EFFECTS OF MODIFIED INSULIN RESISTANCE ON GLUCOSE AND FATTY ACID METABOLISM DURING LATE GESTATION IN DAIRY CATTLE

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Katie Marie Schoenberg

January 2011

EFFECTS OF MODIFIED INSULIN RESISTANCE ON GLUCOSE AND FATTY ACID METABOLISM DURING LATE GESTATION IN DAIRY CATTLE

Katie Marie Schoenberg, Ph. D.

Cornell University 2011

In dairy cattle, insulin resistance of peripheral tissues during the transition from pregnancy to lactation is a normal adaptation to spare glucose for the gravid uterus and mammary gland. Exaggerated insulin resistance, a potential factor in overfed dry cows, may contribute to excess mobilization of adipose tissue in early lactation. The objectives were to determine the mechanism by which an insulin sensitizing agent (2,4-thiazolidinedione; TZD) and plane of nutrition (PON) can affect the metabolic health of transition cows. In experiment one, plasma leptin and tumor necrosis factor-α, and adipose tissue peroxisome proliferator activated-γ (PPARγ) mRNA were increased by prepartum TZD administration. The effects of PON and feed deprivation on insulin responses were measured in experiment two via glucose tolerance test (GTT) and hyperinsulinemic-euglycemic clamp (HEC) techniques. Cows subjected to high and low PON and then subjected to feed deprivation had different responses in glucose and lipid metabolism as measured by both GTT and HEC. The effects of feed deprivation were much greater than PON. Cows deprived of feed had much slower clearance of glucose during GTT, suggesting greater insulin resistance. Following feed deprivation, cows had greatly attenuated insulin response to GTT. In the final experiment, the effects of both PON and TZD were investigated using GTT, insulin challenge (IC), and adipose tissue mRNA analysis. There were differential effects of PON on glucose and fatty acid metabolism such that cows fed a

lower PON had smaller glucose but larger NEFA responses following GTT. The only significant interactions of PON and TZD administration were that plasma NEFA responses were most dramatic for cows treated with TZD and fed lower PON. While TZD administration or diet did not affect fatty acid synthase, leptin, TNFα, or adiponectin mRNA expression, the higher energy level diet increased mRNA of PPARγ and lipoprotein lipase. It is possible that these effects and interactions of diet and TZD would be more dramatic closer to the time of calving. Results from GTT and IC indicate that PON and insulin-sensitizing agents affect glucose and lipid metabolism during the dry period, which may have implications for the transition period.

BIOGRAPHICAL SKETCH

Katie Marie (Nelson) Schoenberg was born September 9, 1981 to Robert and Elaine Nelson in Rome, NY. She and her older brother, Dan, benefited from growing up in the outskirts of town. Attending Transfiguration Elementary School (Rome, NY) as well as Vernon-Verona-Sherrill (VVS) public schools, Katie knew from a very early age that she wanted a career involving animals. Only when she attended Cornell University as an undergraduate in Animal Science did she realize her passion would involve dairy cattle. During her time as an undergraduate, Katie enjoyed working three years for the Cornell Cooperative Extension office of Tompkins County, NY in youth education as well as an internship with Agway Feed and Nutrition working with dairy farms in Western New York State. Katie graduated with her Bachelor of Science degree *Magna Cume Laude* in 2003 and started an internship at the National Zoo in Washington, D.C., where she worked on a desert tortoise nutrition project. Her next experience was in poultry nutrition working as a Faculty Research Assistant in the Animal and Avian Sciences Department at the University of Maryland, College Park, MD. Katie then earned a Master's degree with Dr. Brian Bequette studying the metabolic control of milk protein synthesis in lactating mice. Two months before defending her M.S. thesis, Katie had the joy of marrying her best friend, Jonathan Schoenberg. Her return to Cornell allowed her to pursue her Doctorate degree in transition cow metabolism with Dr. Thomas Overton. In addition to pursuing her Ph.D, Katie had opportunity to explore interests in teaching, alumni involvement, intellectual property, and research in the animal health industry. Her research in dairy cattle metabolism will continue upon completion of her degree as a post-doctoral research associate in the laboratory of Dr. Yves Boisclair.

ACKNOWLEDGMENTS

There is not enough room to thank all of the people who have supported me through this journey, but I will try my best.

First, to Tom, who has been an unbelievably supportive and encouraging advisor. You have welcomed me not only into your scientific world, but also your family, and for all of that and much more I am extremely grateful. The lessons you have taught me will stick with me forever and I cannot imagine having a better PhD advisor.

To the rest of my committee: Drs. Dale Bauman, Yves Boisclair, Qiaoming Long, and Ling Qi, thank you for your time, support, and stimulating scientific discussion. A special thank-you to Dr. Bauman who has, in one form or another, served as my advisor since I arrived on campus as a "wet behind the ears" Freshman over 10 years ago. Thanks also to Dr. Alan Bell who not only lit my fire for animal nutrition, but my passion for teaching as well. Many thanks to the Department of Animal Science for its continued support of my program, and for selection as the McClure Assistantship recipient. It's been an honor.

To those who have been amazing to work alongside such as Charlene, Ramona, Lisa, and Gladys and the rest of the staff at T&R: your time, patience, and support make the work so much easier. I have also had the extreme pleasure to work alongside some amazing undergraduates: Chelsea, Mark, Paul, Jennine, Brianna, and Veronica. Thank you for your time and enthusiasm.

To my family and especially my Mom and Dad who taught me the benefits of perseverance, attention to detail, loyalty, and compassion for all things living.

To my husband, Jon, for all of his loving support that continually goes above and beyond the call of duty. WE can do anything. Thank you too, for always being

my loudest cheer leader. To our four-legged family Dutch, Anabel, and Bailey who have been my escape from all things stressful, never judge, and are always there for a cuddle.

Finally, I have developed friendships throughout this journey that not only got me through the hard times, but gave me some of the most joyous times of my life thus far. We have created memories that will last a lifetime. Again there are too many to mention, but first, to those who were not only friends but people that I've spilled blood, sweat, and tears (literally) with in the lab and in the barn: Laurie, Julie, R.B., Manolo, Ryan, Takashi, and Mollie, I am lucky not only to have you as friends but also as colleagues. It has been a dream. I could not live without you. To Pep: for always giving us a place to get away from it all. Also to T.C., Kelly and Jim, Sarah and Jay, Age and Eva, and the Lynah Faithful, here's to all of the amazing times ahead. I cannot wait.

"We can always look back at what we did ….
you know we could do better than anything that we did
you know that you and me we could do anything" - Dave Matthews

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AUC	Area under the curve
ADF	Acid detergent fiber
BCS	Body condition score
BHBA	β-hydroxybutyrate
BW	Body weight
CP	Crude protein
CR	Clearance rate
DMI	Dry matter intake
ELISA	Enzyme-linked immunosorbent assay
FAS	Fatty acid synthase
FD	Feed-deprived
GTT	Glucose tolerance test
HEC	Hyperinsulinemic-euglycemic clamp
HSL	Hormone sensitive lipase
IC	Insulin challenge
IRS	Insulin receptor substrate
Jak2	Janus kinase 2
LARTU	Large animal research and teaching unit
LPL	Lipoprotein lipase
MAPK	Mitogen-activated protein kinase
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
PEPCK	Phosphoenolpyruvate carboxykinase
PPAR	Peroxisome proliferator-activated receptor

RQUICKI	Revised quantitative insulin sensitivity check index
RT-PCR	Reverse transcriptase-polymerase chain reaction
STAT3	Signal transducer and activator of transcription 3
TNFα	Tumor necrosis factor-α
TZD	Thiazolidinedione

CHAPTER 1: INTRODUCTION

The overall profitability and health of a dairy cow benefits from successful management during the transition period. The metabolic adaptations that support the onset of lactation include increased mobilization of fatty acids from adipose tissue and increased hepatic gluconeogenesis (Bell, 1995). Orchestration of nutrient partitioning is necessary to meet new metabolic demands coinciding with a simultaneous decrease in dry matter intake around calving, which requires mobilization of body fat reserves (Bauman and Currie, 1980). These responses increase circulating non-esterified fatty acids (NEFA), which when taken up by the liver in excess generally result in triglyceride accumulation and increase the risk for fatty liver and other disorders such as ketosis, milk fever, retained placenta, and mastitis (Goff and Horst, 1997).

Insulin resistance during pregnancy and early lactation is a normal homeorhetic adaptation, the purpose of which is to spare glucose for the gravid uterus and mammary gland (Bauman and Currie, 1980). While some mobilization of adipose tissue is necessary to support lactation, some animals will experience excessive mobilization which is detrimental to the metabolic health of dairy cattle, and is a major concern for the dairy industry (Drackley, 1999). Cows that are overfed and gain excessive body condition during the dry period often experience prolonged negative energy balance in early lactation (Douglas et al., 2006; Dann et al., 2006). Additionally, overfed cows were also determined to have increased insulin resistance (Holtenius et al., 2003).

Although previous research has focused on the role of the liver during the transition period (Drackley et al., 2001), regulation of adipose tissue metabolism may be an appropriate approach to understanding transition cow metabolism. It may be possible to regulate adipose tissue mobilization without compromising production.

Previous work has demonstrated that administration of insulin sensitizing agents to prepartum dairy cattle can have positive effects on transition cow metabolism (Smith et al., 2007; 2009). The mechanisms of these responses are yet to be determined.

Therefore, one objective of this work was to further elucidate the role of insulin resistance in dry cows and its potential influence on transition cow metabolism. Furthermore, the influence of plane of nutrition on these responses was examined. An additional objective was to determine potential mechanisms for the effects of insulin sensitizing agents observed in transition cows. In order to meet these objectives, changes in glucose and fatty acid metabolism were investigated.

CHAPTER 2: LITERATURE REVIEW
THE ROLES OF PLANE OF NUTRITION, INSULIN SIGNALING, AND ADIPOSE TISSUE IN TRANSITION COW METABOLISM

Introduction

The overall profitability and health of a dairy cow benefits from successful management during the transition period (Drackley, 1999). The metabolic adaptations that support the onset of lactation include increased mobilization of fatty acids from adipose tissue and increased hepatic gluconeogenesis (Bell, 1995). Orchestration of nutrient partitioning is necessary to meet new metabolic demands coinciding with a simultaneous decrease in dry matter intake around calving, which requires mobilization of body fat reserves (Bauman and Currie, 1980). These responses increase circulating NEFA, which when taken up by the liver in excess generally result in triglyceride accumulation and increase risk for fatty liver and other disorders such as ketosis, milk fever, retained placenta, and mastitis (Goff and Horst, 1997).

Insulin is a protein hormone secreted by the β-cells of the pancreas which stimulates translocation of glucose transporters such as GLUT4, resulting in glucose uptake (Sasaki, 2002). In ruminants, volatile fatty acids from the gastrointestinal tract are the major energy sources for oxidative metabolism and biosynthesis rather than glucose per se. Thus, insulin plays a slightly different role in ruminants versus nonruminants, though many aspects of insulin function are the same. Over the past two decades, the concept of insulin resistance has garnered much research attention with the increasing prevalence of obesity, Type II Diabetes, and metabolic syndrome in humans (Mokdad et al., 2003). Insulin resistance can involve changes in sensitivity (the amount of hormone required to elicit a response) and/or responsiveness (the

maximum response to a hormone; (Kahn, 1978)). As in humans, insulin resistance in ruminants is potentially related to changes in both sensitivity and responsiveness. For the purpose of this review, the term insulin resistance will be used with the implication that the relative importance of sensitivity or responsiveness is not known in most cases. The role of insulin resistance in ruminants may prove to be an important metabolic regulator or potential intervention point for management.

Insulin resistance of peripheral tissues is a normal homeorhetic adaptation to late pregnancy and early lactation (Bauman and Currie, 1980; Vernon, 1989). The purpose of insulin resistance of peripheral tissues is to spare glucose for the gravid uterus and eventually milk production (Bauman and Currie, 1980; Bell and Bauman, 1997). Early lactation is characterized by decreased lipogenesis and increased lipolysis in order to meet the needs for the rapid increase in milk production, which is in part controlled by insulin resistance of adipose tissue (Bauman and Currie, 1980; Vernon and Pond, 1997). However, when excessive mobilization of adipose tissue occurs and the animal is in a period of prolonged negative energy balance, there can be negative effects on metabolic health (Drackley, 1999; Drackley et al., 2001)

Excessive insulin resistance may help to explain why cows that enter the transition period have greater risk for metabolic disorders. It has been shown that cows overfed during the dry period lose more body weight (BW), experience a more dramatic decrease in dry matter intake (DMI), and have higher circulating NEFA around calving (Douglas et al., 2006; Dann et al., 2006). In cows fed at 178% of predicted energy requirements prepartum, Holtenius et al. (2003) implicated increased insulin resistance (as measured by decreased clearance rate of glucose following glucose infusion) as a mechanism for increased lipolysis resulting in greater NEFA and body condition score (BCS) loss post-calving. Since these discoveries, research has concentrated on dietary strategies to mitigate transition cow problems potentially

related to insulin resistance (Dann et al., 2005; Douglas et al., 2006; Dann et al., 2006). It is clear that fundamentally different approaches to management focusing on the biology of the cow may be beneficial for improvement of transition cow health and performance related to nutrition during the dry period.

Modulation of insulin resistance in periparturient dairy cattle independent or in coordination with altered dietary energy has included the use of chromium as a dietary supplement (Spears, 2010). Chromium supplementation has improved insulin sensitivity as measured by improved responses to glucose infusions, metabolic health and production variables (Yang et al., 1996; McNamara and Valdez, 2005; Spears et al., 2010; Spears, 2010). More recently, the use of a pharmacological insulin sensitizer employed in human diabetes treatment has been investigated in ruminants (Kushibiki et al., 2001; Smith et al., 2007; Smith et al., 2009). A pharmacological intervention used in humans to improve insulin resistance are the thiazolidinediones (TZD) (Yki-Jarvinen, 2004). The overall mode of action of TZD is up-regulation of peroxisome proliferator-activated receptor-γ (PPARγ), which has been shown to be abundant in adipose tissue (Houseknecht et al., 2002; Hammarstedt et al., 2005). The first use of the insulin-sensitizing agent TZD in ruminants was in steers with insulin resistance induced by administration of tumor necrosis factor-α (TNFα) (Kushibiki et al., 2001). Subsequent studies in periparturient dairy cattle suggested potential for use of prepartum TZD treatment to improving insulin signaling (Smith et al., 2007; 2009).

