the HS group may have been in response to the increased AIRg, as a decrease in insulin-independent glucose uptake may be expected to offset an increase in insulin-dependent glucose uptake (Ahrén and Pacini, 1998). In the HF group, however, the reduction in Sg may have been in response to the higher SI, as lower SI in human

subjects with obesity or Type II diabetes is accompanied by a compensatory increase in Sg (Henriksen et al., 1994).

Experiment 2.

Although it is known that forced exercise improves SI in the horse (Freestone et al., 1992b; Powell et al., 2002), many horses kept primarily as companion animals receive little forced exercise. These animals may be at a greater risk for obesity if their caloric intake is not reduced and as such may be at a greater risk for IR and metabolic disease. However, the degree to which SI in the obese horse or the horse fed a concentrate high in starch and sugar is further impaired by a sedentary existence is unknown.

During the current study, the horses were exercised at a walk for 35 min twice daily at a speed of 1.2 m/s, which is equivalent to 5.12 km of walking per day, a distance within the range estimated by Holland et al. (1996) in horses stalled overnight and allowed access to paddocks during the day (4 – 10 km/d). Following the 2 wk minimal exercise period during which the horses were kept in stalls except during routine stall cleaning, SI remained unchanged in the HS horses, indicating that the lower level of SI achieved following adaptation to the treatment concentrate was not further impacted by the level of exercise. However, SI was reduced by 55% in the HF horses, indicating that the protective effect of a fat and fiber diet on SI is at least partially dependent on the ability of the animal to carry out some amount of regular activity. In addition, the HF horses exhibited a significant increase in BW during the minimal exercise period (+ 9 kg) which

was not seen in the HS group (+ 1 kg), despite the fact that both groups were being fed at maintenance levels. Because changes in SI have been observed at BCS ≥7 (Hoffman et al., 2003), there may be a point in the continuum of BW gain where SI may be reduced regardless of diet. The reduction in SI within HF may indicate that the protective effect of diet may have ceased once the horses had reached a theoretical upper limit of 'safe' BW gain.

Exercise has been previously shown to differentially impact SI in horses fed a diet high in starch and sugar versus a diet high in fat and fiber (Treiber, 2006). In endurance-trained Arabian geldings, SI measured during a standard exercise test consisting of trotting at 57% of the lactate threshold was increased to a greater extent in high fat and fiber-fed horses (12-fold) versus high starch and sugar-fed horses (7-fold). It was hypothesized that the high fat and fiber-fed horses were better able to adapt to the energy demands of exercise by increasing their insulin-sensitive glucose uptake (Treiber, 2006). An increased 'metabolic flexibility (Treiber, 2006)' in high fat and fiber-fed horses, while beneficial when exercise is increased to the level of an endurance-trained athlete, may be responsible for the reduction in SI in the HF group in the current study.

While SI was reduced in the HF group, neither AIRg nor Sg were affected, perhaps because SI may not have been reduced to a level requiring compensation by the primary mechanisms involved in glucose uptake. As in Exp. 1, SI was not reduced to the level of obese horses (BCS ≥ 7; Hoffman et al., 2003), overweight ponies or laminitic ponies (BCS 6 – 8; Treiber et al., 2005b), indicating that the risk of metabolic disease was most likely low.

Results of this study provide evidence that SI in the Thoroughbred may be more affected by dietary energy source and exercise than BW gain to a BCS < 7. Diets high in fat and fiber are recommended for horses and ponies at risk for metabolic disease, such as those with chronic laminitis or equine Cushing's disease (Kronfeld and Harris, 2003). However, diets high in starch and sugar with NSC levels comparable to those in the current study may not be detrimental to the healthy horse. While the HS diet was associated with a reduction in SI in the current study, SI did not reach the level previously associated with an increased risk of laminitis in ponies (Treiber et al., 2005b). This indicates that such diets may not increase the risk of metabolic disease in the healthy horse when it gains weight between BCS 4.0 and 7.0. Additionally, while exercise restriction was associated with reduced SI in the HF group, the final SI value was above that previously associated with a risk of laminitis in the pony (Treiber et al., 2005b). Therefore, horses not subjected to regular forced exercise may not be at increased risk for metabolic disorder when fed concentrates high in starch and sugar as long as they are maintained at a BCS < 7.0.

In conclusion, feeding a concentrate high in fat and fiber to Thoroughbred geldings was associated with a higher SI than that of Thoroughbred geldings fed a concentrate high in starch and sugar. A novel finding of the current research is the divergent effect of exercise restriction on SI in the two treatment groups. While this may indicate that the effects of the HF diet on SI may be partially dependent on the animals carrying out some level of exercise, the contribution of weight gain cannot be ignored. However, even though SI was reduced in the HF group at the end of the minimal exercise period, it did not drop below that observed in laminitic ponies. This may indicate that the sedentary

horse, if gaining weight between BCS 4.0 and 7.0, may not be at an increased risk for metabolic disease regardless of dietary energy source fed. However, horses which achieve a BCS higher than 7.0 or those which are sustained near or above BCS 7.0 for an extended period of time might exhibit a decline in SI that may increase their risk for metabolic disease.

LITERATURE CITED

Araujo-Vilar, D., D.A. Garcia-Estevez and J. Cabezas-Cerrato. 1998. Both a reduced acute insulin response to glucose and lower glucose effectiveness are responsible for the worsening of intravenous glucose tolerance in healthy subjects independently of the degree of obesity. Metab. Clin. Exp.. 47:313-320

Ahren, B. and G. Pacini. 1988. Age-related reduction in glucose elimination is accompanied by reduced glucose effectiveness and increased hepatic insulin extraction in man. J. Clin. Endocrinol. Metab. 83:3350–3356.

Bergman, R.N. 1997. The minimal model: yesterday, today and tomorrow. Pages 3 – 50 in: The minimal model approach and determinants of glucose tolerance. Ed: G.A. Bray and D.H. Ryan. Louisiana State University Press, Baton Rouge, LA**.**

Forhead, A.J. and H. Dobson, 1997. Plasma glucose and cortisol responses to exogenous insulin in fasted donkeys. Res. Vet. Sci. 62:265-269.

Frank, N., F.M. Andrews, S.B. Elliot, J. Lew and R.C. Boston. 2005. Effects of rice bran oil on plasma lipid concentrations, lipoprotein composition and glucose dynamics in horses. J. Anim. Sci. 83:2509-2518.

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

Freestone, J.F., K. Shoemaker, R. Bessin, and K.J. Wolfsheimer. 1992. Insulin and glucose response following oral glucose administration in well-conditioned ponies. Equine Vet. J. Suppl. 11:13-17.

Harris, P.A. 1999. Comparison of the the digestible energy (DE) and net energy (NE) systems for the horse. Pages 199-216 in: Proc. Equine Nutr. Conf. Feed Manuf.

Harris, P.A. and D.S. Kronfeld. 2003. Influence of dietary energy sources on health and performance. Curr. Ther. Equine Nutr. 5:698-704.

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

Henricksen, J.E., F. Alford, A. Handberg, A. Vaag, G. Ward, A. Kalfas and H. Beck-Neilson. 1994. Increased glucose effectiveness in normoglycemic but insulin resistant relatives of patients with non-insulin dependent diabetes mellitus: a novel compensatory mechanism. J. Clin. Invest. 94:1196-1204.

Heusner, G.L. 1993. Ad libitum feeding of mature horses to achieve rapid weight gain. Pages 86-87. In: Proc. 8th Equine Nutr. Physiol. Symp., Lexington, KY.

Hoenig, M., K. Thomaseth, J. Brandao, M. Waldron and D.C. Ferguson. 2006. Assessment and mathematical modeling of glucose turnover and insulin sensitivity in lean and obese cats. Domest. Anim. Endocrinol. 31:373–389.

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

Hoffman, R.M., D.S. Kronfeld, W.L. Cooper and P.A. Harris. 2003b. Glucose clearance in grazing mares is affected by diet, pregnancy and lactation. J. Anim. Sci. 81:1764-1771.

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

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

Johnson, P.J. 2002. The equine metabolic syndrome Peripheral Cushing's syndrome. Vet. Clin. North Am. Equine Pract.. 18:271-293.

Kane, R.A., M. Fisher, D. Parrett, and L.M. Lawrence. 1987. Estimating fatness in horses. Pages 127-131. In: Proc. 10th Equine Nutr. Phsyiol. Symp., Ft. Collins, CO.

Lawrence, L.M. 2000. Advances and gaps in energy nutrition. In: Advances in Equine Nutrition II. J.D. Pagan and R.J. Geor, ed. Nottingham Univ. Press, p.227-236.

Lindberg, J.E. and C. Palmgren-Karlsson. 2001. Effect of partial replacement of oats with sugar beet pulp and maize oil on nutrient utilization in horses. Equine Vet. J. 33:585-590.

NRC. 1989. Pages 90 - 110 in Nutrient Requirements of the Horse. 6th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2007. Pages 90 - 110 in Nutrient Requirements of the Horse. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Pagan, J.D., B. Essen-Gustavsson, A. Lindholm and J. Thornton. 1987. The effect of dietary energy source on blood metabolites in Standardbred horses during exercise. Pages 425-430 In: Proc. 11th Equine Nutr. Physiol. Symp., Fort Collins, CO.

Pagan, J.D., P.A. Harris, M.A.P. Kennedy, N. Davidson and K.E. Hoekstra. 1999. Feed type and intake effects glycemic response in Thoroughbred horses. Pages 149-152. In: Proc. 16th Equine Nutr. Phsyiol. Symp., Raleigh N.C.

Powell, D., S. Reedy, D. Sessions, and B. Fitzgerald. 2002. Effect of short-term exercise training on insulin sensitivity in obese and lean mares. Equine Vet. J. 34:81-84.

Pratt, S.E., R.J. Geor and L.J. McCutcheon. 2005. Repeatability of 2 methods for assessment of insulin sensitivity and glucose dynamics in horses. J. Vet. Intern. Med. 19:883-888.

Quinn, R.W., A.O. Burk, T.G. Hartsock, K.H. Treiber and R.C. Boston. 2007. Effects of weight gain and diet on insulin sensitivity in Thoroughbred geldings. Pages 9-10. In: Proc. 20th Equine Sci. Soc., Hunt Valley, MD.