Adipose tissue plays an important role in metabolic regulation of the transition from late pregnancy to early lactation. In addition to regulation of enzymes responsible for lipolysis and lipogenesis, adipose tissue hormones and cytokines such as leptin, adiponectin, TNFα, and others are secreted from the tissue and have metabolic effects throughout the body. It is possible that these mechanisms are factors

in the apparent dysregulation that occurs in overfed or over-conditioned dry cows. Though much attention has been focused on liver and other tissues (Bell, 1995; Drackley et al., 2001), the role of adipose tissue in metabolic regulation of transition cows may be critically important.

Thus, questions still exist regarding the interactions of insulin resistance, the role of plane of nutrition, and adipose tissue during the dry and transition periods. How does metabolic regulation of each of these affect the success of an animal through calving and early lactation? If these relationships can be more clearly defined, there may be potential to target the use of insulin-sensitizing agents during the transition period based on BCS, energy status, or other measures.

Metabolic adaptation during the transition period

The transition period has been defined as the three weeks preceding and following calving (Grummer, 1993), but the implications from a management perspective begin well before that. Once a cow is dried off, the process of preparing for the next lactation begins. As the dry period progresses, the growing fetus will eventually increase metabolic demands on the dam. The shifts in metabolic priorities and regulatory changes that occur during both pregnancy and the onset of lactation are examples of homeorhesis (Bauman and Currie, 1980). In this manner, metabolic set points change and nutrient partitioning adjusts accordingly.

During the dry period, fetal growth is not a major nutrient drain until the third trimester during which mammary cell proliferation and differentiation is also at its peak (Bauman and Currie, 1980). In animals carrying multiple offspring, this metabolic drain can be quite significant and results in metabolic disorders such as pregnancy toxemia in ewes (Vernon et al., 1981). In dairy cattle, especially with

successful management of nutrition during the dry period, occurrence of metabolic disorders during late gestation is rare. Instead, the dramatic increase in metabolic demand in dairy cattle occurs after calving when milk production doubles energy demands within a few days during the immediate periparturient period (Drackley et al., 2001).

Cows during the far-off dry period (generally considered to be from dry off at approximately 60 d before expected parturition until three to four weeks before expected calving) are experiencing relatively low metabolic demands as fetal growth is just beginning to accelerate and cows are no longer lactating. However, it is known that glucose turnover is faster in pregnant ruminants (0.32 g/hr/kg$^{3/4}$ for pregnant ewes vs. 0.24 g/hr/kg$^{3/4}$ for nonpregnant ewes (Bergman et al., 1974)). The bulk (86%) of the glucose turnover is accounted for by hepatic gluconeogenesis (Bergman et al., 1974). Fetal and uterine tissues are insulin-independent and so as energy demands increase with days of pregnancy, maternal peripheral tissues will need to become more insulin resistant, reducing dependence on glucose utilization in order to support fetal growth (Bell, 1995). Investigation of mRNA levels for various glucose transporters correlate with these responses. Insulin-independent GLUT1 is the major glucose transporter in the mammary gland, and its mRNA expression is increased significantly during lactation compared to the dry period (Komatsu et al., 2005). GLUT1 mRNA decreases substantially in adipose tissue during peak lactation, while GLUT4 adipose tissue mRNA expression stays relatively constant throughout lactating and nonlactating periods (Komatsu et al., 2005). Given these results, the authors concluded that insulin concentration and GLUT4 activation were important during lactation, but it would be interesting to speculate on the implications during the prepartum period (Komatsu et al., 2005). These changes in the role of insulin likely have effects on energy metabolism in dry cows.

Metabolic adaptations in periparturient dairy cattle become most obvious during the last three weeks prior to calving. Adaptations include reduced glucose utilization by peripheral tissues and increased hepatic gluconeogenesis (Bell and Bauman, 1997). These adaptations occur in the face of potentially decreasing DMI (Ingvartsen and Andersen, 2000; Hayirli et al., 2002). It is still unclear what the major factors regulating intake are during this time, but it is likely a combination of physical and metabolic signals. Hayirli et al. (2002) summarized dry matter intake data from 49 different diets within 16 experiments and determined that 56.1% of variation in DMI can be attributed to day of gestation. As reviewed by Allen et al. (2005), there is evidence for increased propionate supply, increased concentrations of NEFA, and gut peptides that may serve to decrease DMI. Increased propionate and fatty acid supply to the liver (such as when high starch diets are fed or when mobilization of adipose tissue is high) is thought to reduce intake through increased hepatic oxidation (Allen, 2000; Oba and Allen, 2003). Secretion of gut peptides may reduce gut motility or have indirect influence on insulin, reducing intake (Choi et al., 2000; Benson and Reynolds, 2001). Though now thought to be previously overemphasized, physical fill was suggested to limit intake in late pregnancy in the transition cow (Stanley et al., 1993; Ingvartsen and Andersen, 2000). Based on more recent evidence, it appears that the coordination of oxidation of metabolic fuels coupled with signaling of other peptides result in central nervous system regulation of intake (Ingvartsen and Andersen, 2000).

Briefly, orchestration of nutrient partitioning is necessary to meet new metabolic demands. Increased demands coupled with decreased DMI requires mobilization of body fat reserves (Bauman and Currie, 1980). These responses increase circulating NEFA, which when taken up by the liver in excess of rates of oxidation and export as very-low density lipoproteins induce triglyceride accumulation

and increased risk for fatty liver and other disorders such as ketosis, retained placenta, and mastitis (Goff and Horst, 1997). Additionally, a proportion of the rise in circulating NEFA may be independent of energy status (Bertics et al., 1992; Grummer, 1995). Bertics et al. (1992) determined that cows force-fed via rumen cannula in the days leading up to calving had lower liver triglycerides as well as increased milk yield during the first 28 d of lactation, thus underscoring the importance of maintaining adequate DMI during the prepartum period. However, force-feeding did not entirely mitigate the rise in circulating NEFA observed during the transition period, suggesting additional mechanisms for NEFA responses (Bertics et al., 1992).

The shift in demand during late pregnancy and early lactation occurs via coordinated responses in different tissues. These changes in tissue responses serve to spare glucose for the gravid uterus and eventually the mammary gland during early lactation (Bell and Bauman, 1997). Tissues involved include the liver, adipose tissue, and muscle. The end results are increased hepatic gluconeogenesis, increased NEFA mobilization from adipose tissue, and decreased use of glucose by peripheral tissues (Bell and Bauman, 1997). Modulation of insulin pathways is a large factor in many of these adaptations.

Adaptations related to insulin include changes in plasma concentration as well as downstream effects. Vernon et al. (1981) reported that both plasma insulin concentrations and the number of insulin receptors on adipocytes were decreased as ewes exceeded 100 d of pregnancy. These metabolic changes corresponded with loss of adipose tissue, which is consistent with the increased lipolysis that is greater during late pregnancy than early lactation in ewes. Although the timing of adipose tissue mobilization relative to parturition differs in sheep and cattle, the mechanisms for regulation are likely to be similar. As already mentioned, there are also tissue-dependent changes in responses to insulin. Figure 2-1 illustrates the coordinated

regulation occurring during the transition period, and the role of insulin in many of these effects is notable.

Insulin activity and peripheral tissues

Insulin action varies by tissue type. Resistance of tissues to insulin can be attributed to changes in sensitivity, responsiveness, or both. Decreased sensitivity occurs when a greater than normal concentration of hormone is required to elicit a normal response and is related to changes in receptor number (Kahn, 1978). Decreased responsiveness suggests changes in the maximal response to a hormone, or changes downstream of receptor-binding (Kahn, 1978). Figure 2-2 from Kahn (1978) illustrates that the normal dose-response curve is shifted to the right in cases of reduced sensitivity, and shifted downward in the case of reduced responsiveness. The distinction between changes in sensitivity and responsiveness is necessary to appropriately describe the mechanisms underpinning the changes in response to insulin, though at times the specific mechanisms are hard to distinguish from phenotypic responses. Based on current literature, in ruminants it is often the case that the exact proportions of insulin resistance that can be allocated to differences in sensitivity or responsiveness is not known. Therefore, unless data can prove otherwise, the general term “insulin resistance” is assumed to involve elements of both.

Additionally, tissues themselves may either be insulin-dependent or independent depending on the presence or absence of insulin-responsive glucose transporters. For example, placental tissue contains insulin-independent glucose transporters GLUT1 and GLUT3 almost exclusively, and the mammary gland contains insulin-independent transporter GLUT1, meaning that glucose uptake by these cells

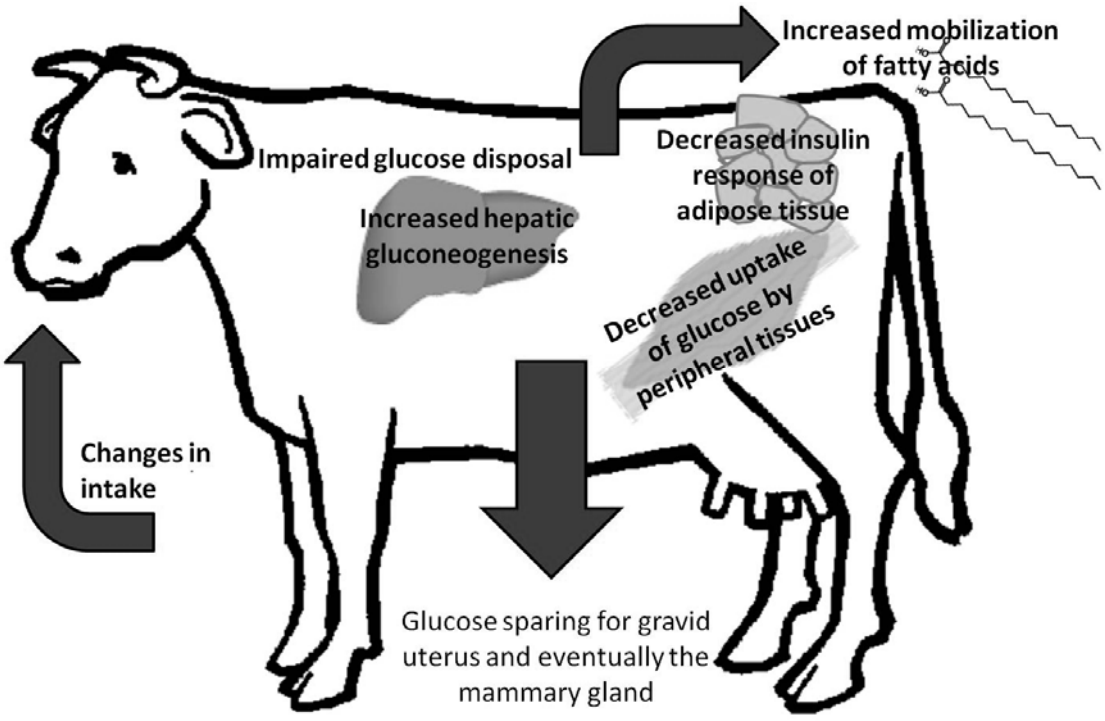

Figure 2-1. Metabolic adaptation during the transition period.

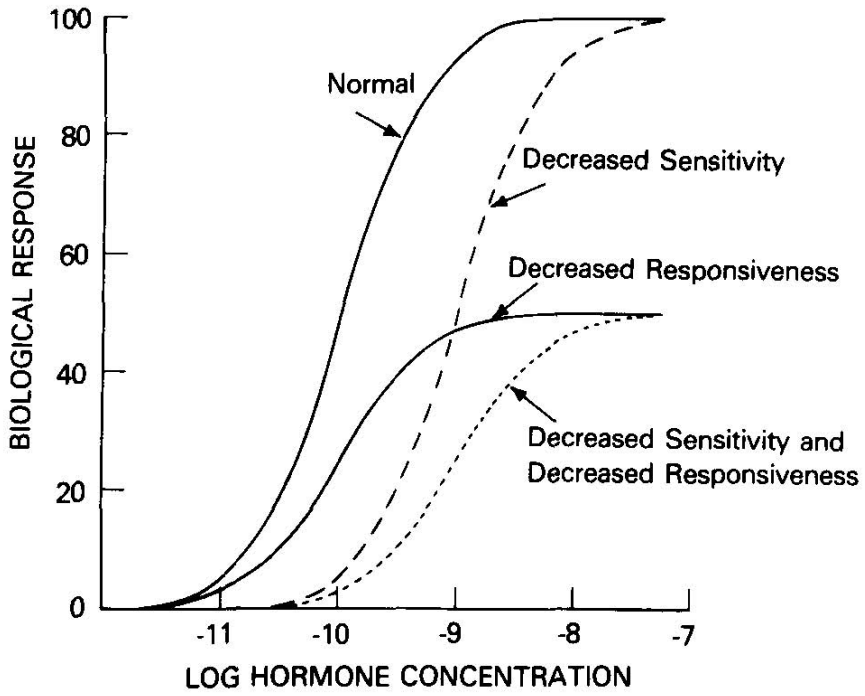

Figure 2-2. Types of resistance to hormone action. From (Kahn, 1978).

will not be mediated by insulin (Bell and Bauman, 1997). Whole-body insulin resistance, therefore, depends on signaling within tissues that are responsive to insulin such as liver, skeletal muscle, and adipose tissue. The term insulin resistance is further generalized at the whole-body level although quantitative aspects of resistance may be different within specific tissues.

In the liver, insulin inhibits gluconeogenesis and therefore the hypoinsulinemia characteristic of early lactation favors hepatic gluconeogenesis (Vernon, 1989). This is another mechanism in order to ensure adequate glucose supply for the mammary gland. Observed in nonruminants, insulin inhibits gluconeogenesis in the liver via suppression of phosphoenol pyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (Barthel and Schmoll, 2003). Though the liver will increase its production of glucose in early lactation to increase glucose supply (Reynolds et al., 2003), absorbed gluconeogenic precursors account for approximately 65% of average mammary glucose uptake (Bell, 1995). The remaining gluconeogenic precursors are accounted for via mobilization of body tissue [adipose tissue and skeletal muscle; (Bell, 1995)].

In ruminants, changes in insulin resistance within skeletal muscle appear to be related to changes in levels of GLUT4 transporter (Petterson et al., 1993). Regardless of mechanism, the result of having tissues such as the mammary gland insensitive to insulin while other insulin-dependent tissues (muscle, adipose) reduce sensitivity ensures that nutrients are properly partitioned to meet increased demands in late pregnancy and early lactation.

Insulin activity, fatty acid metabolism, and adipose tissue

Additional roles of adipose tissue in metabolic regulation during the transition period will be discussed in later sections while the specific relationship between

insulin signaling and adipose tissue is most relevant in this section. Insulin promotes fatty acid synthesis and lipid accretion in adipose tissue. In the transition from late pregnancy to early lactation, not only do circulating concentrations of insulin decrease, but adipocytes also become insulin resistant (Bell and Bauman, 1997). The end results during the third trimester are increased transcription of enzymes such as lipoprotein lipase (LPL), acetyl CoA carboxylase, and increased activity of pyruvate dehydrogenase and stimulation of glucose transport (Vernon and Pond, 1997). Increased expression of hormone sensitive lipase (HSL) has also been observed in early lactation (Vernon et al., 1981). Similarly, in humans with gestational diabetes there is reduced ability of insulin to suppress NEFA as gestation progresses (Catalano et al., 2002). The end result is mobilization of body reserves and a rise in circulating NEFA.