Ropp, J.K., R.H. Raub and J.E. Minton. 2003. The effect of dietary energy source on serum concentration of insulin-like growth factor-I, growth hormone, insulin, glucose, and fat metabolites in weanling horses. J. Anim. Sci. 81:1581-1589.

Sticker, L.S., D.L. Thompson, L.D. Bunting, J.M. Fernandez and C.L. DePew. 1995. Dietary protein and(or) energy restriction in mares: plasma glucose, insulin, non-esterified fatty acid and urea nitrogen responses to feeding, glucose and epinephrine. J. Anim. Sci. 73:136-144.

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

Treiber, K.H., R.C. Boston, D.S. Kronfeld, W.B. Staniar, and P.A. Harris. 2005. Insulin resistance and compensation in Thoroughbred weanlings adapted to high-glycemic meals. J. Anim. Sci. 83:2357-236.

Treiber, K.H., T.M. Hess and D.S. Kronfeld. 2005b. Insulin resistance and compensation in laminitis-predisposed ponies characterized by the minimal model. Pferdeheilkunde. 21:91-92

Treiber, K.H., D.S. Kronfeld, T.M. Hess, B.M. Byrd, R.K. Splan and W.B. Staniar. 2006. Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. J. Am. Vet. Med. Assoc. 228:1538-1545.

Treiber, K.H. 2006. 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. Ph.D. Diss. Virginia Polytechnic and State University, Blacksburg, VA.

Taniguchi, A., Y. Nakai, M. Fukushima, H. Imura, H. Kawamura, I. Nagata, G. L. Florant and K. Tokuyama. 1994. Insulin sensitivity, insulin secretion and glucose effectiveness in subjects with impaired glucose tolerance: a minimal model analysis. Metab. Clin. Exp.. 43:714-718.

Vick, M.M., A.A. Adams, B.A. Murphy, D.R. Sessions, D.W. Horohov, R.F. Cook, B.J. Shelton and B.P. Fitzgerald. 2007. Relationship between inflammatory cytokines, obesity and insulin sensitivity in the horse. J. Anim. Sci. 85:1144-55.

Wilcox, G. 2005. Insulin and insulin resistance. Clin. Biochem. 26:19-39.

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

GENERAL SUMMARY AND CONCLUSIONS

Body weight gain in the Thoroughbred gelding fed a diet high in starches and sugars was not associated with a change in SI that would be consistent with declining glucose tolerance (Hoffman et al., 2003). However, when compared to a cohort of horses fed a diet high in fat in fiber, a divergent effect of dietary energy source on SI can be observed. The substitution of fats and fermentable fibers for starches and sugars in equine diets has been recommended to reduce the risk of developing or exacerbating colic, laminitis, polysaccharide storage myopathy and other conditions, as well as spare glycogen during athletic activities and stimulate calm, quiet behavior in stall-kept horses (Kronfeld and Harris, 2003). More recently, evidence has emerged that diets high in fat and fiber can increase SI in both weanling and mature Thoroughbreds (Hoffman et al., 2003; Treiber et al., 2005). Coupled with the observation that Thoroughbreds of BCS $\geq$ 7 have reduced SI (Hoffman et al., 2003) and that IR is an underlying and perhaps predisposing factor for laminitis in ponies (Treiber et al., 2006), we hypothesized that the provision of a high fat and fiber diet to Thoroughbreds gaining weight would attenuate any negative weight gain-associated fluctuations in SI and thus reduce the risk of metabolic disease.

Although SI did not appreciably decline with BW gain in either study, SI was significantly lower in those horses fed the high starch and sugar concentrate following acclimation to the diet and remained numerically lower through the end of weight gain. However, this level of SI was not associated with metabolic disease, although one horse on the HS diet did show anecdotal evidence of polydypsia, polyurea and foot soreness.

While no standard values for SI in the healthy and non-healthy Thoroughbred gelding exist, the SI values in the current series of experiments were 6.5 times higher than those reported by Hoffman and colleagues (2003) in Thoroughbred geldings of BCS ≥ 7, likely indicating that the risk of metabolic disease was lower in the current research.

Because no appreciable weight-gain associated effect on glucose and insulin dynamics were found in this study, the observed changes in AIRg and Sg are most likely attributable to dietary energy source. Insulin resistance in humans is often accompanied by a compensatory, although inadequate, increase in insulin secretion, (Aurajo-Vilar et al., 1988). In the current study, the lower SI associated with the HS diet may have been countered by the increase in AIRg for the purpose of maintaining glucose tolerance. However, the progression of IR often culminates in inadequate insulin secretion and glucose intolerance, previously observed in the Thoroughbred of BCS ≥ 7 (Hoffman et al., 2003).

The unique response of the treatment groups to cessation of exercise underscores the need for some level of spontaneous, if not forced, daily exercise in all horses. While laminitis was not observed in this study, the fact that SI declined in the HF group during the minimal exercise period indicates that the risk of laminitis may be increased in sedentary horses. Perhaps a further decline in SI would have been observed in the HS horses if the minimal exercise period had been lengthened. The divergent impact of exercise on SI in the two treatment groups may be reflective of the ability of the horse to utilize stored glucose and fatty acids more efficiently when adapted to the HF diet.

During exercise, a high SI is necessary for adequate glucose uptake into muscles while low insulin levels are required to facilitate lipolysis. The higher basal insulin and AIRg

in high starch and sugar fed horses may negatively impact the animals' ability to utilize stored adipose tissue for energy (Treiber, 2006). The increased flexibility of the fat and fiber-fed horse to choose the appropriate substrate for exercise, be it glucose for high intensity or NEFA for low intensity, may also make the animal more sensitive to a reduction in exercise.

Research in humans finds that IR is a risk factor for metabolic disease in obese, genetically predisposed individuals following exposure to a dietary trigger such as a diet high in starch and sugar (Kopp, 2003). However, the development of laminitis as opposed to diabetes in the horse indicates that the horse responds to excessive adiposity and highly insulinogenic diets in a unique way which warrants further research. We and others (Hoffman et al., 2003; Treiber et al., 2005) have found that equine SI can be affected by BW, BCS and diet and it is likely that the horse will respond similarly to the pony, albeit with a slower progression. In the horse, however, the rapidity at which SI deteriorates to a level where the laminitis risk is increased is unknown and is most likely related to the length of sustained obesity as well as the individual animal's ability to compensate for changing glucose tolerance. Because the likelihood of an individual horse developing IR and laminitis following the attainment of obesity cannot yet be predicted, light breed horses should be maintained at a moderate BCS (4 – 6) for the duration of its life.

APPENDIX

Additional tables and figures

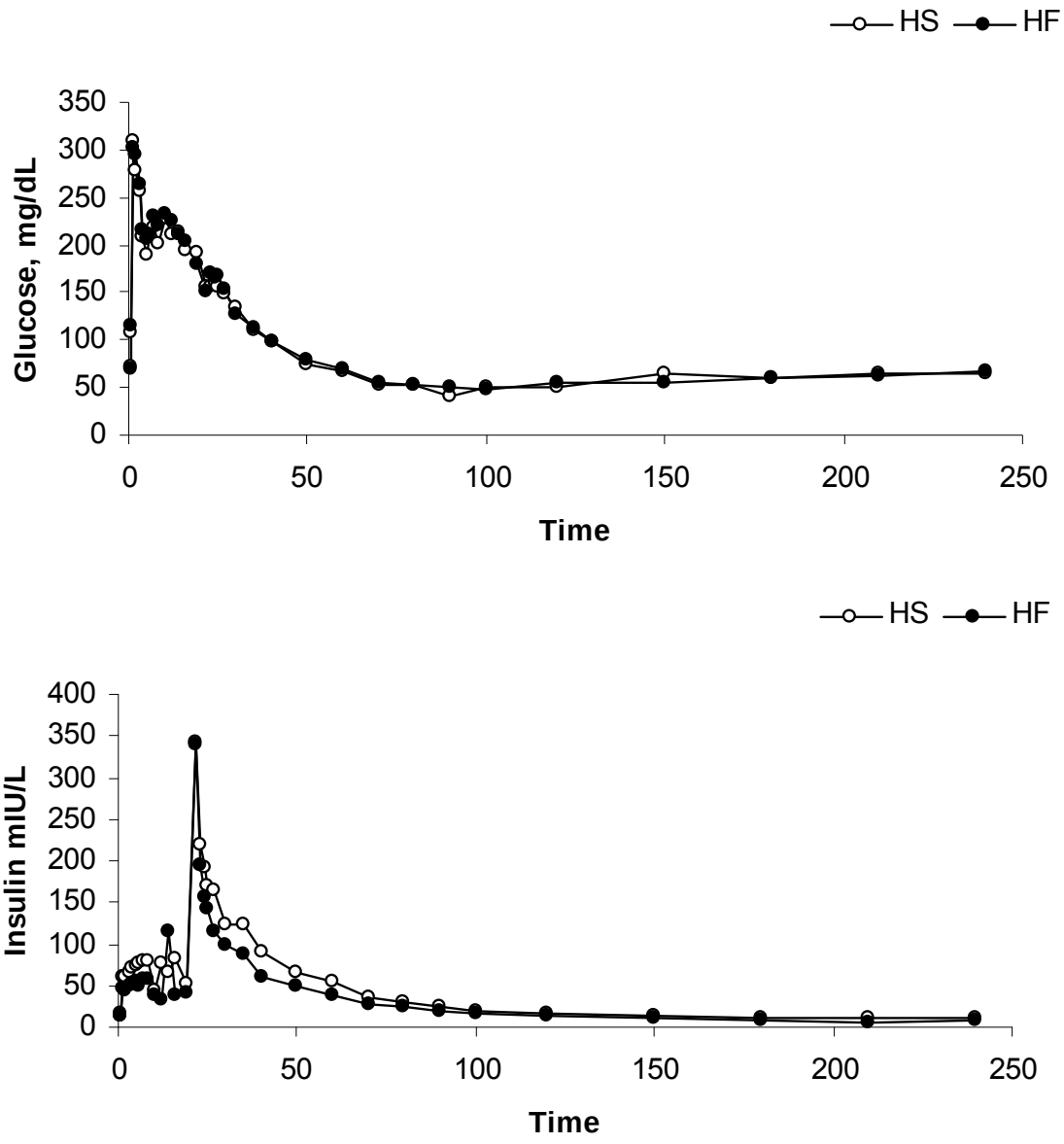


Figure I. (Chapter II, III). Raw glucose and insulin responses of Thoroughbred geldings to a frequently sampled i.v. glucose tolerance test following the third wk of commercial concentrate (CFMM).