While the overall effects of decreased lipogenic and increased lipolytic activity of adipose tissue are to supply milk fat precursors to the mammary gland and to provide oxidative fuels to tissues during negative energy balance (Pullen et al., 1989), there can be negative consequences throughout the body. Primarily, the liver is affected if uptake of fatty acids exceeds the liver's ability to oxidize them or export them in the form of very low density lipoproteins (Bobe et al., 2004). The resulting accumulation of triglycerides in the liver, known as fatty liver, decreases the gluconeogenic capacity of the liver (Drackley et al., 2001; Bobe et al., 2004) . There are several control points, as described by Drackley (1999), that result in fatty liver: delivery of NEFA to the liver, uptake of NEFA into mitochondria via carnitine palmitoyltransferase I (CPT-1), regulation of mitochondrial ketogenesis, regulation esterification steps, portioning of newly synthesized triglycerides between secretion and storage, and export of very low density lipoproteins. One way to improve the metabolic health of transition dairy cattle, and especially of those over-conditioned

during the dry period, would be to decrease the delivery of NEFA to the liver while maintaining milk production. Modulation of insulin signaling could be a key factor.

An additional approach to decrease triglyceride accumulation in liver would be to improve the liver's ability to oxidize or export liver triglycerides; several possibilities have been proposed in the literature. Fatty acid oxidation in the liver is inhibited by propionate and insulin and stimulated by carnitine (Jesse et al., 1986a; Jesse et al., 1986b). Limited choline supply reduces the liver's ability to secrete fatty acids in the form of very low density lipoproteins (Santos and Lima, 2009). Rumen protected choline has been used in an attempt to improve fatty liver in dairy cattle (Hartwell et al., 2000; Piepenbrink and Overton, 2003; Cooke et al., 2007). Pipenbrink and Overton (2003) reported that choline supplementation increased liver glycogen concentrations and decreased capacity for esterification of fatty acids in liver slices. Cooke et al. (2007) determined that choline supplementation decreased liver triglycerides in cows with dietary-induced fatty liver. However, Hartwell et al. (2000) observed that the effects of choline supplementation were dependent upon dietary rumen undegradable protein supply as well as BCS prepartum. More recently, the use of clofibrate in liver slices from weaned calves suggested that the use of a PPARα agonist may decrease triacylglycerol content due increased expression of genes regulating fatty acid oxidation (Litherland et al., 2010).

Insulin may have greater influence on triglyceride accumulation in the liver through its ability to reduce fatty acid supply to the liver. Andersen et al. (2002) subjected cows to hyperinsulinemic-euglycemic clamps (HEC) in order to determine the effects of insulin on hepatic lipidosis based upon stage of lactation. Based on decreased plasma NEFA levels and lower triglyceride content of liver in cows administered insulin, the authors concluded that the anti-lipolytic effect of insulin on adipose tissue was more important than insulin's ability to alter hepatic long-chain-

fatty acid oxidation (Andersen et al., 2002). This would support the concept that effects on transition cow health may be greater if efforts focused on adipose tissue metabolism and reducing the supply of NEFA to the liver.

Interestingly, cows with higher BCS prepartum experience more dramatic decreases in DMI during the transition period and thus have higher NEFA (Agenas et al., 2003; Dann et al., 2006). In a meta-analysis of over 5,000 cows, Garnsworthy (2006) determined that the optimal BCS at calving is 2.1-2.5 and that a BCS > 2.5 results in greater loss in BCS post-calving. Likewise, Bernabucci (2005) found that cows that calved at a BCS > 3.0 had higher NEFA and oxidative stress. This provides support that it is the oxidation of fuels, and thus circulating levels of NEFA that are a regulator of intake and metabolism in transition dairy cattle. In this manner, cows that have higher BCS at calving, have higher circulating NEFA concentrations and thus have a more dramatic decrease in intake resulting in further loss of body condition. Thus, many began investigating the role of plane of energy during the dry period on transition cow metabolic health, more examples of which are found in more detail in the next section.

Effects of plane of nutrition during the dry period

The NRC determines energy requirements during the dry period based on maintenance and pregnancy requirements (NRC, 2001). Energy for maintenance is defined as 0.08 Mcal/kg $BW^{0.75}$(NRC, 2001). Energy requirements for pregnancy in late gestation are assumed to be dependent mostly on day of gestation (and efficiency of energy use by the gravid uterus) but also calf birth weight (Bell, 1995; NRC, 2001).

The equation for calculated energy requirement for pregnancy is:

$$\text{Term B } (NE_L/\text{day}) = \frac{[(0.00318 \times \text{day} - 0.0352) \times (\text{calf birth weight}/45)]}{0.218}$$

Therefore, the final energy requirement during pregnancy is the sum of both the energy required for maintenance (Term A) and the energy requirement of pregnancy (Term B). Animals consuming energy above and beyond that necessary to support maintenance and pregnancy could be consuming excess energy, are overfed, or at a high plane of nutrition.

In the past ten years researchers have become increasingly aware of the effect of feeding management during the dry period on transition dairy cattle. Management involves not only monitoring intake and energy level of the diet, but the timing of different nutritional strategies during the dry period. Historically, many believed energy intake should be maximized during the dry period (NRC, 2001). Curtis et al. (1984; 1985) reported improved performance and decreased correlation with metabolic disorders in cows fed higher planes of nutrition prior to calving. However, the idea that cows should be fed a high energy "steam-up" ration just prior to calving has slowly begun to lose ground (Drackley et al., 2006).

It was shown that cows fed high energy diets during the dry period are more likely to have fatty liver after parturition, experience a more dramatic decrease in intake after calving, and have higher incidences of metabolic disorders in early lactation (Rukkwamsuk et al., 1998; Rukkwamsuk et al., 1999a; Agenas et al., 2003; Dann et al., 2006). Furthermore, Dann et al. (2006) determined that dietary energy level during the far-off dry period has a significant effect on metabolism during the transition period regardless of close-up dry cow nutrition. In cows that were fed a far-off dry cow diet that met 80%, 100% or 150% of NRC energy requirements and were then switched to a close-up diet fed at *ad libitum* or restricted levels, the cows that

were overfed during the far-off period had higher NEFA and lower DMI postpartum independent of close-up diet (Dann et al., 2006). Furthermore, cows that were restricted to only 80% of predicted NE_L in their close-up diets had higher NEFA prepartum but higher energy balance in the first 56 days in milk (Dann et al., 2006). Many of these responses are likely related to changes in insulin signaling.

Insulin resistance as a potential mediator

The mechanism for the effects of dry period nutrition on the metabolic health of cows as they approach calving and early lactation needs investigation. Analysis of adipose tissue collected from cows fed at restricted and excessive energy levels during the dry period revealed that adipose tissue from overfed cows had higher rates of esterification (Rukkwamsuk et al., 1999b). Overfed cows also had greater rates of lipolysis post-calving than those fed restricted diets (Rukkwamsuk et al., 1999b). They concluded that adipose tissue from overfed cows had a lower basal lipolytic rate prior to parturition and a higher rate postpartum compared to cows fed diets at restricted amounts (Rukkwamsuk et al., 1999b). As in dairy cattle, there is a decline in the ability of insulin to suppress NEFA during late gestation in humans (Catalano et al., 2002). Interestingly enough, the ability is more severely depressed when individuals have gestational diabetes. These humans with gestational diabetes also have decreased activation of PPARγ, and greater NEFA concentrations (Catalano et al., 2002).

Overfed cows (consuming 178% of calculated energy requirements for eight weeks prior to calving) also had increased insulin responses to glucose challenge three weeks prior to calving, suggesting that overfed cows are more insulin resistant (Holtenius et al., 2003). Additionally the overfed cows had a 20% slower glucose clearance rate measured three weeks postpartum. This implicates postpartum insulin

resistance which the authors suggest contributed to these cows having higher NEFA and greater BCS loss postpartum (Holtenius et al., 2003). Likewise, over-conditioned ewes had decreased insulin sensitivity as measured by the HEC technique (Bergman et al., 1989). Dann et al. (2006) determined that cows fed 150% of NRC energy requirements during the far-off dry period had higher NEFA and β-hydroxybutyrate (BHBA) and lost more body weight postcalving despite having higher insulin levels during the dry period. In this way, the adipose tissue of overfed cows is geared up for lipogenesis in the dry period and will switch to excessive rates of lipolysis post-calving. Higher rates of lipolysis lead to higher circulating NEFA and eventually fatty liver (Van den Top et al., 1995). Additionally, higher NEFA serve as an additional signal to decrease DMI in early lactation, further driving down negative energy balance (Emery et al., 1992; Allen, 2000).

It is also likely that increased adipose deposition in ruminants results in changes in regulation of adipose tissue hormones related to insulin resistance such as leptin, TNFα, adiponectin, and others. For example, Holtenius et al. (2003) reported that overfed cows had higher circulating levels of leptin, which is a regulator of energy metabolism and feed intake. Briefly, but to be discussed in further detail, leptin serves as signal of adiposity in many species, and does so via activation of the central nervous system (Vernon et al., 2001; Ingvartsen and Boisclair, 2001). Also to be discussed are the potential positive effects of adiponectin on insulin action, as well as the negative effects of TNFα on insulin action (Arner, 2003; Kadowaki et al., 2006). An additional factor from adipose tissue in ruminants is resistin. Resistin increases insulin resistance and it was shown by Komatsu et al. (2003) that lactating animals have 13-fold greater resistin mRNA expression in adipose tissue versus non-lactating animals. These adipokines may serve as links between overfed cows, insulin resistance, and metabolic disorders.

The use of insulin-sensitizing agents

Many of the effects discussed related to oxidative metabolism, intake regulation, and overall metabolic health are related to insulin action. Chromium deficiency has been linked to insulin resistance and treatment of cows with chromium may be a mediator of insulin resistance during the transition period (Hayirli et al., 2001; Sumner et al., 2007; Spears, 2010). Lower plasma insulin concentration following a glucose tolerance test (GTT) suggested that growing heifers were more insulin sensitive when they were fed diets supplemented with chromium propionate (Spears et al., 2010). The effects of chromium on insulin sensitivity are partially mediated through increased sensitivity of adipose tissue resulting in greater lipogenic rates and a tendency for decreased lipolysis (McNamara and Valdez, 2005). Chromium propionate supplementation was shown to increase lipogenesis in adipose tissue and reduce lipolysis in periparturient dairy cattle (McNamara and Valdez, 2005). In growing heifers, chromium propionate supplementation increased basal glucose concentrations and decreased basal insulin and NEFA concentrations, as well as increased glucose clearance rate following GTT (Sumner et al., 2007). Older cows with higher BCS were shown to benefit from chromium supplementation as measured by decreased circulating BHBA and NEFA, which the authors concluded may result in lower rates of metabolic disorders (Yang et al., 1996). There may be opportunity to explore additional insulin-sensitizing agents.

The period of mild to moderate insulin resistance during late pregnancy in dairy cattle may not be unlike humans in late gestation (Catalano et al., 2002). Insulin-sensitizing agents have been used for the treatment of Type II diabetes in humans for a number of years (Hammarstedt et al., 2005). In Type II diabetes, rather than an inability to properly produce insulin, individuals have tissue resistance to

insulin resulting in a higher concentration of insulin required to elicit a normal response. Common drugs used are those in the family of TZD (Guo and Tabrizchi, 2006). The TZDs are synthetic ligands for PPARγ; natural ligands include unsaturated fatty acids and eicosanoids (Stienstra et al., 2006). In general, PPARs are ligand-activated transcription factors which are involved in energy status and also modulate inflammatory responses (Yki-Jarvinen, 2004). Abundant in adipose tissue, PPARγ is a major regulator of adipogenesis (Stienstra et al., 2006). It has been characterized and found to be highly expressed in adipose tissue of ruminants as well (Sundvold et al., 1997; Harvatine and Bauman, 2007).

When a ligand binds to PPARγ, heterodimerization with retinoid X receptor occurs and there is a conformational change that leads to repressor release and activator binding which then allows PPARγ to interact with several transcription factors (Hammarstedt et al., 2005; Guo and Tabrizchi, 2006). Example transcription factors that bind PPARγ include CAAT/enhancer binding protein responsible for PPARγ's role in adipocyte differentiation and sterol response-element binding protein 1 responsible for changes in fatty acid metabolism (Hammarstedt et al., 2005). Within adipocytes, PPARγ activation upregulates fatty acid-binding protein, acyl-CoA synthase, and LPL and represses TNFα, leptin, and other genes implicated in insulin resistance (Guo and Tabrizchi, 2006). Upregulation of PPARγ has several effects, as shown in Table 2-1, which ultimately lead to an overall increase in insulin sensitivity.

Thus, it is clear that TZD-effects on insulin sensitivity can be attributed to direct [i.e., improved β-cell function; (Gastaldelli et al., 2007)] or indirect mechanisms (i.e., adipose tissue remodeling and altered levels of adipokines). Treatment of nonruminants with TZD caused a restructuring of adipose tissue such that there were a greater number of smaller adipocytes that are more insulin-sensitive (Arner, 2001). Related effects of this remodeling are changes in adipokine secretion and activity. For

Table 2-1. Effects of PPARγ upregulation.

Increased adipogenesis and adipose tissue remodeling:	Additional effects:
Increases in lipogenic genes (LPL, PEPCK)	Increased intake
Decreases in lipolysis	Decreased circulating NEFA
Increase in small adipocytes	Inhibition of TNFα, leptin, resistin
Decreased visceral adipose tissue	Increased adiponectin
Increased mitochondrial mass in adipocytes	Improved pancreatic β-cell function

Adapted from (Picard and Auwerx, 2002)

instance, PPARγ activation increases adiponectin expression, and adiponectin is known to be lower in individuals who are Type II Diabetic (Maeda et al., 2001). Administration of TZD in humans increased mRNA for adiponectin by 1.72-times, decreased resistin by almost half, and decreased leptin by 28% in adipose tissue (Tonelli et al., 2004). Response to TZD treatment also varies based on specific adipose tissue depots. For example, visceral adipose tissue depots are decreased or not changed with TZD treatment while subcutaneous depots increase (Yang and Smith, 2007). Additionally, response to TZD treatment both *in vivo* and *in vitro* is greater when the ratio of visceral fat to subcutaneous adipose tissue is reduced (Yang and Smith, 2007). This is due to TZD's ability to increase adiponectin secretion from visceral fat (even when the volume of visceral adipose tissue deposits may be reduced), it's ability to control adipocyte cell size and distribution, and control lipolytic and lipogenic genes (Yang and Smith, 2007) .