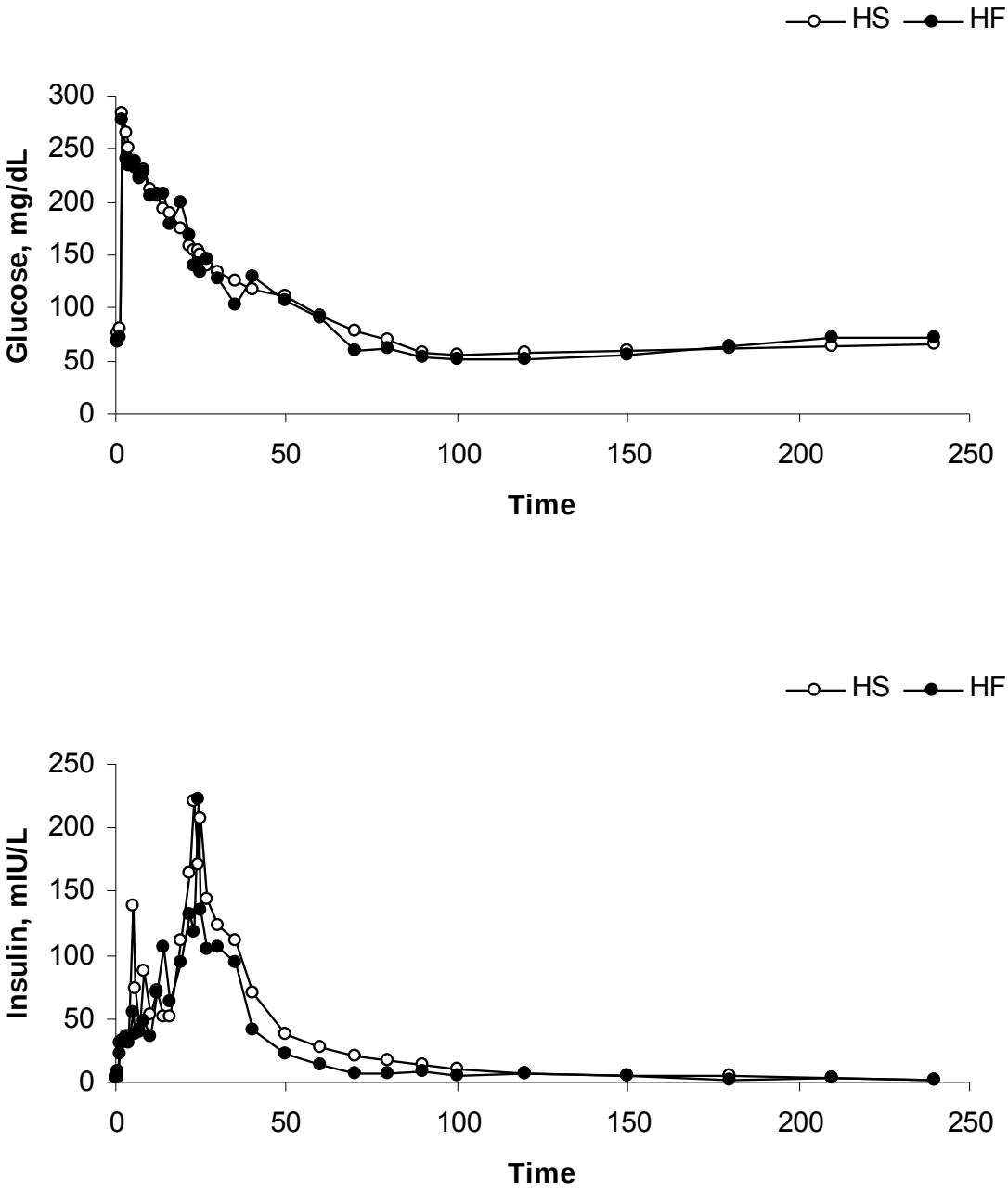


Figure 2. (Chapter II, III). Raw glucose and insulin responses to a frequently sampled i.v. glucose tolerance test following the fourth wk of treatment concentrate (TXMM).

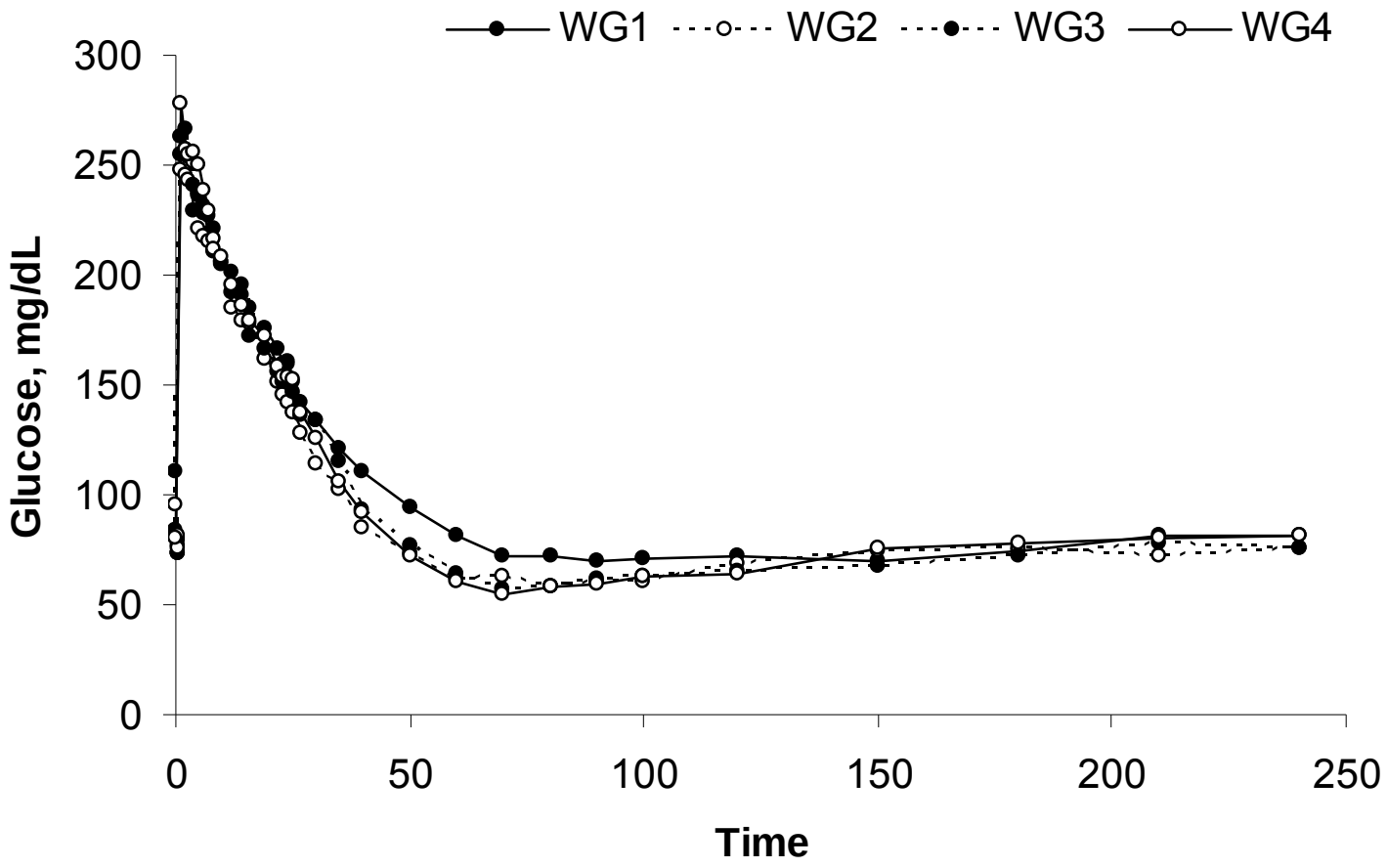


Figure 3. (Chapter II). Raw plasma glucose responses in Thoroughbred geldings to a frequently sampled i.v. glucose tolerance test following each 22.7 kg BW gain, WG1, WG2, WG3 and WG4.