It has been shown that the main effects of TZD treatment on insulin resistance are related to fatty acid esterification (Tordjman et al., 2003a). In rats, glyceroneogenesis accounts for 75% of the TZD-effect (Tordjman et al., 2003a). Through activation of this pathway, release of fatty acids to circulation is decreased since they are essentially now trapped in adipose tissue via upregulation of the cytosolic form of PEPCK. There may be additional positive effects of TZD-treatment during pregnancy. TZD treatment was shown to reduce the incidence of diabetes in women with a history of gestational diabetes as well as slow decline in β-cell function (Xiang et al., 2006). The positive impact TZD treatment has on fatty acid metabolism, as illustrated in applications such as gestational diabetes, suggests that it could serve as a potential therapy in transition cows.

Treatment with TZD also has been shown to have effects on energy balance. In a study of 20 males, energy balance for those receiving placebo was 72% of those

treated with TZD (Joosen et al., 2006) . Larsen et al. (2003) reported increased intake and feed efficiency resulting in increased weight gain in rats treated with TZD. Some of the effects of TZD on energy balance may be indirect, such as its effect on adipokine circulation. Type II diabetics treated with TZD experience increased body mass index concurrent with increased hunger (Shimizu et al., 1998). These same subjects had decreased circulating leptin (Shimizu et al., 1998). Though increased weight gain might not be desirable in the situation of human therapy, improved energy status might be beneficial in the transition dairy cow in early lactation.

2, 4-thiazolidinedione treatment in ruminants

The first group to treat ruminants with TZD used it to reverse TNFα-induced insulin resistance (Kushibiki et al., 2001). After treating steers with TNFα, the treated animals had smaller glucose area under the curve (AUC) in response to an insulin challenge and insulin AUC was smaller following a glucose challenge suggesting that steers treated with TNFα were insulin resistant (Kushibiki et al., 2001). Treatment with TZD partially reversed the insulin resistance caused by TNFα administration as demonstrated by lower glucose and insulin responses to glucose and insulin challenges (Kushibiki et al., 2001). This paper illustrated the potential use of TZD in ruminants to alter insulin resistance. Additionally, TZDs appeared to enhance peripheral tissue sensitivity to insulin without dramatically affecting circulating levels of insulin.

In the first study with transition dairy cattle, cows were treated once daily from 25 d before expected parturition until parturition (Smith et al., 2007). The results of this initial trial indicated that cows treated prepartum with TZD had decreased circulating NEFA concentrations during the pre and peri-partum periods and increased DMI during the post-partum period (Smith et al., 2007). Additionally, cows in this study were administered an insulin challenge at two doses (0.8 μg/kg of BW and 2.4

μg/kg BW) both pre- and postpartum. There were no differences in responses to the insulin challenges based on TZD dose as measured by glucose and insulin AUC, which the authors attributed to a lack of effect of TZD treatment on glucose utilization in skeletal muscle as expected (Smith et al., 2007). The results of this study suggested the potential for the use of TZD in transition dairy cattle to mitigate the dramatic decrease in intake and concurrent rise in NEFA that occurs postcalving.

In a second study in transition dairy cattle, cows were treated with two levels of TZD (0, 2.0 and 4.0 mg/kg of BW) prepartum and followed through early lactation (Smith et al., 2009). As in the first TZD-transition cow study, plasma NEFA were lower in the postpartum period in TZD-treated cows and there was increased DMI in the cows during the peripartum period (Smith et al., 2009). There were also linear decreases in postpartum liver triglycerides and glycogen (Smith et al., 2009). Administration of TZD linearly increased BCS postpartum, suggesting more favorable energy balance in TZD-treated cows (Smith et al., 2009). As described, the results in this study are likely an effect of both direct and indirect effects of TZD administration. For instance, increases in DMI can not only be attributed to PPARγ activation, but also decreased NEFA.

The first published study involving ruminants and TZD administration illustrated the potential effects on insulin resistance in ruminants in general (Kushibiki et al., 2001). The second and third studies from our laboratory suggested the potential applied application of TZD administration in transition dairy cattle (Smith et al., 2007, 2009). The reason that TZD administration might be beneficial for transition dairy cattle is related to the changes in insulin signaling that occur during the transition period, as already described. Evidence that insulin resistance occurs in transition dairy cattle, especially those overfed during the dry period, in combination with results from ruminant TZD trials suggests opportunity for further exploration of the role of insulin

resistance in metabolic disorders. Furthermore, there may be opportunity to use TZDs to treat or prevent disease in these animals. Upregulation of PPARγ likely has its greatest effects in adipose tissue, and thus there is opportunity to explore the specific roles of adipose tissue in these situations.

The role of adipose tissue

Excessive adiposity has been linked strongly with Type II Diabetes and metabolic syndrome (Stienstra et al., 2006). Additionally, the location of adipose tissue depots is correlated with insulin resistance. Visceral (vs. subcutaneous) adipose tissue depots release more NEFA, with more direct access to the liver, and secrete more adipokines (Arner, 2003; Sharma and Staels, 2007). In 18 non-pregnant, non-lactating cows fed either low or moderate levels of energy for 56 d, cows that were fed the moderate energy level had upwards of 75% increase in omental and mesenteric adipose tissue despite no significant differences in BCS (Nikkhah et al., 2008). This suggests that rather than changes in BCS alone, changes in adipose tissue depot size and architecture may be factors in overfed cows. Adipocyte size also appears to relate to insulin resistance in that large adipocytes are more insulin resistant, release more inflammatory cytokines, less adiponectin, and are found in higher proportion in insulin-resistant individuals (Yang and Smith, 2007). Many of the genes involved in fatty acid metabolism as well as the hormones secreted from adipose tissue have already been discussed as being implicated in insulin resistance. What additional roles does adipose tissue play in the development of insulin resistance, especially in transition dairy cattle?

Regulation of lipolysis and lipogenesis

There are a few major genes implicated in regulation of fat metabolism in adipose tissue of dairy cattle. Lipoprotein lipase is the rate-limiting factor in fatty acid uptake the endothelium from very low density lipoproteins and chylomicrons. Cows and ewes that were feed-restricted and then refed had large responses in LPL (19-25-fold increase) and fatty acid synthase (FAS; 6-8-fold increase) mRNA levels after refeeding, but less dramatic responses in hormone sensitive lipase (HSL; slight increase in feed-restricted cows, no effect in ewes) (Bonnet et al., 1998). Activity of LPL is highly correlated with mRNA level, though the relative activity is much lower than in nonruminant species (Hocquette et al., 1998). Treatment with TZD increases LPL expression in white adipose tissue of mice and humans (Kageyama et al., 2003; Bogacka et al., 2004). Fatty acid synthase, involved in de novo fatty acid synthesis, also is increased by TZD administration (Bogacka et al., 2004). It is not surprising that key genes involved with fat storage are regulated by TZDs, given the responses seen in fat metabolism and the reduction of circulating fatty acids.

The balance of lipolysis versus lipogenesis in adipose tissue is a large factor in regulating circulating NEFA levels. Expression of HSL mRNA in adipose tissue increases with the onset of lactation and is a key enzyme involved in fat mobilization (Sumner and McNamara, 2007). Lipogenic rates in adipose tissue of dairy cattle in early lactation can be almost 40 times lower than rates in mid to late lactation while lipolytic rates are also lower (McNamara and Hillers, 1986a; McNamara and Hillers, 1986b). Furthermore, effects on lipogenic rates are more related to energy balance whereas lipolytic rates seem to vary more based on level of milk production (McNamara and Hillers, 1986a; McNamara and Hillers, 1986b). More recently, cows fed moderate versus low levels of dietary energy has higher expression of genes related to lipolysis such as HSL (Ji et al., 2010). Insulin activates enzymes involved in

lipid synthesis (LPL, FAS) and downregulates those related to lipolysis (HSL), which corresponds with decreased concentration of insulin in ruminants in early lactation (Vernon et al., 1981). Resistance of adipose tissue to insulin therefore removes the negative signals insulin has upon lipolytic genes and the result is release of NEFA. Thus the relationship of adipose tissue lipogenesis and lipolysis and regulation via alteration in response to insulin is a potential area of research.

Adipose tissue also has an indirect effect on perpetuating insulin resistance through the release of these NEFA (Guo and Tabrizchi, 2006). Most importantly, excess NEFA from adipose tissue further increases insulin resistance. NEFA enter the cell via fatty acid transport proteins and result in increased fatty acid metabolism through fatty acyl-CoA and diacylglycerol (Morino et al., 2006). These changes upregulate protein kinase C, and the serine/threonine kinases. More serine/threonine phosphorylation of insulin receptor substrate (IRS) reduces activity, reducing the activity of PI_3 kinase activity. With less PI_3 kinase activity, glucose uptake is slowed, resulting in insulin resistance (Petersen and Shulman, 2006). Additionally, the excess NEFA from adipose tissue results in increased liver triglycerides, reducing the gluconeogenic capacity of the liver (Petersen and Shulman, 2006). Women with gestational diabetes had decreased IRS-1 as well as PPARγ (Catalano et al., 2002).

The same result of high NEFA is seen in dairy cattle. Dairy cattle with elevated NEFA in response to a 48-h fast had decreased response to glucose infusion and reduced rates of glucose clearance were reported (Pires et al., 2007a). The cattle with high NEFA were compared to cows that were treated with nicotinic acid in order to reduce plasma NEFA, and the nicotinic acid-treated cows appeared to be more insulin sensitive as measured by improved glucose clearance rates (Pires et al., 2007a). Furthermore, cows that were infused with tallow emulsion in order to induce hyperlipidemia also had increased insulin resistance as measured by impaired glucose

clearance during GTT (Pires et al., 2007b). This implicates high levels of NEFA during early lactation as a cause for more severe insulin resistance.

Excessive NEFA from adipose tissue as a result of insulin resistance can also have effects on insulin resistance of peripheral tissues. Increased circulating NEFA result in increased fatty acid oxidation by muscle and thus inhibits glucose metabolism (Schinner et al., 2005). Thus, in humans there is emphasis on alleviating excess fatty acids as a means of reducing insulin resistance (Delarue and Magnan, 2007). There may also be effects on glucose uptake via impairment of IRS and PI_3 kinase or increased serine phosphorylation (Schinner et al., 2005). In this manner, there is cross-talk between adipose tissue (more specifically, excessive lipolysis) and peripheral tissues upsetting normal glucose and fatty acid homeostasis.

Excessive lipolysis in adipose tissue also may have direct effects on the pancreas. In starved cows, there is noted absence of the normal biphasic insulin secretion in response to a glucose challenge, and an overall attenuated insulin response (Hove, 1978). Similarly, in ketotic cows, GTT administration resulted in lower insulin responses versus non-ketotic cows (Sakai et al., 1996). In ketotic cows subjected to both glucose and insulin challenges, there were effects of long-term hyperketonemia on insulin secretion and metabolism (Kerestes et al., 2009). Finally, in cows with metabolic acidosis achieved through adjustment of the dietary cation-anion difference, glucose utilization as measured by as reduced insulin response to GTT is altered (Bigner et al., 1996).

Much of the discussion has already focused on regulation of lipolysis and lipogenesis as it is relates to situations of over-feeding during the dry period. As stated, cows that are overfed during the dry period have altered adipose tissue metabolism causing them to eat less and lose more body condition post-partum. Much of this is linked to the imbalance of lipolysis vs. lipogenesis described above, and the

effects of high concentrations of NEFA on other tissues. Additional factors include hormones released from adipose tissue depots.

Leptin and other adipokines

The role of adipose tissue in relation to insulin during the transition periods has already been discussed; the effects of additional hormones and cytokines secreted from adipose tissue also play important roles during the transition period. The molecules include but are not limited to: leptin, adiponectin, TNFα, and resistin. While some hormones secreted from adipose tissue such as leptin serve as a marker of energy status and intake control, others such as adiponectin can have direct and indirect effects on insulin signaling.

Leptin and its role in ruminant metabolism has received much attention in recent years (Chilliard et al., 2001; Ingvartsen and Boisclair, 2001; Chilliard et al., 2005). Known as a signal of adiposity in many species, leptin has the ability to increase energy expenditure and protect tissues from accumulation of excess lipid (Vernon et al., 2001). It does so via activation of the central nervous system as mediated by cytoplasmic tyrosine kinase Janus kinase-2 (Jak2), IRS-I, the mitogen-activated protein kinase (MAPK) pathway, and the signal transducer and activator of transcription-3 (STAT3), all of which are related to insulin signal transduction (Ingvartsen and Boisclair, 2001).

In dairy cattle, leptin decreases as much as 50% less than two weeks prior to calving and remains low throughout early lactation (Block et al., 2001; Liefers et al., 2003). If the negative energy balance seen during early lactation is prevented by not milking cows, plasma leptin concentrations are twice that of cows in early lactation (Block et al., 2001). Plasma leptin has been shown to be responsive to periods of negative energy balance and refeeding (Chelikani et al., 2004).

A major factor regulating leptin during the transition period is likely to be insulin (Block et al., 2003). If insulin is a positive regulator of leptin, this may partially explain the consequent decrease in plasma leptin and insulin during the late prepartum period in dairy cattle (Block et al., 2003). Additionally, it appears that during the negative energy balance experienced during early lactation, the effects of leptin may be more on energy conservation than regulation of DMI (Leury et al., 2003). Furthermore, the ability of insulin to regulate leptin is decreased in early lactation as compared to late pregnancy (Leury et al., 2003). Leptin mRNA in adipocytes is decreased by TZD treatment (Hammarstedt et al., 2005). Leptin may therefore be a key hormone in the regulation of insulin resistance in transition cows with or without TZD administration.

Another adipokine implicated in insulin resistance is TNFα. Tumor necrosis factor-α is mainly secreted by macrophages but also by other tissue types including adipose tissue, and is involved in the acute phase response of inflammation (Carswell et al., 1975). The effects of increased TNFα are decreased lipogenesis, increased lipolysis, and increased circulating leptin concentrations (Arner, 2003). Additionally, TNFα also increases oxidative species in the pancreas and has negative effects directly on β-cell function (Greenberg and McDaniel, 2002). Tumor necrosis factor-α has been shown to be higher in cows with greater insulin resistance and fatty liver (Ohtsuka et al., 2001). As already mentioned, steers treated with TNFα had smaller glucose AUC in response to an insulin challenge, and insulin AUC was smaller following a glucose challenge suggesting insulin resistance (Kushibiki et al., 2001). The TZDs have been shown to affect TNFα activity by decreasing its circulating levels, antagonizing TNFα inhibition of insulin signaling, and reducing TNFα stimulation of lipolysis (Arner, 2003). The effects of TZD treatment on TNFα levels in dairy cattle are not known.

Adiponectin, which increases insulin sensitivity, is increased by TZD-treatment (Hammarstedt et al., 2005). Administration of TZD has also been shown to mitigate the reduction in plasma adiponectin that occurs prepartum in transition dairy cattle (Kim et al., 2008). Adiponectin is considered to be an insulin sensitizer because it activates pathways related to insulin sensitivity and also inhibits hepatic gluconeogenesis, resulting in reduced glucose concentrations (Kadowaki et al., 2006). Circulating concentrations of adiponectin were shown to be reduced in women with gestational diabetes (Ranheim et al., 2004). Though not studied extensively in ruminants, adiponectin receptors were reported by Lemor et al. (2009) to be decreased in dairy cattle post-calving while adiponectin mRNA was not different. More research is required for the full characterization of adiponectin in transition dairy cattle and the potential relationship with insulin resistance.

An additional factor from adipose tissue in ruminants is resistin, which is shown to have greater mRNA expression in lactating versus non-lactating dairy cattle (Komatsu et al., 2003). However, the cows in this study were low in number (4 per group) and had large variation in period of time during the dry period (3 to 10 weeks). Originally identified as being overexpressed in obese mice, resistin is also downregulated by the insulin sensitizing TZDs (Steppan et al., 2001), though the data in mouse models has been contradictory (Way et al., 2001). More recently, researchers failed to find a link between resistin and insulin resistance in a group of humans despite obese individuals having higher circulating levels of the protein (Owecki et al., 2010). Further work in both ruminants and nonruminants is necessary to determine the true relationship between resistin and insulin resistance.

Finally, perilipin may be another factor related to adipose tissue metabolism and the transition cow. Phosphorylation of perilipin is necessary for HSL to interact with lipids (Shen et al., 2009). Recently, it has been hypothesized that

phosphorylation of perilipin may be an important regulator in both lipolysis occurring in early lactation as well as basal lipolysis in late lactation (Sumner and McNamara, 2007; Elkins and Spurlock, 2009). Sumner and McNamara (2007) reported increased perilipin mRNA in lactation (90 DIM). Elkins and Spurlock (2009) did not observe differences in either mRNA or protein level in early (5-14 DIM) versus later (176-206 DIM) lactation, though they did report increases in phosphorylation of perilipin. The roles of leptin, adiponectin, TNFα, and other proteins secreted from adipose tissue in metabolic regulation of insulin resistant ruminants require further investigation.

Integration of plane of nutrition and insulin-sensitizing agents

As outlined above, characterization of the effects of plane of nutrition and insulin-sensitizing agents on insulin resistance in periparturient ruminants has been limited. Furthermore, interactions of plane of nutrition and administration of these agents also have not been investigated. In order to properly measure effects on insulin signaling, however, the appropriate method for evaluating insulin resistance should be selected. As described, insulin resistance includes a combination of both sensitivity and responsiveness and if possible, experimental methods chosen must attempt to distinguish between the two. However, practicality of experimental procedures also need be considered as well. Throughout the literature, there are three major methods for evaluating insulin resistance: the hyperinsulinemic-euglycemic clamp (HEC), the intravenous glucose tolerance test (GTT), and an intravenous insulin challenge (IC). There is only one steady-state calculation-based measure of insulin sensitivity that has been used in ruminants and that is the revised quantitative insulin sensitivity check index (RQUICKI) (Holtenius and Holtenius, 2007).

The HEC is known in medical fields as the “gold standard” for measuring insulin resistance (DeFronzo et al., 1979; Muniyappa et al., 2008). The reason it is considered the “gold standard” is because of its ability to measure effects of elevated levels of insulin without the interference of altered glucose concentrations (Trout et al., 2007). During the HEC, insulin is infused at a constant rate and glucose is infused simultaneously at variable rates in order to maintain euglycemia. The goal is to raise plasma insulin levels to a plateau, and maintain the animal at those elevated insulin levels while maintaining glucose levels at basal concentration +/- 10% (DeFronzo et al., 1979; Griinari et al., 1997). Blood is sampled frequently and analyzed for glucose immediately so that the rate of glucose infusion can be adjusted accordingly. Thus the rate of glucose required to maintain euglycemia is essentially an estimate of whole-body glucose disposal rate given that the greater concentration of insulin should, in theory in nonruminants, suppress hepatic glucose production (Muniyappa et al., 2008). Accordingly, an individual that requires more glucose to maintain euglycemia is more insulin sensitive as they have a greater glucose disposal rate.

Short-term clamps that last one and one-half to a few hours (of plateau or steady-state) in duration are used to measure changes in insulin resistance while longer-term clamps (generally 96 h in the case of ruminants) are used to measure longer-term regulation of insulin on metabolism. The HEC technique has also been used to compare differences in insulin sensitivity of fatty acid metabolism in lactating versus nonlactating sheep (Faulkner and Pollock, 1990) and in lactating versus nonlactating dairy cattle (Andersen et al., 2002). As described, the assumptions for the HEC must be as follows: 1. Steady-state conditions are achieved, 2. Hepatic glucose production is completely suppressed, and 3. Steady-state is achieved within an insulin concentration where glucose disposal rate can vary based on insulin

sensitivity (Muniyappa et al., 2008). Only when these three criteria are met can one be confident in the conclusions drawn from an HEC about relative insulin sensitivity.

The GTT, though much easier to administer than the HEC, has results that must be interpreted indirectly. During the GTT, an intravenous dose of glucose is given and rapid blood samples are taken in order to determine the time response of plasma glucose and insulin. It is an indirect measure of insulin response since there is the added complication that there is a proportion of glucose clearance that is related to direct glucose effects (glucose effectiveness) (Bergman et al., 1979; Bergman et al., 1981). It gives an overall comparison of insulin sensitivity, glucose effectiveness, and β-cell function. Furthermore, responses in circulating fatty acids can be used to evaluate insulin action on fatty acid metabolism (Sumner et al., 2004; Moate et al., 2007; Boston et al., 2008).

If a bolus insulin injection is given shortly after the glucose bolus, there is opportunity to make calculations based on the minimal model developed by Bergman (1979). However, if the objective is to measure responses in fatty acid metabolism to GTT, it has been observed that adipose tissue will be nonresponsive to exogenous insulin given within 20 minutes of the glucose infusion due to hyperinsulinemia caused by the response to glucose (Sumner et al., 2004). Therefore, in terms of measuring responses in fatty acid metabolism, it would be beneficial to not include the insulin infusion, or wait until endogenous insulin has begun to decline following GTT.

Unlike the HEC, steady-state is never reached and thus the GTT model has the following assumptions: 1. Instantaneous distribution of the glucose dose, 2. Glucose disappearance is assumed to occur at a monoexponential rate, 3. Glucose concentration at the end of the GTT is assumed to be identical to that at the beginning, 4. Insulin is assumed to act extravascularly to promote glucose disappearance, and 5. The effects of insulin to promote glucose disposal and suppress hepatic glucose

production are lumped together (Muniyappa et al., 2008). If a GTT alone, without simultaneous insulin infusion and minimal model analysis is completed, it can be thought of as an evaluation of "glucose tolerance", not necessarily insulin sensitivity (Bergman et al., 1979; Bergman et al., 1981). Additionally, hyperglycemia has also been found to have direct effects on fatty acid metabolism in dogs (Park et al., 1990). During GTT, it may be possible to make relative comparisons of peak concentration of glucose relating to insulin responsiveness as well as glucose clearance rate reflecting insulin sensitivity (Kahn, 1978). The GTT may be a more appropriate measure of insulin resistance in non-lactating animals as lactating animals have the large non-insulin dependent glucose utilization by the mammary gland (Bossaert et al., 2009).

Finally, the IC is a measure of insulin responsiveness as long as the dose of insulin given is large enough. In the IC, a bolus dose of insulin is given intravenously and rapid blood samples are collected in order to determine changes in plasma glucose and insulin over time. The rate and magnitude of decline of plasma glucose following the insulin infusion provides an estimate of insulin sensitivity (Muniyappa et al., 2008). Disadvantages of IC are that it may induce a state of hypoglycemia which may cause counterregulatory mechanisms and confound insulin sensitivity estimates (Muniyappa et al., 2008). Responses in fatty acid metabolism to IC can be observed, as long as the animal is not already in a hyperinsulinemic state (Sumner et al., 2004).

The RQUICKI is a relative measure of insulin sensitivity based on basal levels of insulin, glucose, and NEFA. Although used extensively in human medicine where steady-state glucose metabolism is relatively common, it is used less in ruminant species (Holtenius and Holtenius, 2007). However, the measure has been tested and used in ruminant animals on a few occasions (Holtenius and Holtenius, 2007; Kerestes et al., 2009; Bossaert et al., 2009). The calculation for RQUICKI is as follows:

$$RQUICKI = 1 / [\log(G_b) + \log(I_b) + \log(NEFA_b)]$$

Where
G_b = Basal glucose (mg/dL)
I_b = Basal insulin (μU/mL)
$NEFA_b$ = Basal NEFA (mmol/L)

The nature of RQUICKI is such that a lower RQUICKI suggests greater insulin resistance. Accordingly, obese cows are found to have lower RQUICKI than those with adequate body condition (Holtenius and Holtenius, 2007). The limitations of RQUICKI are that it is more applicable in nonruminants where steady-state (fasting) is commonly achieved and that it is a single-point-in-time calculation. Its robustness as a relative measure needs further testing in ruminant animals.

Summary and major research questions

As outlined in this review, though much is already known about transition cow metabolism, the mechanistic details of many aspects have yet to be explored. It is clear that insulin plays a major role in many of the metabolic adaptations that occur during the transition period. The concept of insulin resistance in humans is much more thoroughly studied and thus it has been beneficial to turn to research in human medicine to make applicable assumptions in dairy cattle. Most interesting has been the correlations with obesity and Type II diabetes and how that metabolic situation may relate to dairy cattle. This is not unlike the situation we see in overfed dry cows. The nature of this dysregualtion likely stems from fatty acid metabolism and the roles of adipose tissue. Early work in dairy cattle using an insulin-sensitizing agent that also serves to target adipose tissue had desirable results and it will beneficial to pursue future application. The exact mechanism of how TZDs affect transition cow metabolism would help target the use of such a therapy. Finally, because energy

status plays a large role in insulin signaling, there is opportunity to explore the potential interaction of TZD and plane of nutrition. The major research questions sought to be answered with this series of experiments include:

1. What are the potential metabolic outcomes for the use of insulin-sensitizing agents in transition cows?
2. Do changes in insulin resistance underpin changes in metabolism during situations of modified energy status in dairy cattle during late gestation?
3. Do the effects of TZD administration depend upon plane of energy intake in dry cows?

It is hypothesized that TZD administration will affect various metabolic outcomes in transition cows, which may include changes in plasma leptin, TNFα and adipose tissue gene expression. Furthermore, it is thought that plane of nutrition will impact insulin resistance in dairy cattle, responses to negative energy balance, and that these responses may interact with TZD administration.

CHAPTER 3: EFFECTS OF PREPARTUM 2,4-THIAZOLIDINEDIONE ON INSULIN SENSITIVITY, PLASMA CONCENTRATIONS OF ADIPOKINES, AND ADIPOSE TISSUE GENE EXPRESSION[1]

Abstract

Administration of thiazolidinediones (TZD), which are potent ligands for peroxisome proliferator-activated gamma (PPARγ), to prepartum dairy cattle has been shown to improve dry matter intake (DMI) and reduce circulating non-esterified fatty acids (NEFA) around the time of calving. The objective of this experiment was to further elucidate the mechanisms of TZD action in transition dairy cattle via investigation of plasma leptin, tumor necrosis factor alpha (TNFα), the revised quantitative insulin sensitivity check index (RQUICKI), and adipose tissue gene expression of leptin, peroxisome proliferator activated receptor-gamma (PPARγ), lipoprotein lipase (LPL) and fatty acid synthase (FAS). Holstein cows (n = 40) entering second or greater lactation were administered 0, 2.0, or 4.0 mg TZD/kg BW by intrajuglar infusion once daily from 21 d before expected parturition until parturition. Plasma samples collected daily from 22 d before expected parturition through 21 d postpartum were analyzed for glucose, NEFA, and insulin. Plasma samples collected on d -14, -3, -1, 1, 3, 7, 14, and 49 relative to parturition also were analyzed for leptin and TNFα. Data from 40 cows were used for prepartum analyses

[1]Research material published in part as abstracts in 2008 and 2010 (Schoenberg et al., 2008; 2010)

and 31 cows were used for peripartal (d -7 to +7) and postpartum analyses. Adipose tissue was collected by tissue biopsy on d -7 prior to expected parturition from a subset of cows and adipose tissue gene expression was examined via quantitative real-time polymerase chain reaction. There was a tendency for a treatment by time effect on plasma leptin prepartum such that values were similar on d -14 but cows receiving 2.0 mg/kg BW of TZD tended to have lower circulating leptin as calving approached. Leptin tended to be increased linearly (2.3, 2.4, 2.5 ng/ml) during the postpartum period in cows treated with TZD prepartum. Plasma TNFα also increased linearly (0.56, 0.67, 0.70 pg/mL) in response to TZD treatment and decreased through the first week postpartum. RQUICKI was calculated (RQUICKI = (1/[log(glucose) + log(insulin) + log(NEFA)])) for 21 d pre- and post-calving and there was an effect of day on RQUICKI. During the peripartal period there was a trend for a quadratic effect of TZD administration on RQUICKI (0.77, 0.75, 0.80), suggesting increased insulin sensitivity in cows administered TZD. Administration of TZD increased adipose tissue expression of PPARγ mRNA (11.0, 13.3, 12.8). There was a quadratic effect of TZD on adipose tissue gene expression of leptin (16.2, 10.7, 17.4). There were no effects of TZD-administration on adipose tissue gene expression of LPL or FAS. Results implicate altered expression and plasma concentrations of leptin, plasma TNFα, potential changes in insulin resistance, and changes in expression of PPARγ but not LPL or FAS in adipose tissue as potential mechanisms for the effect of TZD administration on transition dairy cattle.

Introduction

The overall profitability and health of a dairy cow benefits from successful management during the transition period (Drackley, 1999). The metabolic adaptations that support the onset of lactation include increased mobilization of fatty acids from adipose tissue and increased hepatic gluconeogenesis (Bell, 1995). Orchestration of nutrient partitioning is necessary to meet new metabolic demands corresponding with a simultaneous decrease in DMI around calving, which requires mobilization of body fat reserves (Bauman and Currie, 1980). These responses increase circulating NEFA, which when taken up by the liver in excess predispose cows to accumulate triglyceride in liver and increase risk for fatty liver and other disorders such as ketosis, milk fever, retained placenta, and mastitis (Drackley et al., 2001).