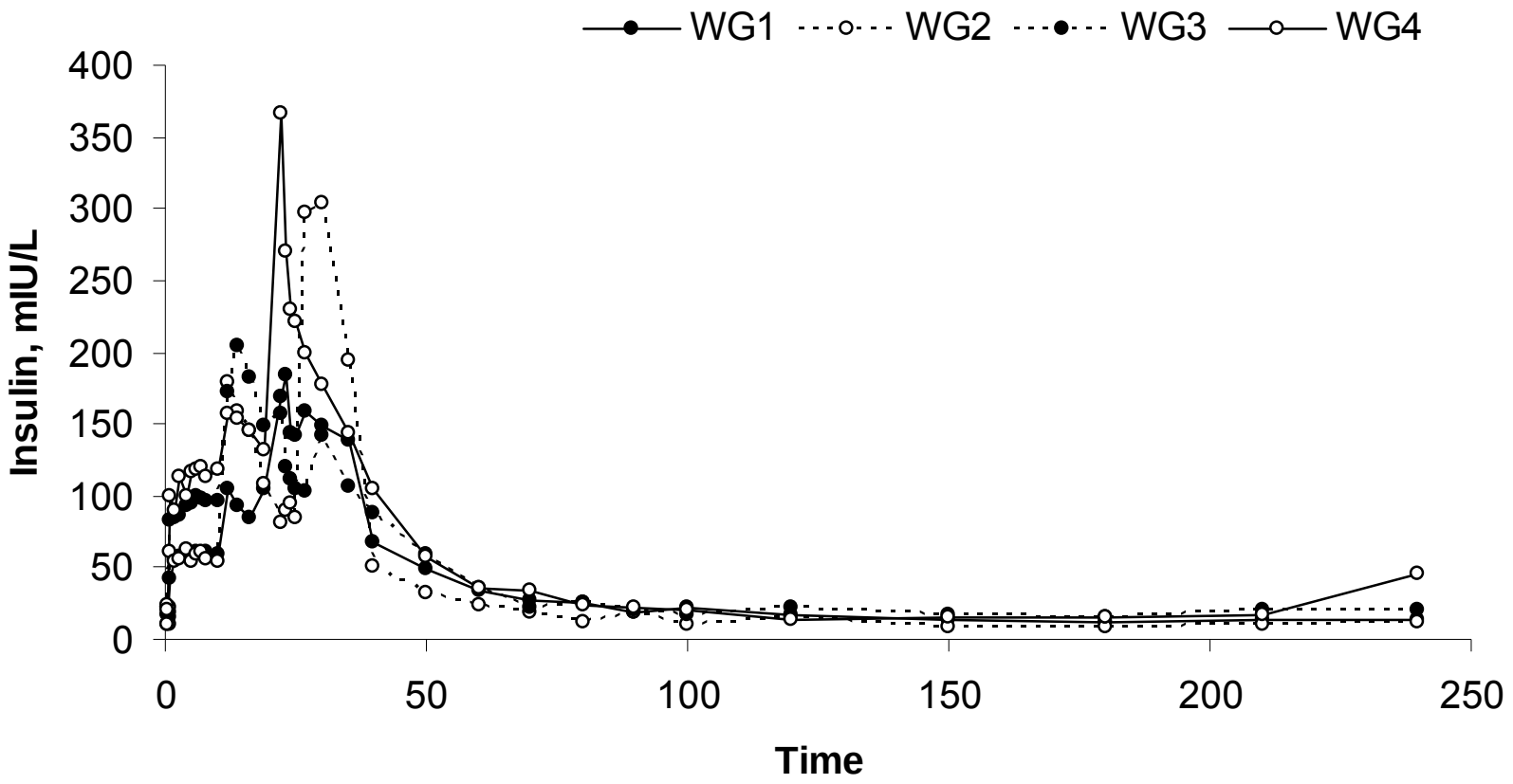
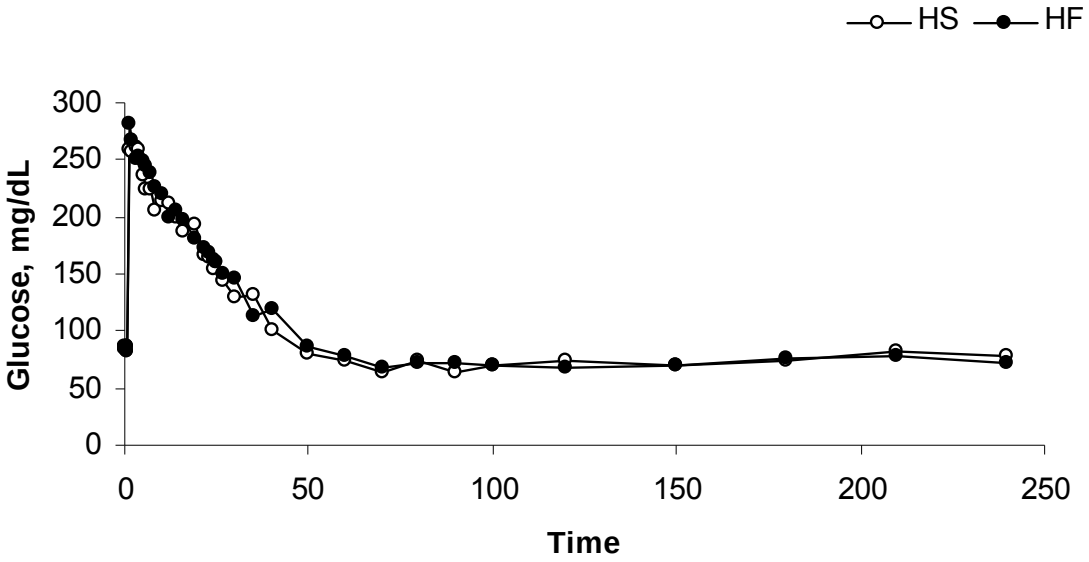


Figure 4. (Chapter II). Raw serum insulin responses in Thoroughbred geldings to a frequently sampled i.v. glucose tolerance test following each 22.7 kg BW gain, WG1, WG2, WG3 and WG4.



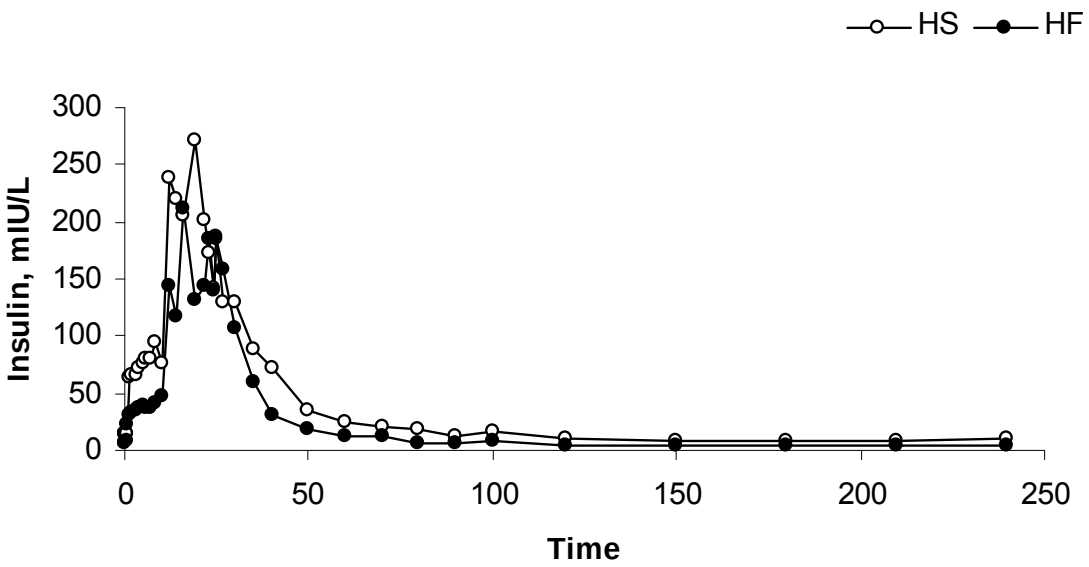


Figure 5. (Chapter III). Raw glucose and insulin responses in Thoroughbred geldings to a frequently sampled i.v. glucose tolerance test following an average 90.8 kg BW gain (ENDMM).

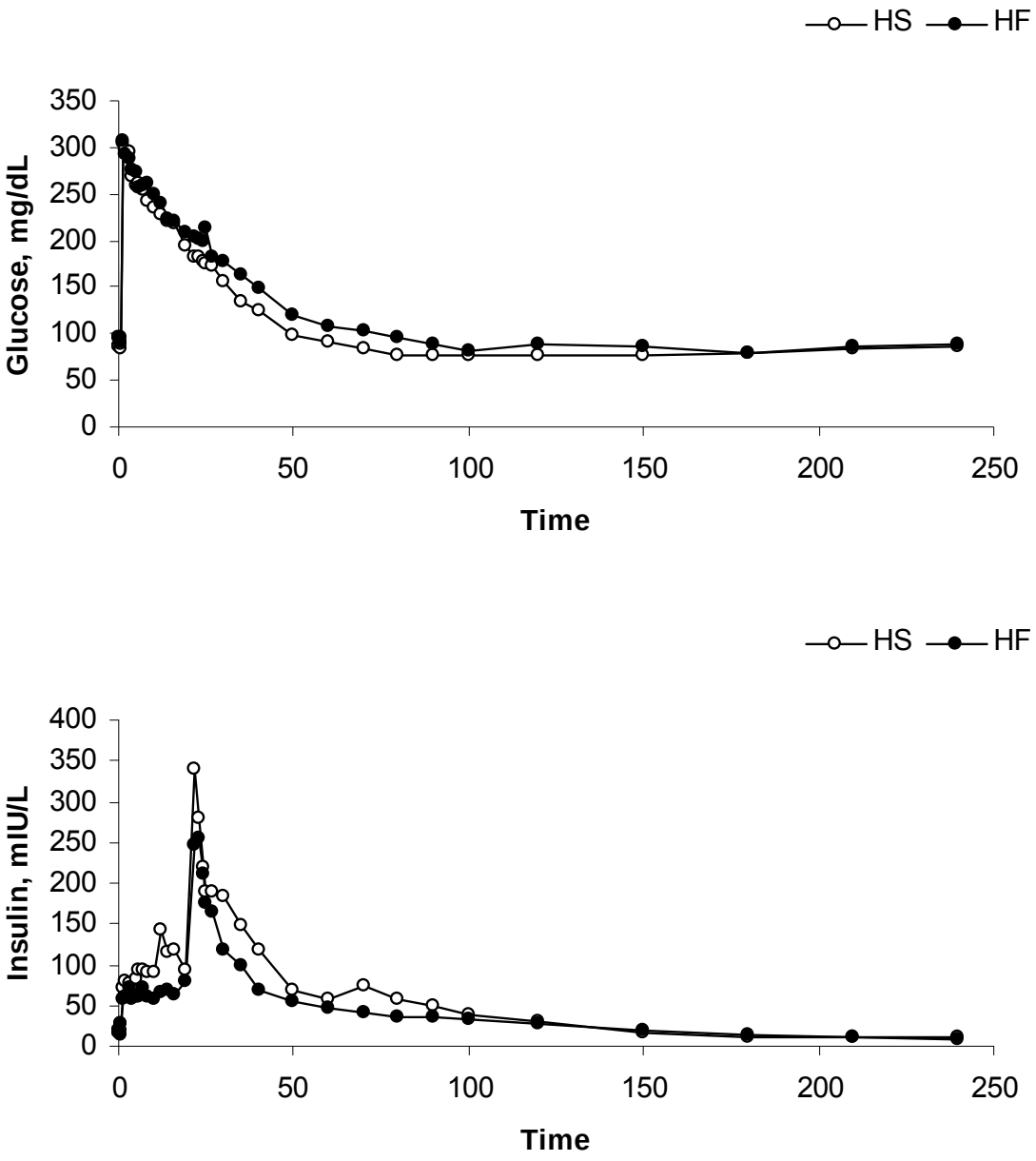


Figure 6. (Chapter III). Raw glucose and insulin responses in Thoroughbred geldings to a frequently sampled i.v. glucose tolerance test following two weeks of minimal exercise (MINEX).

LITERATURE CITED

Ader, M. 1997. Physiologic principles underlying glucose effectiveness. Pages 159 – 186 in: The minimal model approach and determinants of glucose tolerance. Ed: G.A. Bray and D.H. Ryan. Louisiana State University Press, Baton Rouge, LA.