Although much research has focused on the ability of the liver to process these excess fatty acids (Bobe et al., 2004), there also may be potential to reduce the fatty acid load to the liver by modulating the spike in circulating NEFA that occurs during the immediate peripartal period. Part of the homeorhetic adaptation that occurs from late pregnancy to early lactation is insulin resistance of peripheral tissues, including adipose, in order to spare glucose for the mammary gland (Bell and Bauman, 1997). Although this is beneficial to support lactation, excessive insulin resistance of adipose tissue likely results in disproportionately high NEFA levels (Vernon, 2005). Whether or not cows experience excessive adipose tissue mobilization postpartum may be related to plane of nutrition during the dry period. Cows that were overfed to 150% of energy requirements during the dry period lost more body weight (BW) and had higher circulating NEFAs despite higher circulating levels of insulin (Dann et al., 2006). Additionally, cows that are overfed during the dry period were suggested to be more insulin resistant as measured by reduced insulin responses to glucose challenge

(Holtenius et al., 2003). These observations combined suggest changes in insulin's ability to stimulate glucose uptake and attenuate lipolysis, as well as changes in insulin release in response to glucose. Especially in relation to altering the influence on lipolysis, opportunities to target adipose tissue in order to mediate potential insulin resistance might be beneficial to transition dairy cows.

The use of insulin-sensitizing agents has been used for the treatment of Type II diabetes in humans for a number of years (Hammarstedt et al., 2005). Popular drugs used are those belonging to the TZD family of synthetic PPARγ ligands (Guo and Tabrizchi, 2006). PPARs in general are ligand-activated transcription factors that are involved in energy status and also modulate inflammatory responses (Yki-Jarvinen, 2004). PPARγ is a major regulator of adipogenesis and is most abundant in adipose tissue (Stienstra et al., 2006). It has been characterized and determined to be most abundant in adipose tissue of ruminants as well (Sundvold et al., 1997; Harvatine and Bauman, 2007).

The first group to treat ruminants with TZD used it to reverse TNFα-induced insulin resistance in steers (Kushibiki et al., 2001). Treatment with TZD partially reversed the insulin resistance caused by TNFα administration as demonstrated by decreased response of plasma insulin to glucose infusion, and reduced response in plasma glucose to insulin challenges (Kushibiki et al., 2001). This paper illustrated the potential use of TZD in ruminants to alter insulin resistance. Additionally, TZDs appeared to enhance peripheral tissue sensitivity to insulin without dramatically affecting circulating levels of insulin.

In the first study with transition dairy cattle, cows were treated with either 0 or 4 mg/kg BW of TZD by intravenous infusion once daily from 25 d before expected parturition until parturition (Smith et al., 2007). The results of this initial experiment indicated that cows treated prepartum with TZD had decreased NEFA during the pre

and peri-partum periods and increased DMI in the post-partum period (Smith et al., 2007). This suggested the potential for the use of TZD in transition dairy cattle to mitigate the dramatic decrease in intake and concurrent rise in NEFA that occur post-calving.

The results discussed below are a follow-up from a second study in transition dairy cattle (Smith et al., 2009). Similar to the first TZD-transition cow study, plasma NEFA were lower in the post-partum period in TZD-treated cows and peripartal DMI was higher (Smith et al., 2009). There were also linear decreases in post-partum liver concentrations of triglycerides and glycogen (Smith et al., 2009). Based on these results, it was of interest to further explore potential mechanisms for these beneficial effects of TZD administration on transition cow metabolism. The objectives of this study were to determine the effect of prepartum TZD administration on plasma leptin, plasma TNFα, a calculated index of insulin sensitivity, and adipose tissue mRNA abundance for genes involved in lipid metabolism.

Materials and methods

All procedures involving animals were approved prior to the onset of the experiment by the Cornell University Institutional Animal Care and Use Committee. This experiment was part of a larger experiment that commenced in September 2006 and was completed in March 2007 (Smith et al., 2009). Detailed Materials and Methods can be found in previously published work (Smith et al., 2009). In brief, Holstein cows (n = 40) entering second or greater lactation that had been dried off at approximately 60 d before expected calving were selected from the Cornell Teaching and Research Center dairy herd and moved to individual tie stalls at approximately 32 d before expected calving.

Cows were fitted with a single indwelling jugular catheter (Micro-Renathane® Implantation Tubing, 2.03 mm o.d. x 1.02 mm i.d.; Braintree Scientific Inc., Braintree MA) 23 d before expected calving date. At 21 d before expected parturition, cows were assigned to one of three treatments in a completely randomized design and administered TZD at one of two doses (2.0 or 4.0 mg/kg BW) or saline (control) by intrajugular infusion once daily at 1200 h until parturition. TZD was obtained as 2,4-thiazolidinedione from Sigma Chemical Co. (St. Louis, MO). Cows were assigned to treatments by balancing for BCS and previous calculated 305-d mature equivalent milk yield. Cows were fed a common TMR for *ad libitum* intake during each of the pre- and postpartum periods formulated to meet or exceed predicted requirements for energy, protein, minerals, and vitamins (NRC, 2001).

Body weights and BCS were recorded once weekly beginning the week prior to treatment initiation until the end of the study. Body condition scores were assigned using a five-point system and the scores of two individuals were averaged as the value assigned to each cow (Wildman et al., 1982).

Plasma and tissue analyses

Blood samples were collected immediately prior to treatment administration once daily at 1200 h via jugular catheter from 22 d prior to expected parturition to parturition. A sterile solution of sodium heparin (200 IU/mL of saline) and Naxcel® (4 mg/mL of saline; Pfizer Inc.; New York, NY) filled the catheter after sampling to prevent blood coagulation and bacterial growth. Following parturition, blood samples were collected daily via venipuncture of the coccygeal vessels until 21 d postpartum and then twice per week from wk 4 through 9 of lactation. Blood samples were transferred into glass test tubes containing sodium heparin (100 IU/mL blood). Plasma was harvested following centrifugation (2,800 x g for 15 min at 4°C), snap-

frozen in liquid N_2, and stored at -20°C until analyses for metabolites. Plasma concentrations of glucose were determined by enzymatic analysis (glucose oxidase) using a commercial kit (kit 510-A; Sigma Chemical). Plasma concentrations of NEFA also were analyzed by enzymatic analysis (NEFA-C; WAKO Pure Chemical Industries; Osaka, Japan) with modifications described by McCutcheon and Bauman (1986). Intra- and interassay coeffiecients of variation for plasma glucose and NEFA assays were as described previously (Smith et al., 2009). All spectrophotometric measurements were conducted using a $Versa_{max}$ tunable microplate reader (Molecular Devices, Sunnyvale, CA). Plasma concentrations of leptin on d -14, -3, -1, 1, 3, 7, 14, and 49 relative to parturition were determined by double-antibody radioimmunoassay (Ehrhardt et al., 2000). Intra- and interassay coefficients of varation for plasma leptin were 3.9 and 9.7%, respectively. Plasma TNFα concentrations from d -14, -3, -1, 1, 3, 7, 14, and 49 relative to parturition were determined in the laboratory of Dr. Barry Bradford (Kansas State University, Manhattan. KS) by enzyme-linked immunosorbent assay (ELISA) (Farney et al., 2010).

Adipose tissue was collected from a subset of cows prior to initiation of treatments and again seven d prior to expected calving date (and thus after approximately 14 d of TZD administration). Six cows were from the group administered saline, five cows were administered 2.0 mg/kg BW TZD and four were administered 4.0 mg/kg BW TZD. Days prior to actual calving date averaged 5.8 +/- 3.8 d and ranged from 1 to 13 days prior to actual calving date. During biopsies, the area was brushed and clipped to remove excess hair and prepared with betadine surgical scrub [Betadine Surgical Scrub (7.5% povidone-iodine); Purdue Frederick; Stamford, CT] and 70% ethanol in water. Cows were administered 20 mg of the sedative zylazine hydrochloride (Rompun 2%, Bayer Inc., Sarnia, Ontario, Canada). A local anesthetic (Lidocaine HCL; 2%; Butler Animal Health; Dublin, OH; 18 cc)

was injected subcutaneously around the biopsy site. The area below the spinal processes between the hips and the pins of the cow was palpated to locate an area with sufficient subcutaneous fat, a 4-8 cm incision was made with a scalpel blade, and a small biopsy (0.5-1.5 g) was removed using sterile forceps and scalpel. The incision was closed with 6-12 surgical skin staples (3M™ Precise™ Vista Disposable Skin Stapler; 3M; St. Paul, MN) and treated with a topical antiseptic (BluKote™ aerosol spray; H.W. Naylor Co.; Morris, NY). Adipose samples were washed with sterile saline, cut into aliquots and placed into sterile and RNAse-free vials, and quenched in liquid N_2.

Quantitative RT-PCR

Analysis of gene mRNA expression was done via quantitative real-time polymerase chain reaction (RT-PCR). RNA was isolated from 150-200 mg of adipose tissue using the RNAeasy mini kit (Qiagen; Valencia, CA) and on-column DNase digestion with the RNase-free DNAse set (Qiagen; Valencia, CA) after homogenization in 1.0 mL of Qiazol (Qiagen; Valencia, CA) reagent. RNA concentration and integrity was determined using an Agilent 2100 BioAnalyzer (Agilent Technologies; Santa Clara, CA). Total RNA was reverse-transcribed using ABIs High Capacity cDNA reverse transcription kit with RNAse Inhibitor (Applied Biosystems; Foster City, CA). PCR reactions were carried out using Power SYBR Green (Applied Biosystems; Foster City, CA), 25 ng total RNA (or 5 ng total RNA for 18S) and 400 nmol/L gene-specific forward and reverse primers in a 25 μl reaction volume using a 2-step program (95°C for 15 seconds followed by 60°C for 60 seconds) on an ABI PRISM 7000 sequence detection system (Applied Biosystems; Foster City, CA). Expression of 18S mRNA was used as a housekeeping gene.

Primers used were published previously (Thorn et al., 2006; Harvatine et al., 2009). Table 3.1 details the primer sequences used.

Calculations

RQUICKI, the relative insulin sensitivity measure used in ruminants was calculated as the following (Holtenius and Holtenius, 2007):

$$RQUICKI = 1 / [\log(G_b) + \log(I_b) + \log(NEFA_b)]$$

Where
G_b = Basal glucose (mg/dL)
I_b = Basal insulin (μU/mL)
$NEFA_b$ = Basal NEFA (mmol/L)

such that a lower RQUICKI suggests greater insulin resistance.

Statistical analysis

Statistical analyses were conducted as analysis of variance (ANOVA) on measures conducted over time (plasma concentrations, BCS) using the PROC MIXED procedure of SAS (2001) for a completely randomized design with repeated measures. Nine cows were removed from the dataset for peri- and post-partum data as detailed in Smith et al. (2009). The statistical model included fixed effects of covariate, treatment, time and the interaction of treatment and time. The random effect was cow nested within treatment. For each variable, six covariance structures were evaluated (first-order autoregressive, heterogeneous first-order autoregressive, compound symmetry, heterogeneous compound symmetry, first-order ante-dependence, and unstructured) and the structure with the smallest Akaike's information criterion was selected. The method of Kenward-Rogers was used for calculation of denominator degrees of freedom. Covariates were dropped from the statistical model if $P > 0.15$

Table 3-1. Primer sequences for adipose tissue gene expression.

Target	Forward Primer Sequence	Reverse Primer Sequence	Source
PPARγ	AAGAGCCTTCCAACTCCCTCA	CCGGAAGAAACCCTTGCAT	(Harvatine et al., 2009)
Leptin	TCACCAGGATCAATGACATCTCA	ACCAGTGACCCTCTGTTTGGA	(Harvatine et al., 2009)
Lipoprotein lipase (LPL)	GAACTGGATGGCGGATGAAT	GGGCCCCAAGGCTGTATC	(Harvatine and Bauman, 2006)
Fatty Acid Synthase (FAS)	CTGATGAGCTGACGGACTCCA	GCGATTGGGCAGGGCT	(Harvatine and Bauman, 2006)

and the data were reanalyzed. In all cases, prepartum (d -21 to -1; or -14 and -3 d in the case of plasma leptin), peripartum (d -7 to +7), and postpartum data were analyzed separately, consistent with Smith et al. (2009). Gene expression data were analyzed using the MIXED procedure of SAS including the effects of treatment. Day relative to actual calving date and BCS were evaluated for significance as covariates and removed if $P > 0.15$. Expression data were evaluated relative to reference gene (18S) expression and with expression from a covariate sample as a covariate in the statistical model.

Results

Plasma

Results related to daily plasma values and production levels were reported previously (Smith et al., 2009). As reported, TZD administration during the prepartum period resulted in a quadratic effect in prepartum plasma NEFA such that cows receiving 2.0 mg TZD/kg of BW had higher NEFA than either the control cows or those receiving 4.0 mg TZD/kg of BW. While there were no effects on plasma NEFA during the peripartum period, prepartum TZD linearly decreased plasma NEFA during the postpartum period. Administration of TZD prepartum resulted in higher glucose concentrations for cows administered 4.0 mg TZD/kg of BW during the pre- and peripartum periods. There was no effect of TZD administration on plasma insulin concentrations in these cows.

Figure 3-1 illustrates the trend for treatment by day interaction ($P = 0.09$) of prepartum plasma leptin such that cows receiving 2.0 mg TZD/kg of BW prepartum had the lowest concentration of plasma leptin as calving approached. There was no

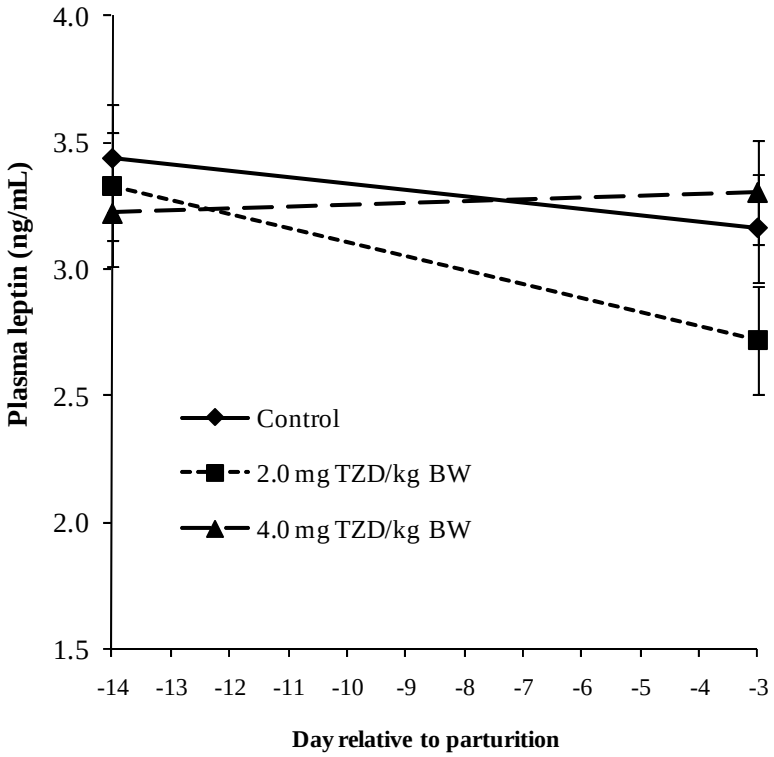

Figure 3-1. Prepartum plasma leptin. Cows were administered increasing amounts of TZD during the prepartum period. Data shown are plasma values from d 14 and 3 prior to expected calving date. Values are least squares means with error bars representing SEM; n = 13 for Control, n = 13 for 2.0 mg TZD/kg BW, n = 14 for 4.0 mg TZD/kg BW. The *P* value for treatment by day interaction was 0.09 and effect of day was 0.04.

treatment effect on plasma leptin ($P = 0.76$) during the peripartum period, but plasma leptin decreased as calving approached and remained low through d +7 (Figure 3-2; $P < 0.0001$). Postpartum plasma leptin tended ($P = 0.14$) to increase linearly in response to TZD-treatment as shown in Figure 3-3. Plasma TNFα was also increased linearly during day -14 through day +49 relative to calving date ($P = 0.01$; Figure 3-4) in response to TZD-treatment and decreased ($P = 0.001$, Figure 3-5) during the first week post-partum.

As shown in Figure 3-6, there was a trend ($P = 0.09$) for an interaction of TZD administration and day during the peripartum period such that cows receiving 2.0 mg TZD/kg of BW had lower RQUICKI prepartum while cows receiving 4.0 mg TZD/kg of BW had higher RQUICKI closer to calving. Additionally, there was a main effect of day ($P < 0.0001$) such that RQUICKI is lowest around the time of calving. Postpartum RQUICKI was not affected by prepartum TZD administration ($P = 0.54$).

Adipose tissue gene expression

Cows that were administered TZD had greater adipose tissue expression of mRNA for PPARγ than control cows ($P = 0.01$; Figure 3-7). There was a quadratic effect of TZD ($P = 0.04$, Trt; $P = 0.07$, Quad; Figure 3-8) on adipose tissue gene expression of leptin such that cows administered 2.0 mg TZD/ kg of BW had the lowest leptin mRNA expression. There were no effects of TZD-administration on adipose tissue gene expression for either LPL or FAS (Figures 3-9, 3-10).

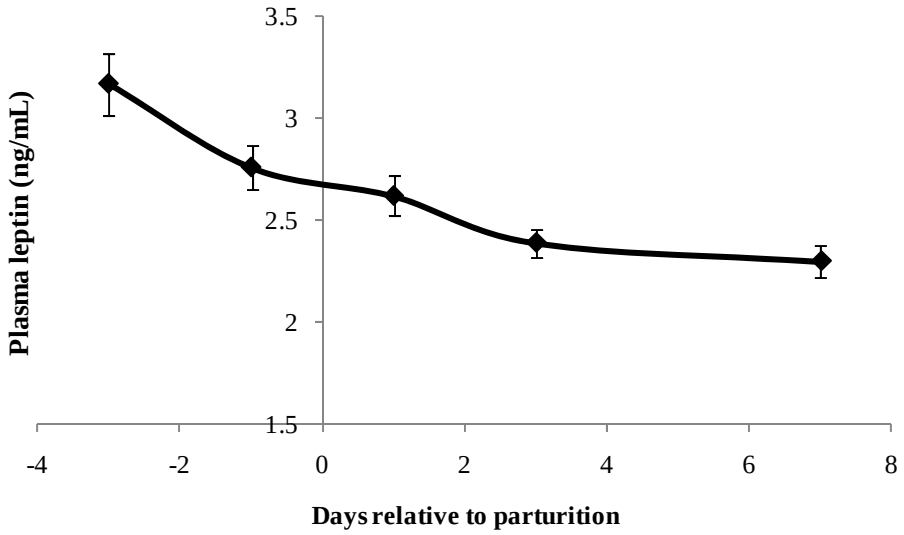

Figure 3-2. Peripartal plasma leptin. Cows were administered increasing amounts of TZD during the prepartum period. Data shown are plasma values from d -3 through d +7 relative to calving date. Values are least squares means with error bars representing SEM; n =31. Only the main effect of day is shown as effect of TZD was not significant ($P = 0.76$). The P value for effect of day was < 0.0001.

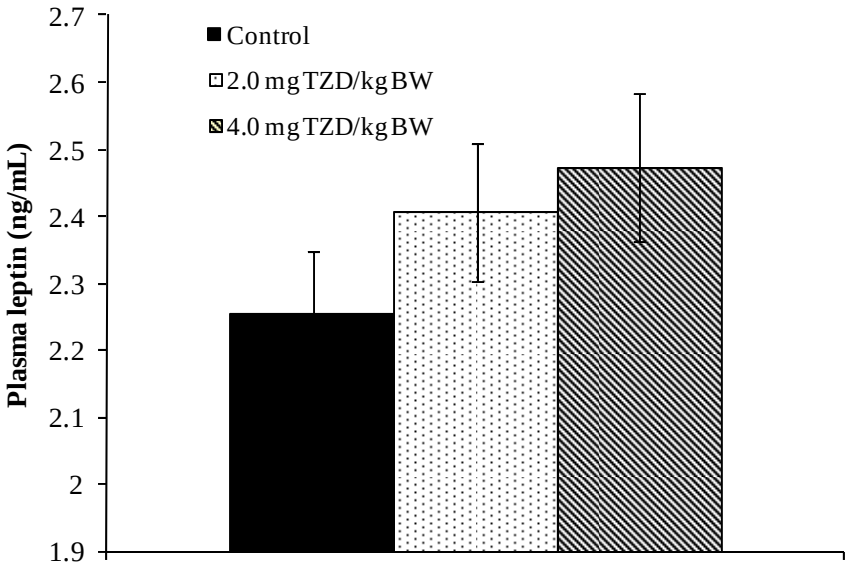

Figure 3-3. Postpartum plasma leptin. Cows were administered increasing amounts of TZD during the prepartum period. Data shown are average plasma values from d +1 through d +49 relative to calving date. Values are least squares means with error bars representing SEM; n = 13 for Control, n = 13 for 2.0 mg TZD/kg BW, n = 14 for 4.0 mg TZD/kg BW. The *P* value for effect of day was 0.008; the *P* value for linear effect of treatment was 0.14.

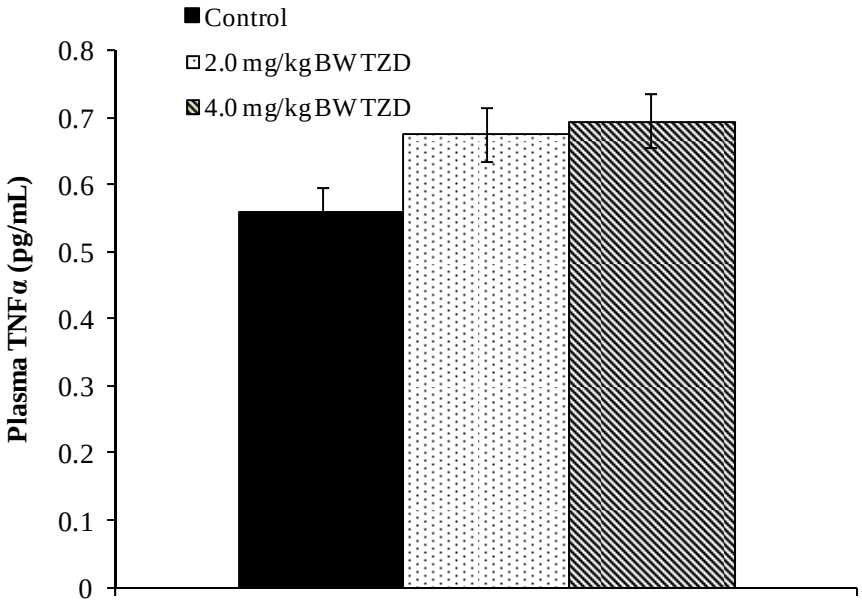

Figure 3-4. Pre- and postpartum plasma TNFα. Cows were administered increasing amounts of TZD during the prepartum period. Data shown are plasma values from d -14 through d +49 relative to calving date. Values are least squares means with error bars representing SEM; n = 12 for Control, n = 10 for 2.0 mg TZD/kg BW, n = 9 for 4.0 mg TZD/kg BW. The *P* value for effect of treatment was 0.03; the *P* value for linear effect of treatment was 0.01; the *P* value for effect of day was 0.001; the *P* value for the interaction of treatment and day was 0.68.

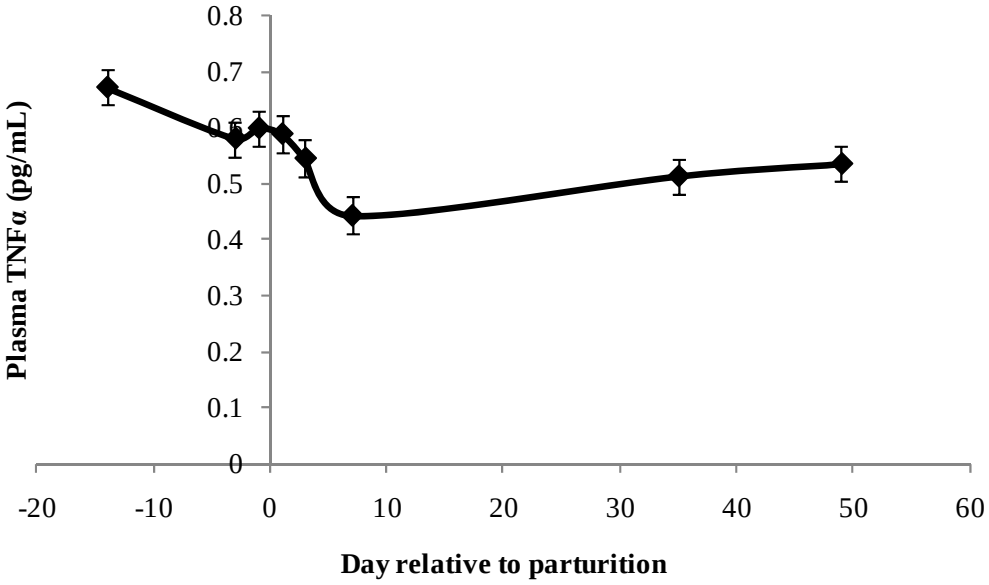

Figure 3-5. Peripartal plasma TNFα. Cows were administered increasing amounts of TZD during the prepartum period. Data shown are plasma values from d -14 through d +49 relative to calving date. Values are least squares means with error bars representing SEM; n = 31. Treatment effects are illustrated in Figure 3-4. The *P* value for effect of day was 0.001; the *P* value for the interaction of treatment and day was 0.68.

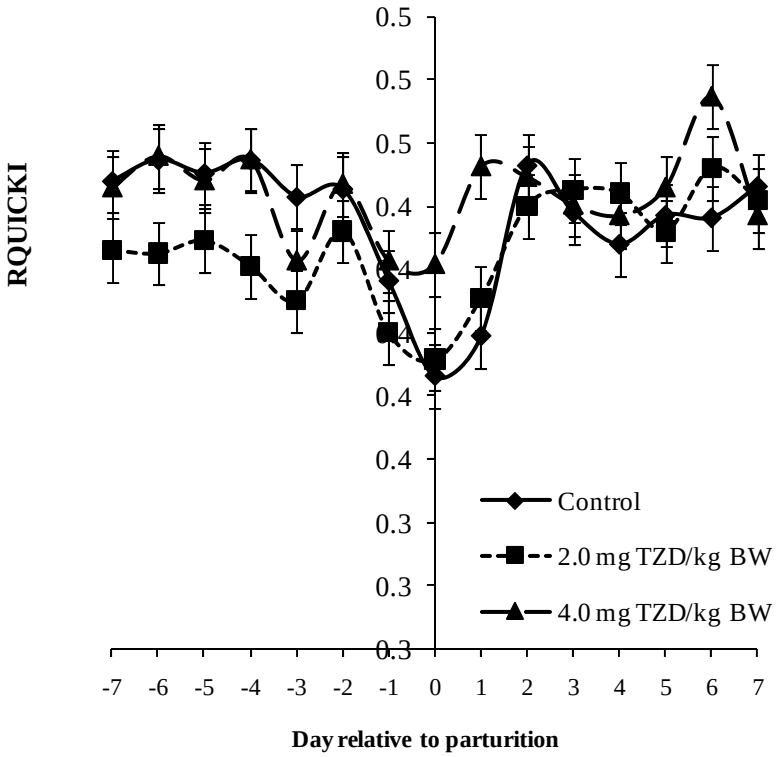

Figure 3-6. Peripartal RQUICKI. Cows were administered increasing amounts of TZD during the prepartum period. RQUICKI was calculated (RQUICKI = (1/[log(glucose) + log(insulin) + log(NEFA)])) for 7 d pre- and post-calving. Values are least squares means with error bars representing SEM; n = 12 for Control, n = 10 for 2.0 mg TZD/kg BW, n = 9 for 4.0 mg TZD/kg BW. The *P* value for effect of treatment was 0.38, for effect of day was < 0.0001; *P* value for interaction of treatment by day was 0.09.