Ahren, B. and G. Pacini. 1988. Age-related reduction in glucose elimination is accompanied by reduced glucose effectiveness and increased hepatic insulin extraction in man. J. Clin. Endocrinol. Metab. 83:3350–3356.

Ahren, B. and G. Pacini. 2005. Islet adaptation to insulin resistance: mechanisms and implications for intervention. Diabetes Obes. Metab. 7:2-8.

Alford, F.P., J.E. Henriksen, C. Rantzau, A. Vaag, L.F. Hew, G.M. Ward and H. Beck-Nielsen. 1998. Impact of family history of diabetes on the assessment of β-cell function. Metab. Clin. Exp. 47:522-528.

Alford, P., S. Geller, B. Richardson, M. Slater, C. Honnas, J. Foreman, J. Robinson, M. Messer, M. Roberts, D. Goble, D. Hood and M. Chaffin. 2001. A multicenter, matched case control study of risk factors for equine laminitis. Prev. Vet. Med. 49:209-222.

Appleton, D.J., J.S. Rand, G.D. Sunvold. 2001. Insulin sensitivity decreases with obesity and lean cats with low insulin sensitivity are at greatest risk of glucose intolerance with weight gain. J. Feline. Med. Surg. 3:211-228.

Arch, J.R.S. 2005. Central regulation of energy balance: inputs, outputs and leptin resistance. Proc. Nutr. Soc. 64:39-46.

Art, T., N. Kirschvink, N. Smith and P. Lekeux. 1999. Indices of oxidative stress in blood and pulmonary epithelium lining fluid in horses suffering from recurrent airway obstruction. Equine Vet. J. 31:397-401.

Art T., N. Kirschvink, N. Smith, D. Votion and P. Lekeux. 1999b. Cardiorespiratory measurements and indices of oxidative stress in exercising COPD horses. Equine Vet. J. Suppl. 30:83-7.

Argenzio, R.A. and H.F. Hintz. 1971. Volatile fatty acid tolerance and effect of glucose and VFA on plasma insulin levels in ponies. J. Nutr. 101:723-730.

Aurajo-Vilar, D., D.A. Garcia-Estevez and J. Cabezas-Cerrato. 1998. Both a reduced acute insulin reponse to glucose and lower glucose effectiveness are responsible for the worsening of intravenous glucose tolerance in healthy subjects independently of the degree of obesity. Metab. Clin. Exp. 47:313-320.

Baetz, A.L. and J.E. Pearson. 1972. Blood constituent changes n fasted ponies. Am. J. Vet. Res. 33:1941-1946.

Baker, J.R. and H.E. Ritchie. 1974. Diabetes mellitus in the horse: a case report and review of the literature. Equine Vet. J. 6:7-11.

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

Bergman, R.N., Y. Ziya Ider, C.R. Bowden and C. Cobelli. 1979. Quantitative estimation of insulin sensitivity. Am. J. Physiol. Endocrinol. Metab. 236:667-677.

Bergman, R.N., R. Preger, A. Volund and J.M. Olefsky. 1987. Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. J. Clin. Invest. 79:790-800.

Bergman, R.N., Y.J. Yang, I.D. Hope, and M. Ader. 1990. The role of transcapillary insulin transport in the efficiency of insulin action: studies with glucose clamps and the minimal model. Horm. Metab. Res. 24:49-56.

Bergman, R.N. 1997. The minimal model: yesterday, today and tomorrow. Pages 3 – 50 in: The minimal model approach and determinants of glucose tolerance. Ed: G.A. Bray and D.H. Ryan. Louisiana State University Press, Baton Rouge, LA**.**

Best, J.D., F.P. Alford, I.K. Martin, R.G. Pestell and G.M. Ward. 1990. Practical application of methods for in vivo assessment of insulin secretion and action. Horm. Metab. Res. Suppl. 24:60-66.

Bianchi, M. 1989. Fat cell size in various body regions. A statistical analysis in *Equus caballus*. Anat. Anz. Jena. 169: 351-366.

Boston, R.C., D. Stefanovski, P.J. Moate, A.E. Sumner, R.M. Watanabe and R.N. Bergman. 2003. MinMod Millenium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. Diabetes Technol. Ther. 5:1003-1015.

Buff, P.R., A.C. Dodds, C.D. Morrison, N.C. Whitley, E.L. McFadin, J.A. Daniel, J. Djiane, and D.H. Keisler. 2002. Leptin in horses: Tissue localization and relationship between peripheral concentrations of leptin and body condition. J. Anim. Sci. 80:2942-2948.

Buff, P.R., P.J. Johnson, C.E. Wiedmeyer, V.K. Ganjam, N.T. Messer and D.H. Keisler. 2006. Modulation of leptin, insulin and growth hormone in obese pony mares under chronic nutritional restriction and supplementation with ractopamine hydrochloride. Vet. Ther. 7:64-72.

Buff, P.R., P.J. Johnson, J.L. Casati, B.A. Abel, J.R. Turk, D.H. Keisler, N.T. Messer and V.K. Ganjam. 2007. Leptin receptor and neuropeptide Y in the medial basal hypothalamus of horses. Pages 124-125 in Proc. 20th Equine Sci. Soc. Symp., Hunt Valley, MD.

Burkholder, W.J. 2002. Use of body condition scores in clinical assessment of the provision of optimal nutrition. J. Am. Vet. Med. Assoc. 217:650-654.

Carroll, C.L., and P.J. Huntington. 1988. Body condition scoring and weight estimation. Equine Vet. J. 20:41-45.

Cartmill, J.A., D.L. Thompson, W.A. Storer, L.R. Gentry, and N.K. Huff. 2003. Endocrine responses in mares and geldings with high body condition scores grouped by high vs. low resting leptin concentrations. J. Anim. Sci. 81:2311-2321.

Castillo, C., C. Bogardus, R. Bergman, P. Thuillez and S. Lillioja. 1994. Interstitial insulin concentrations determine glucose uptake rates but not insulin resistance in lean and obese men. J. Clin. Invest. 93:10-16.

Caumo, A., P. Vicini and C. Cobelli. 1996. Is the minimal model too minimal? Diabetologia. 39:997-1000.

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

Ceddia, R.B. 2005. Direct metabolic regulation in skeletal muscle and fat tissue by leptin: implications for glucose and fatty acids homeostasis. Int. J. Obes. 29:1175-1183.

Chang, L., S. Chiang and A.R. Saltiel. 2004. Insulin signaling and the regulation of glucose transport. J. Mol. Med. 10:65-71.

Clarkson, P.M. and H.S. Thompson. 2000. Antioxidants: what role do they play in physical activity and health? Am. J. Clin. Nutr. 72:637S–46S.

Coffman, J.R., and C.M. Colles. 1983. Insulin tolerance in laminitic ponies. Can. J. Comp. Med. 47:347-351.

Coppack, S.W. 2001. Pro-inflammatory cytokines and adipose tissue. Proc. Nutr. Soc. 60:349-356.

Cymbaluk, N.F., and G.I. Christison. 1990. Environmental effects on thermoregulation and nutrition of horses. Vet. Clin. North Am. Equine Pract. 6:355-372.

Dandona, P., A. Ahmad, P. Mohanty, H. Ghanim, A. Bandyopadhyay and A. Chaudhuri. 2003. Insulin suppresses plasma concentration of vascular endothelial growth factor and matrix metalloproteinase-9. Diabetes Care. 26:3310–3314.

De Moffarts, B., N. Kirschvink, T. Art, J. Pincemail and P. Lekeux. 2005. Effect of oral antioxidant status in trained Thoroughbred horses. Vet. J. 169:65-74.

Defronzo, R.A., J.D. Tobin and R. Andres. 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am. J. Physio. Endocrinol. Metab. 237:214-223.

Droge, W. 2002. Free radicals in the physiological control of cell function. Annu. Rev. Physiol. 82:48-80.

Eiler, H., N. Frank, F.M. Andrews, J.W. Oliver and K.A. Fecteau. 2005. Physiologic assessment of blood glucose homeostasis via combined intravenous glucose and insulin testing in horses. Am. J. Vet. Res. 66:1598-1604.

Elenkov, I.J., D.G. Lezzoni, A. Daly, A.G. Harris, G.P. Chrousos. 2005. Cytokine dysregulation, inflammation and well-being. Neuroimmunomodulation. 12:255-269.

Expert Committee, 2003. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 26:S5-20.

Ferrannini, E., S. Camastra, A. Gastaldelli, A. Sironi, A. Natali, E. Muscelli, G. Mingrone and A. Mari. 2004. Beta-cell function in obesity: effects of weight loss. Diabetes 53:S26–S33.

Filho, H.C.M., K. H. McKeever, M. E. Gordon, H.E.C. Costa, M. Watford. 2006. Novel findings regarding Glut-4 expression in adipose tissue and muscle in horses – a preliminary report. Vet. J. DOI:10.1016/j.tvjl.2006.10.022.

Finegood, D.T. and D. Tzur. 1996. Reduced glucose effectiveness associated with reduced insulin release: an artifact of the minimal model method. Am. J. Physio. Endocrinol. Metab. 271:485-495.

Finegood, D. M. 1997. Application of the minimal model of glucose kinetics. Pages 51 – 122 in: The minimal model approach and determinants of glucose tolerance. Ed: G.A. Bray and D.H. Ryan. Louisiana State University Press, Baton Rouge, LA.

Fitzgerald, B.P. 2004. Insulin resistance and inflammatory challenges. J. Equine Vet. Sci. 24:128.

Fitzgerald, B.P. and C.J. McManus. 2000. Photoperiodic versus metabolic signals as determinants of seasonal anaestrus in the mare. Biol. Reprod. 3:335-340.

Fitzgerald, B.P., S.E. Reedy, D.R. Sessions, M.M. Vick, and B.A. Murphy. 2003. Obesity disrupts the duration of the estrous cycle in the mare. J. Anim. Sci. (Suppl. 1):102.

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

Flores, C.A., P.M. Brannon, S.A. Bustamante, J. Bezerra, K.T. Butler, T. Goda and O. Koldovsky. 1988. Effect of diet on intestinal and pancreatic enzyme activities in the pig. Pediatr. Gastroenterol. Nutr. 7:914-21.

Forhead, A.J. and H. Dobson, 1997. Plasma glucose and cortisol responses to exogenous insulin in fasted donkeys. Res. Vet. Sci. 62:265-269.

Frank, N., J.E. Sojka and N.T. Messer. 2002. Equine thyroid dysfunction. Vet. Clin. North Am. Equine Pract. 18:305-319.

Frank, N., F.M. Andrews, S.B. Elliot, J. Lew and R.C. Boston. 2005. Effects of rice bran oil on plasma lipid concentrations, lipoprotein composition and glucose dynamics in horses. J. Anim. Sci. 83:2509-2518.

Frank, N., C.S. Sommardahl, H. Eiler, L.L. Webb, J.W. Denhart and R.C. Boston. 2005b. Effects of oral administration of levothyroxine sodium on concentrations of plasma lipids, concentration and composition of very-low-density lipoproteins, and glucose dynamics in healthy adult mares. Am. J. Vet. Res. 66:1032-1038.

Frank, N., S.B. Elliot, L.E. Brandt and D.H. Keisler. 2006. Physical characteristics, blood hormone concentrations and plasma lipid concentrations in obese horses with insulin resistance. J. Am. Vet. Med. Assoc. 228:1383-1390.

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

Freestone, J.F., K. Shoemaker, R. Bessin, and K.J. Wolfsheimer. 1992. Insulin and glucose response following oral glucose administration in well-conditioned ponies. Equine Vet. J. Suppl. 11:13-17.

Freestone, J.F., R. Beadle, K. Shoemaker, R.T. Bessin, K.J. Wolfsheimer, and C. Church. 1992b. Improved insulin sensitivity in hyperinsulinemic ponies through physical conditioning and controlled feed intake. Equine Vet. J. 24:187-190.

French, K.R., and C.C. Pollitt. 2004. Equine laminitis: Glucose deprivation and MMP activation induce dermo-epidermal separation in vitro. Equine Vet. J. 36:261-266.

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

Gayet, E. Bailhache, H. Dumon, L. Martin, B. Siliart and P. Nguyen. 2004. Insulin resistance and changes in plasma concentration of TNFa, IGF1, and NEFA in dogs during weight gain and obesity. J. Anim. Physiol. Anim. Nutr. (Berl.) 88:157–165.

Gentry, L. 2001. Body condition, leptin and reproductive characteristics in horses. Ph.D. Diss. Louisiana State University, Baton Rouge, LA.

Gentry, L., D.L. Thompson, G.T. Gentry, K.A. Davis, R.A. Godke, and J.A. Cartmill. 2002. The relationship between body condition, leptin, and reproductive and hormonal

characteristics of mares during the seasonal anovulatory period. J. Anim. Sci. 80:2695-2703.

Giraudet, A., K.W. Hinchcliff, C.W. Kohn and K.H. McKeever. 1994. Early insulin response to an intravenous glucose tolerance test in horses. Am. J. Vet. Res. 55:379-381.

Grill, V. and E. Qvigstad. 2000. Fatty acids and insulin secretion. Br. J. Nutr. 83:S79-84.

Gunn, H.M. 1986. Muscle, bone and fat proportions and the muscle distribution of Thoroughbreds and other horses. Pages 253-264 in Proceedings of the 2nd International Conference on Equine Exercise Physiology. San Diego, CA.

Halaas, J.L., C. Boozer, J. Blair-West, N. Fidahusein, D.A. Denton, and J.M. Freidman. 1997. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc. Natl. Acad. Sci. U.S.A. 94:8878-8883.

Hales, C.N. and D.J.P. Barker. 1992. Type 2 (non-insulin dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetalogia 35:595-601.

Hallebeek, J.M. and A.C. Beynen. 2003. Influence of dietary beetpulp on the plasma level of triacylglycerols in horses. J. Anim. Physiol. Anim. Nutr. (Berl.) 87:181-187.

Harris, P.A. 1999. Comparison of the the digestible energy (DE) and net energy (NE) systems for the horse. Pages 199-216 in: Proc. Equine Nutr. Conf. Feed Manuf.

Harris, P.A. and D.S. Kronfeld. 2003. Influence of dietary energy sources on health and performance. Curr. Ther. Equine Nutr.5:6;98-704.

Heilig, C.W., L.A. Concepcion, B.L. Riser, S.0. Freytag, M. Zhu and P. Cortes. 1995. Overexpression of glucose transporters in rat mesangial cells cultured in a normal glucose milieu mimics the diabetic phenotype. J. Clin. Invest. 96:1802-1814.

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

Henricksen, J.E. 2002. Exercise effects of muscle insulin signaling and action invited review: Effects of acute exercise and exercise training on insulin resistance. J. Appl. Physiol. 93: 788–796.

Henricksen, J.E., F. Alford, A. Handberg, A. Vaag, G. Ward, A. Kalfas and H. Beck-Neilson. 1994. Increased glucose effectiveness in normoglycemic but insulin resistant relatives of patients with non-insulin dependent diabetes mellitus: a novel compensatory mechanism. J. Clin. Invest. 94:1196-1204.

Heusner, G.L. 1993. Ad libitum feeding of mature horses to achieve rapid weight gain. Pages 86-87. In: Proc. 8th Equine Nutr. Physiol. Symp., Lexington, KY.

Hinckley, K.A., S. Fearn, B.R. Howard and I.W. Henderson. 1996. Nitric oxide donors as treatment for grass-induced acute laminitis in ponies. Equine Vet. J. 28:17-28.

Hochberg, Z., M. Friedberg, L. Yaniv, T. Bader and D. Tiosano. 2004. Hypothalamic regulation of adiposity: the role of 11-β-hydroxysteroid dehydrogenase type I. Horm. Metab. Res. 36:365-369.

Hoenig, M., K. Thomaseth, J. Brandao, M. Waldron and D.C. Ferguson. 2006. Assessment and mathematical modeling of glucose turnover and insulin sensitivity in lean and obese cats. Domest. Anim. Endocrinol. 31:373–389.

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

Hoffman, R.M., D.S. Kronfeld, W.L. Cooper and P.A. Harris. 2003b. Glucose clearance in grazing mares is affected by diet, pregnancy and lactation. J. Anim. Sci. 81:1764-1771.

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

Hollenbeck, C.B. and G.M. Reaven. 1987. Treatment of patients with non-insulin-dependent diabetes mellitus: diabetic control and insulin secretion and action after different treatment modalities. Diabet. Med. 4:311-316.

Hornick, J.L., C. Van Eenaeme, O. Gérard, I. Dufrasne and L. Istasse. 2000. Mechanisms of reduced and compensatory growth. Domest. Anim. Endocrinol. 19: 121–132.

Hughes, K.J., D.R. Hodgson, and A.J. Dart. 2004. Equine hyperlipaemia: a review. Aust. Vet. J. 82:136-142.

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

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

Johnson, P.J., S.C. Tyagi, L.C. Katwa, V.K. Ganjam, L.A. Moore, J.M. Kreeger and N.T. Messer. 1998. Activation of extracellular matrix metalloproteinases in equine laminitis. Vet. Rec. 142:392-396.

Johnson, P.J. 2002. The equine metabolic syndrome Peripheral Cushing's syndrome. Vet. Clin. North Am. Equine Pract.. 18:271-293.

Johnson, P.J., V.K. Ganjam, S.H. Slight, J.M. Kreeger, and N.T. Messner. 2004. Tissue-specific dysregulation of cortisol metabolism in equine laminitis. Equine Vet J. 36:41-45.

Johnson, P.J., N.T. Messer and E.Kellon. 2004b. Treatment of equine metabolic syndrome. Compendium on Continuing Education for the Practicing Veterinarian. February:122-130.

Johnson, P.J., N.C. Scotty, C. Wiedmeyer, N.T. Messer and J.M. Kreeger. 2005. Diabetes mellitus in a domesticated Spanish Mustang. J. Am. Vet. Med. Assoc. 226:584-588.

Julian, L.M., J.H. Lawrence, N.I. Berlin and G.M. Hyde. 1956. Blood volume, body water and body fat of the horse. J. Appl. Physiol. 8:651-653.

Kahn, C.R., 1978. Insulin resistance, insulin insensitivity and insulin unresponsiveness: a necessary distinction. Metab. Clin. Exp..27:1893-1902.

Kahn, S.E. 2003. The relative contributions of insulin resistance and β-cell function to the pathophysiology of type 2 diabetes. Diabetologia. 46:2-19.

Kahn, SE., R.L. Prigeon, D.K. McCulloch, E.J. Boyko, R.N. Bergman, M.W. Schwartz, J.L. Neifing, W.K. Ward, J.C. Beard, J.P. Palmer and D. Porte. 1994. The contribution of insulin-dependent and insulin-independent glucose uptake to intravenous glucose tolerance in healthy human subjects. Diabetes. 43:587-592.

Kahn, S.E., R.L. Prigeon, R.S. Scwartz, W.Y. Fujimoto, R.H. Knopp, J.D. Brunzell and D. Porte, Jr. 2001. Obesity, Body Fat Distribution, Insulin Sensitivity and Islet b-Cell Function as Explanations for Metabolic Diversity. J. Nutr. 131: 354S–360S.

Kaiyala, K.J., R.L. Prigeon, S.E. Kahn, S.C. Woods and M.W. Schwartz. 2000. Obesity induced by a high-fat diet is associated with reduced brain insulin transport in dogs. Diabetes. 49:1525-1533.

Kane, R.A., M. Fisher, D. Parrett, and L.M. Lawrence. 1987. Estimating fatness in horses. Pages 127-131. In: Proc. 10th Equine Nutr. Phsyiol. Symp., Ft. Collins, CO.

Kearns, C.F., K.H. McKeever, K. Malinowski, M.B. Struck, and T. Abe. 2001. Chronic administration of therapeutic levels of clenbuterol acts as a repartitioning agent. J. Appl. Physiol. 91:2064-2070.

Kearns, C.F., K.H. McKeever and T. Abe. 2002. Overview of horse body composition and muscle architecture: implications for performance. Vet. J. 164:224-234.

Kearns, C.F., K.H. McKeever, V. Roegner, S.M. Brady, and K. Malinowski. 2005. Adiponectin and leptin are related to fat mass in horses. Vet. J. Epub. ahead of print. www.elsevier.com/locate/tvjl.

Keen, J.A., M. McLaren, K.J. Chandler and B.C. McGorum. 2004. Biochemical indices of vascular function, glucose metabolism and oxidative stress in horses with equine Cushing's disease. Equine Vet. J. 36:226-229.

Kinnunen, S., S. Hyyppa, A. Lehmuskero, N. Oksala, P. Maenpaa, O. Hannienen and M. Atalay. 2005. Oxygen radical absorbace capacity and exercise-induced oxidative stress in trotters. Eur. J. Appl. Physiol. 95:550-556.

Kopp, W. 2003. High-insulinogenic nutrition - an etiologic factor for obesity and the metabolic syndrome? Metab. Clin. Exp.. 52:40-844.

Koranyi, L.I., R.E. Bourey, H. Vuorinen-Markkola, Y.A. Kovisto, M. Mueckler, M.A. Permutt and H. Yki-Järvinen. 1991. Level of skeletal muscle glucose transporter protein correlates with insulin-stimulated whole body glucose disposal in man. Diabetologia. 34:763-765.

Kronfeld, D.S. 2003. Equine syndrome X, the metabolic disease and equine grain-associated disorders: nomenclature and dietetics. J. Equine Vet. Sci. 23:567-569

Kronfeld, D.S. and P.A. Harris. 2003. Equine grain-associated disorders. Compendium on Continuing Education for the Practicing Veterinarian. 25:974-982.

Kronfeld, D.S. R.J. Geor, J. Beech and R.C. Boston. 2005. Questions findings in Spanish Mustange study. J. Am. Vet. Med. Assoc. 226:1476.

Kronfeld, D.S., K.H. Treiber, T.M. Hess, and R.C. Boston. 2005b. Insulin resistance in the horse: Definition, detection and dietetics. J. Anim. Sci. 83(Suppl.):E22-E31.

Lawrence, L.M. 2000. Advances and gaps in energy nutrition. In: Advances in Equine Nutrition II. J.D. Pagan and R.J. Geor, ed. Nottingham University Press, p.227-236.

Lehnhard, R.A., K.H. McKeever, C.F. Kearns, and M.D. Beekley. 2004. Myosin heavy chain profiles and body composition are different in old versus young Standardbred mares. Vet. J. 167:59-66.

Lindberg, J.E. and C. Palmgren-Karlsson. 2001. Effect of partial replacement of oats with sugar beet pulp and maize oil on nutrient utilization in horses. Equine Vet. J. 33:585-590.

Lovejoy, J.C., S.R. Smith, C.M. Champagne, M.M. Most, M. Lefevre, J.P. Delany, Y.M. Denkins, J.C. Rood, J. Veldhuis and G.A. Bray. 2002. Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or trans (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. Diabetes Care. 25:1283–1288.

Lyon, C.J., R.E. Law and W.A. Hsueh. 2003. Minireview: adiposity, inflammation and atherogenesis. Endocrinology. 114:2195-2200.

Marangou, A.G., K.M. Weber, R.C. Boston, P.M. Aitken, J.C.P. Heggie, R.L.G. Kirsner, J.D. Best and F.P. Alford. 1986. Metabolic consequences of prolonged hyperinsulinemia in humans. Diabetes. 35:1383-1389.

Marlin, D.J., K. Fenn, N. Smith, C.D. Deaton, C.A. Roberts, P.A. Harris, C. Dunster and F.J. Kelly. Changes in circulatory antioxidant status in horses during prolonged exercise. J. Nutr. 132:1622S-1627S.

Martin-Rosset, W. and M. Vermorel. 1991. Maintenance energy requirement variations determined by indirect calorimetry and feeding trials in light horses. J. Equine Vet. Sci. 11:42 – 45.

McCoy, D.J. 1986. Diabetes mellitus associated with bilateral granulosa cell tumors in a mare. J. Am. Vet. Med. Assoc. 188:733-735

McCue, P.M. 2002. Equine Cushing's disease. Vet. Clin. North Am. Equine Pract.. 18:533-543.

Meier, U., and A.M. Gressner. 2004. Endocrine regulation of energy metabolism: Review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin. Chem. 50:1511-1525.

Mills, P.C., N.C. Smith, I. Casas, P. Harris, R.C. Harris and D.J. Marliln. 1996. Effects of exercise intensity and environmental stress on indices of oxidative stress and iron homeostasis during exercise in the horse. Eur. J. Appl. Physiol. 74:60-66.

Mueller, W.M., F.M. Gregoire, K.L. Stanhope, C.V. Mobbs, T.M. Mizuno, C.H. Warden, J.S. Stern and P. J. Havel. 1998. Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. Endocrinology. 139:551-558.

Mungall, B.A., C.C. Pollitt and R. Collins. 1998. Localisation of gelatinase activity in epidermal hoof lamellae by in situ zymography. Histochem. Cell Biol. 110:535–540.

Muylle E., C. van den Hende, P. Deprez, J. Nuytten and W. Oyaert. 1986. Non-insulin dependent diabetes mellitus in a horse. Equine Vet.J. 18:145-6.

Neville, R.F., T. Hollands, S.N. Collins and F.V. Keyte. 2004. Evaluation of urinary TBARS in normal and chronic laminitic ponies. Equine Vet. J. 36:292-294.

NIH. 1998. Clinical guidelines on the identification, evaluation and treatment of overweight and obesity in adults. National Institutes of Health and National Heart, Lung and Blood Institute. NIH Publication #98-4083.

NRC. 1989. Pages 90 - 110 in Nutrient Requirements of the Horse. 6th rev. ed. Natl. Acad. Press, Washington, DC.

NRC. 2007. Pages 90 - 110 in Nutrient Requirements of the Horse. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Ott, E.A. and J. Kivipelto. 1999. Influence of chromium tripicolinate on growth and glucose metabolism in yearling horses. J. Anim. Sci. 77:3022-3030.

Pagan, J.D., B. Essen-Gustavsson, A. Lindholm and J. Thornton. 1987. The effect of dietary energy source on blood metabolites in Standardbred horses during exercise. Pages 425-430 In: Proc. 11th Equine Nutr. Physiol. Symp., Fort Collins, CO.

Pagan, J.D., P.A. Harris, M.A.P. Kennedy, N. Davidson and K.E. Hoekstra. 1999. Feed type and intake effects glycemic response in Thoroughbred horses. Pages 149-152 In: Proc. 16th Equine Nutr. Physiol. Symp., Raleigh, NC.

Pan, S.J., J. Hancock, Z. Ding, D. Fogt, M. Lee, and J.L. Ivy. 2001. Effects of clenbuterol on insulin resistance in conscious obese Zucker rats. Am. J. Physiol. Endocrinol. Metab. 280:554-561.

Petersen, K.F. and G.I. Shulman. 2006. Etiololgy of insulin resistance. Am. J. Med. 119: 10S-16S.

Polonsky, K.S., B.D. Given, L. Hirsch, E.T. Shapiro, H. Tilli, C. Beebe, J.A. Galloway, B.H. Frank, T. Kerrison and E. Van Cauter. 1988. Quantitative study of insulin secretion and clearance in normal and obese subjects. J. Clin. Invest. 81:435-441.

Powell, D.M., L.M. Lawrence, D.F. Parrett, J. DiPietro, L.R. Moser, M.G. Fisher and K.D. Bump. 1989. Body composition changes in broodmares. Pages 91-95 In: Proc. 11th Equine Nutr. Physiol. Symp., Fort Collins, CO.

Powell, D.M., L.M. Lawrence, B.P. Fitzgerald, K. Danielsen, A. Parker, P. Siciliano and A. Crum. 2000. Effect of short-term feed restriction and calorie source on hormonal and metabolic responses in geldings receiving a small meal. J. Anim. Sci. 78:3107-3113.

Powell, D.M., S.E. Reedy, D.R. Sessions, and B.P. Fitzgerald. 2002. Effect of short-term exercise training on insulin sensitivity in obese and lean mares. Equine Vet. J. 34:81-84.

Potter, G.D., J.W. Evans, G.W. Webb and S.P. Webb. 1987. Digestible Energy Requirements of Belgian and Percheron Horses. Pages 133-138 In: Proc. 11th Equine Nutr. Physiol. Symp., Fort Collins, CO

Pratt, S.E., R.J. Geor and L.J. McCutcheon. 2005. Repeatability of two methods for assessment of insulin sensitivity and glucose dynamics in horses. J. Vet. Intern. Med. 19:883-888.

Prigeon, R.L., M.E. Roder, D. Porte and S.E. Kahn. 1996. The effect of insulin dose on the measurement of insulin sensitivity by the minimal model technique. J. Clin. Invest. 97:501-507.

Quinn, R.W., A.O. Burk, T.G. Hartsock, K.H. Treiber and R.C. Boston. 2007. Effects of weight gain and diet on insulin sensitivity in Thoroughbred geldings. Pages 9-10. In. Proc. 20th Equine Sci. Soc., Hunt Valley, MD.

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

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

Reaven, G.M. 2006. The metabolic syndrome: is this diagnosis necessary? Am. J. Clin. Nutr. 83:1237-1247.

Ren, J., J. Ping, E. Wang, E. Liu, D.M. Harlan, X. Li and D.F. Stroncek. 2007. Pancreatic islet cell therapy for type I diabetes: understanding the effects of glucose stimulation on islets in order to produce better islets for transplantation. J. Transl. Med. 5:1-15.

Robb, J., R.B. Harper, H.F. Hintz, J.T. Reid, J.E. Lowe, H.F. Schryver, and M.S.S. Rhee. 1972. Chemical composition and energy value of the body, fatty acid composition of adipose tissue and liver and kidney size in the horse. Anim. Prod. 14:25-34.

Rocchini, A.P., P. Marker and T. Cervenka. 1997. Time course of insulin resistance associated with feeding dogs a high-fat diet. Am. J. Physio. Endocrinol. Metab. 272:E147-E154.

Ropp, J.K., R.H. Raub and J.E. Minton. 2003. The effect of dietary energy source on serum concentration of insulin-like growth factor-I, growth hormone, insulin, glucose, and fat metabolites in weanling horses. J. Anim. Sci. 81:1581-1589.

Ross, M.W., J.E. Lowe, B.J. Cooper, T.J. Reimers and B.A. Froscher. 1983. Hypoglycemic seizures in a Shetland pony. Cornell Vet. 73:151-169.

Ruoff, W.W., D.C. Baker and S.J. Morgan. 1986. Type II diabetes mellitus in a horse. Equine Vet. J. 18:143-144.

Saad, M.F., G.M. Steil, M. Riad-Gabriel, A. Khan, A. Sharma, R. Boyadjian, S.D. Jinagouda and R.N. Bergman. 1997. Method of insulin administration has no effect on insulin sensitivity estimates from the insulin-modified minimal model protocol. Diabetes. 46.2044-2048.

Saladin, R., P. DeVos, M. Guerre-Millo, A. Leturque, J. Girard, B. Staels and J. Auwerx. 1995. Transient increase in obese gene expression after food intake or insulin administration. Nature. 377:527-529.

Schmidt, O., E. Deegen, H. Fuhrmann, R. Duhlmeier and H.P. Salimann. 2001. Effects of fat feeding and energy level on plasma metabolites and hormones in Shetland ponies. J. Vet. Med. Assoc. 48:39-49.

Schulman, G.I. 2000. Cellular mechanisms of insulin resistance. J. Clin. Invest. 106:171-176.

Schwartz, M.W., S.C. Woods, D.Porte, Jr., R.J. Seeley and D.G. Baskin. 2000. Central nervous system control of food intake. Nature. 404:661-670.

Sleeper, M.M., C.F. Kearns and K.H. McKeever. 2002. Chronic clenbuterol administration negatively alters cardiac function. Med. Sci. Sports. Exerc. 34:643-50.

Sommardahl, C.S., N. Frank, S.B. Elliott, L.L. Webb, K.R. Refsal, J.W. Denhart, and D.L. Thompson. 2005. Effects of oral administration of levothyroxine sodium on serum concentrations of thyroid gland hormones and responses to injections of thyrotropin-releasing hormone in healthy adult mares. Am. J. Vet. Res. 66:1025-1031.

Souza, C.T., E.P. Araujo, S. Bordin, R. Ashimine, R.L. Zollner, A.C. Boschero, M.J.A. Saad and L.A. Velloso. 2005. Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology. 146:4192–4199.

Stannard, S.R. and N.A. Johnson. 2003. Insulin resistance and elevated triglycerides in muscle: more important for survival than thrifty genes? J. Appl. Physiol. 554:595-607.

Steil, G.M., J. Murray, R.N. Bergman, T.A. Buchanan. 1994. Repeatability of insulin sensitivity and glucose effectiveness from the minimal model: implications for study design. Diabetes. 43:1114-1121.

Sticker, L.S., D.L. Thompson, L.D. Bunting, J.M. Fernandez and C.L. DePew. 1995. Dietary protein and(or) energy restriction in mares: plasma glucose, insulin, non-esterified fatty acid and urea nitrogen responses to feeding, glucose and epinephrine. J. Anim. Sci. 73:136-144.

Sticker, L.S., D.L. Thompson, L.D. Bunting and J.M. Fernandez. 1996. Dietary protein and energy restriction in mares: Plasma metabolite and hormone concentrations during dietary alteration. J. Anim. Sci. 74:1326-1335.

Stogdale, L. 1985. Definition of diabetes mellitus. Cornell Vet. 76:156-174.

Storlien, L., N.D. Oakes and D.E. Kelley. 2004. Metabolic flexibility. Proc. Nutr. Soc. 63:363-8.

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

Suagee, J.K. 2007. Effects of diet and weight gain on subcutaneous body fat accretion patterns and adipocytokine production in Thoroughbred geldings. M.S. Thesis, University of Maryland, College Park, MD.

Taniguchi, A., Y. Nakai, M. Fukushima, H. Imura, H. Kawamura, I. Nagata, G. L. Florant and K. Tokuyama. 1994. Insulin sensitivity, insulin secretion and glucose effectiveness in subjects with impaired glucose tolerance: a minimal model analysis. Metab. Clin. Exp. 43:714-718.

Treiber, K.H., R.C. Boston, D.S. Kronfeld, W.B. Staniar and P.A. Harris. 2005. Insulin resistance and compensation in Thoroughbred weanlings adapted to high-glycemic meals. J. Anim. Sci. 83:2357-2364

Treiber, K.H., T.M. Hess and D.S. Kronfeld. 2005b. Insulin resistance and compensation in laminitis-predisposed ponies characterized by the minimal model. Pferdeheilkunde. 21:91-92

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

Treiber, K.H., T.M. Hess, D.S. Kronfeld, R.C. Boston, R. Geor and P.A. Harris. 2005d. Dietary energy sources effect insulin sensitivity and β-cell responsiveness of trained Arabian geldings during endurance exercise. J. Anim. Physiol. Anim. Nutr. (Berl.) 89:427-433.

Treiber, K.H., D.S. Kronfeld, T.M. Hess, B.M. Byrd, R.K. Splan and W.B. Staniar. 2006. Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. J. Am. Vet. Med. Assoc. 228:1538-1545.

Treiber, K.H. 2006. 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. Ph.D. Diss. Virginia Polytechnic and State University, Blacksburg, VA.

Treiber, K.H., R.A. Carter, L.A. Gay, C.A. Williams and R. Geor. 2007. Oxidative stress and inflammatory markers in laminitis-prone ponies. Pages 142-143. In: Proc. 20th Equine Sci. Soc., Hunt Valley, MD.

Ueki, K., T. Kondo, Y-H. Tseng and R.C. Kahn. 2004. Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance and the metabolic syndrome in the mouse. Proc. Natl. Acad. Sci. U.S.A. 101:10422-10427

Vettor, R., G. Milan, M. Rossato, and G. Rederspil. 2005. Review article: Adipocytokines and insulin resistance. Aliment. Pharmacol. Ther. 22:3-10.

Vick, M.M., D.R. Sessions, B.A. Murphy, E.L. Kennedy, S.E. Reedy and B.P. Fitzgerald. 2006. Obesity is associated with altered metabolic and reproductive activity in the mare: effects of metformin on insulin sensitivity and reproductive cyclicity. Repro. Fertil. Dev. 18:609-17.

Vick, M.M., A.A. Adams, B.A. Murphy, D.R. Sessions, D.W. Horohov, R.F. Cook, B.J. Shelton and B.P. Fitzgerald. 2007. Relationship between inflammatory cytokines, obesity and insulin sensitivity in the horse. J. Anim. Sci. 85:1144-55.

Vincent, H.K. and A.G. Taylor. 2006. Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. Int. J. Obes. 30:400-418.

Wajchenberg, B.L. 2000. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr. Rev. 21:697-738.

Wang, Z.Q., X.H. Zhang, J.C. Russell, M. Hulver and W.T. Cefalu. 2006. Chromium picolinate enhances skeletal muscle cellular insulin signaling in vivo in obese, insulin-resistant JCR:LA-cp Rats. J. Nutr. 136: 415–420.

Ward, G.M., S.S.P. Gebhart, R.N. Bergman and L.S. Phillips. 1990. Effects of prolonged pulsatile hyperinsulinemia in humans: enhancement of insulin sensitivity. Diabetes. 29:501-507.

Ward, G.M., J.M. Walters. J. Barton, F.P. Alford and R.C. Boston. 2001. Physiologic modeling of the intravenous glucose tolerance test in type 2 diabetes: A new approach to the insulin compartment. Metab. Clin. Exp.. 50:512-519

Webb, S.P., G.D. Potter, J.W. Evans, and G.W. Webb. 1989. Influence of body fat content on digestible energy requirements of exercising horses in temperate and hot environments. Pages 279-284. In: Proc. 11th Equine Nutr. Physiol. Symp., Stillwater, OK.

Webb, A.I. and B.M.Q. Weaver. 1979. Body composition of the horse. Equine Vet. J. 11:39-47.

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

Westervelt, R.G., J.R. Stouffer, H.F. Hintz, and H.F. Schryver. 1976. Estimating fatness in horses and ponies. J. Anim. Sci. 43:781-785

WHO. 1999. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Geneva: World Health Organization.

Wilcox, G. 2005. Insulin and insulin resistance. Clin. Biochem. 26:19-39.

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

Williams C.A., R.M. Hoffman, D.S. Kronfeld, T.M. Hess, K.E. Saker and P.A. Harris. 2002. Lipoic acid as an antioxidant in mature thoroughbred geldings: a preliminary study. J. Nutr. 132:S1628-1631.

Yang, Y.J., J.H. Youn and R.N. Bergman. 1987. Modified protocols improve insulin sensitivity estimation using the minimal model. Am. J. Physio. Endocrinol. Metab. 253:E595-602.

Yang YJ, I.D. Hope, M. Ader, R.N. Bergman. 1989. Insulin transport across capillaries is rate limiting for insulin action in dogs. J. Clin. Invest. 84:1620-1628.

Ye, J. 2007. Role of insulin in the pathogenesis of free fatty acid-induced insulin resistance in skeletal muscle. Endocr. Metab. Immune Disord. Drug Targets. 7:65-74.

Youngren, J. 2007. Regulation of insulin receptor function. Cell. Mol. Life Sci. 64:873-891.

Yu, C., Y. Chen, G.W. Cline, D. Zhang, H. Zong, Y. Wang, R. Bergeron, J.K. Kim, S.W. Cushman, G.J. Cooney, B. Atcheson, M.F. White, E.W. Kraegen and G.I. Shulman. 2002. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. J. Biol. Chem. 52:50230-50236.

Zhao, Y., D.D. Feng and C. Chen. 2006. Contribution of adipocyte-derived factors to β-cell dysfunction in diabetes. Intl. J. Biochem. Cell Bio. 38:804-808.

Vitae

Rachael Weaver Quinn graduated from the Pennsylvania State University in 2000 with a B.S. degree in Dairy and Animal Science. She received her M.S. degree in Animal Science from the University of Maryland in 2002, focusing on animal behavior and swine production, under the direction of Dr. Thomas G. Hartsock. Among her accomplishments, Rachael is most proud of her work contributing to her dissertation, as well as her involvement in the renovations of the former swine unit at the Central Maryland Research and Extension Center into the current Equine Research Unit and her involvement with the Birthing Center at the Maryland State Fair. She was recognized by the attendees of the Mid-Atlantic Nutrition Conference as the highest rated speaker in the Equine Nutrition section in 2006 and has given numerous professional, departmental and extension presentations. Following her successful defense, Rachael will begin employment with the Kansas City Children's Mercy Hospital as Laboratory Supervisor for the Pediatric Cardiac Research satellite facility, located at Thomas D. Morris, Inc in Reisterstown, MD. She plans to continue to enjoy horses as an owner and competitor in the field of dressage.