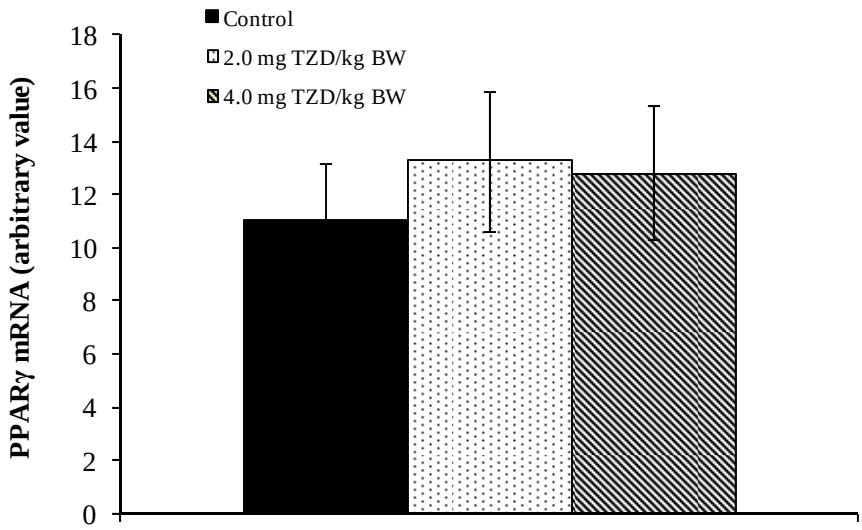

Figure 3-7. PPARγ mRNA expression. Cows were administered increasing amounts of TZD during the prepartum period. Adipose biopsies were conducted 7 d prior to expected calving date. Values are least squares means of arbitrary units in reference to a reference gene (18S) with error bars representing SEM; n = 6 for Control, n = 5 for 2.0 mg TZD/kg BW, n = 4 for 4.0 mg TZD/kg BW. The *P* value for effect of treatment was = 0.01.

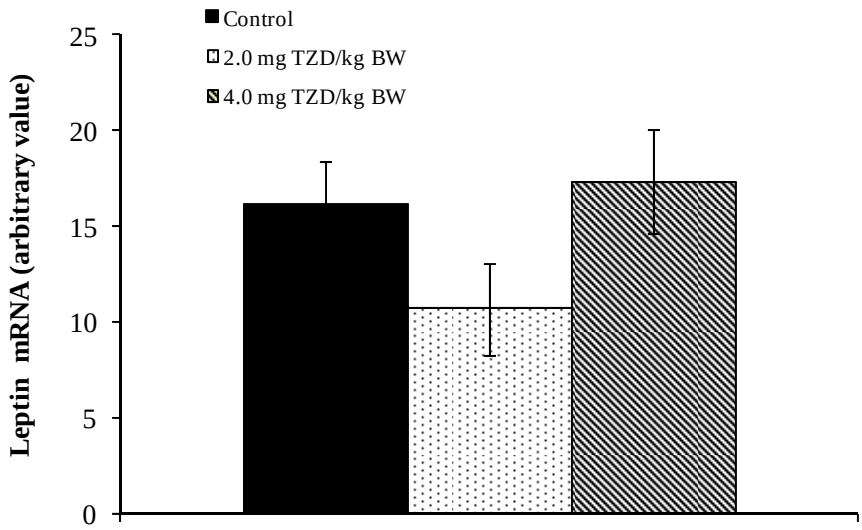

Figure 3-8. Leptin mRNA expression. Cows were administered increasing amounts of TZD during the prepartum period. Adipose biopsies were conducted 7 d prior to expected calving date. Values are least squares means of arbitrary units in reference to a reference gene (18S) with error bars representing SEM; n = 6 for Control, n = 5 for 2.0 mg TZD/kg BW, n = 4 for 4.0 mg TZD/kg BW. The *P* value for effect of treatment was 0.04; the *P* value for quadratic effect of treatment was 0.07.

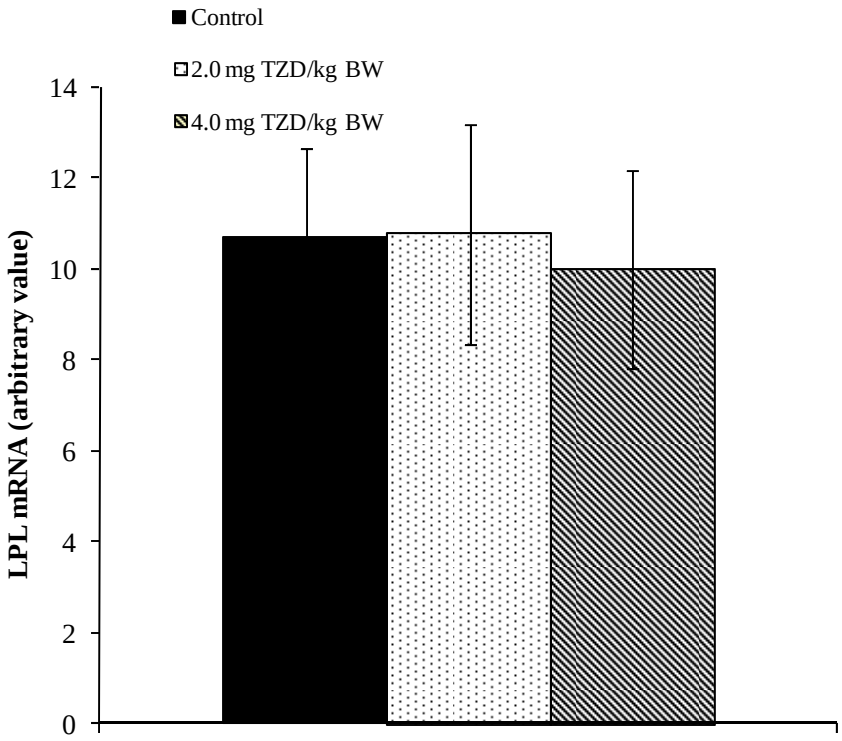

Figure 3-9. Lipoprotein lipase (LPL) mRNA expression. Cows were administered increasing amounts of TZD during the prepartum period. Adipose biopsies were conducted 7 d prior to expected calving date. Values are least squares means of arbitrary units in reference to a reference gene (18S) with error bars representing SEM; n = 6 for Control, n = 5 for 2.0 mg TZD/kg BW, n = 4 for 4.0 mg TZD/kg BW. The *P* value for effect of treatment was not significant (0.20).

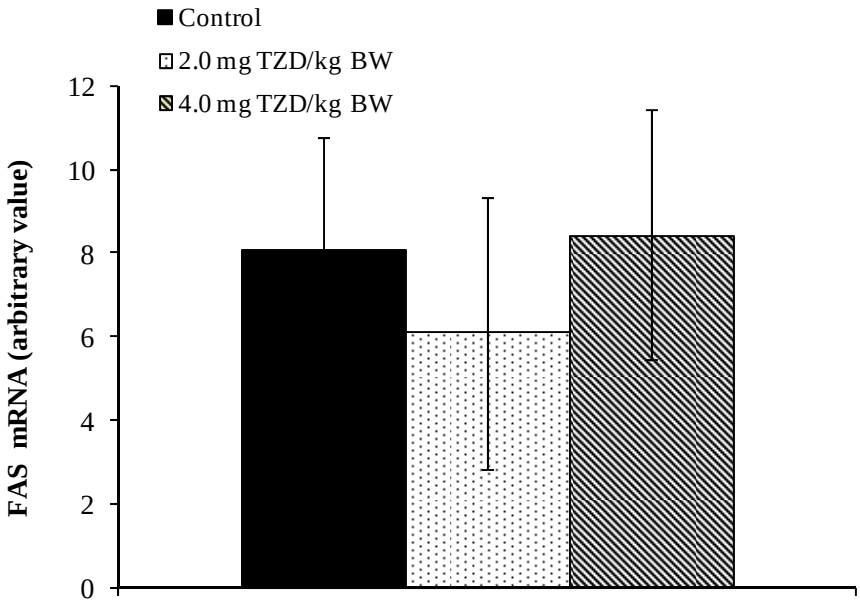

Figure 3-10. Fatty acid synthase (FAS) mRNA expression. Cows were administered increasing amounts of TZD during the prepartum period. Adipose biopsies were conducted 7 d prior to expected calving date. Values are least squares means of arbitrary units in reference to a reference gene (18S) with error bars representing SEM; n = 6 for Control, n = 5 for 2.0 mg TZD/kg BW, n = 4 for 4.0 mg TZD/kg BW. The *P* value for effect of treatment was not significant (0.21).

Discussion

Dynamic metabolic changes occur around the time of parturition in dairy cattle (Bell, 1995). These changes include altered levels of plasma hormones such as leptin, changes in insulin resistance, and regulation of adipose tissue gene expression. In this study prepartum TZD administration increased peripartal DMI and decreased postpartum plasma NEFA indicative of improved metabolic health (Smith et al., 2009). The results reported herein indicate additional effects on plasma leptin, relative changes in insulin sensitivity, and lesser effects on adipose tissue mRNA expression of key genes involved in lipid metabolism.

Known as a signal of adiposity in many species, leptin has the ability to increase energy expenditure and protect tissues from accumulation of excess lipid (Vernon et al., 2001). Plasma leptin decreases substantially as calving approaches and remains low throughout early lactation (Block et al., 2001; Liefers et al., 2003). During the prepartum period, cows treated with the 2.0 mg TZD/kg BW dose had the most substantial decrease in plasma leptin and cows treated at the highest dose remained at a similar level to Control cows (Figure 3-1). Leptin mRNA in adipocytes has shown to be decreased by TZD (Hammarstedt et al., 2005), so the reason for differential effects based on treatment in this case are unclear. Although insulin was reported to increase plasma leptin during the transition period (Block et al., 2003), changes in insulin are not likely to be a factor here as circulating insulin levels prepartum were not different in these cows (Smith et al., 2009). Prepartum energy balance, calculated as net energy balance according to the NRC, was reported previously as 150% for Control cows, 154% for 2.0 mg TZD/kg BW cows, and 162% for cows receiving the highest dose despite no significant differences in prepartum DMI (Smith et al., 2009). It is possible then that there is some interaction prepartum

with energy balance and TZD administration, though energy balance differences here might not be significant. During the peripartum period, effects of treatment were not significant, but the effect of day illustrates the normal plasma leptin response reported during this period (Block et al., 2001), the effects of which may overshadow treatment effects (Figure 3-2).

Although there was no effect of TZD administration on plasma leptin during the peripartum period, there were differences in plasma leptin in the postpartum period. The linear increase in postpartum plasma leptin for TZD-treated cows (Figure 3-3) also may be related to energy balance and BCS. Figure 3-11 shows plasma leptin plotted with postpartum BCS. Circulating leptin has been shown to be higher in cattle with higher body condition score (Ehrhardt et al., 2000). It is possible then, that the higher plasma leptin is manifested through indirect effects that TZD administration had on energy balance. This may also explain in part how TZD administration prepartum can have lasting effects through early lactation. Further discussion regarding indirect effects on leptin is relevant to changes in adipose tissue gene expression discussed in future sections.

Another potentially relevant adipokine is TNFα. Thiazolidinediones have been shown to decrease TNFα circulation and activity (Arner, 2003). In this study, however, TZD administration increased plasma TNFα (Figure 3-4). The relationship of TZD administration on TNFα in ruminants has not been determined and TNFα itself remains relatively unstudied in dairy cattle. It has been implicated in insulin resistance and fatty liver in transition cows (Ohtsuka et al., 2001), as well as during acute mastitis challenges in cows in early lactation (Blum et al., 2000). Interestingly, circulating concentrations of TNFα did decrease based on day relative to parturition and were relatively higher prepartum versus postpartum, as depicted in Figure 3-5. Increased circulating levels of TNFα have been associated with increased circulating

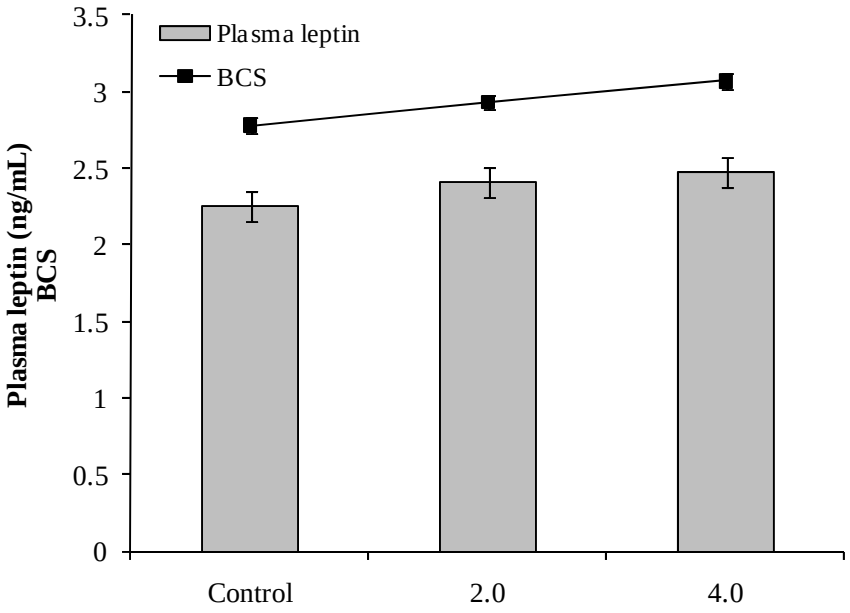

Figure 3-11. Postpartum plasma leptin and BCS. Cows were administered increasing amounts of TZD during the prepartum period. Data shown are average plasma values from d +1 through d +49 relative to calving date and the average BCS from two scorers, based on a 5-point system (Smith et al., 2009). Values are least squares means with error bars representing SEM (0.02); The *P* value for linear effect of treatment was 0.14 for plasma leptin and 0.001 for BCS.

levels of leptin (Arner, 2003), and so it is not surprising that we see similar relationships over time. It is worth noting that the TNFα analysis completed here was completed with a relatively new assay designed to detect much lower levels of TNFα than previously measured by bioassay and radioimmunoassay (Blum et al., 2000; Ohtsuka et al., 2001). Basal levels of TNFα are relatively low with the absence of infection (Blum et al., 2000). As few studies have been able to examine the patterns of TNFα at these lower levels, the pattern shown around the time of calving is worth further investigation.

A lower RQUICKI value has implications of greater insulin resistance (Holtenius and Holtenius, 2007). In this study, RQUICKI values decreased as calving approached and then increased again slightly through early lactation (Figure 3-6). This is in agreement with past statements regarding increased insulin resistance in the late prepartum period, as well as RQUICKI calculations in transition cows (Kerestes et al., 2009). Regarding the effects of TZD administration on RQUICKI, RQUICKI was highest around calving for cows administered the 4.0 mg TZD/kg of BW dose of TZD and lowest precalving for cows receiving the 2.0 mg TZD/kg of BW dose (Figure 3-7). Although RQUICKI has been associated with changes in BCS, further research is required to confirm that cows with lower RQUICKI do indeed have greater insulin resistance. Authors of a previous paper attempted to correlate RQUICKI results with those during GTT or IC and concluded that RQUICKI is a poor indicator of insulin resistance, especially in cows experiencing poor metabolic health (Kerestes et al., 2009). Given the nature of the calculation, changes in plasma NEFA are likely to drive changes in RQUICKI. We would expect changes in plasma NEFA to have direct and indirect effects on insulin resistance already, so RQUICKI may be an applicable relative measure of insulin resistance. It would be premature to use RQUICKI to quantitatively measure insulin resistance in cows under different

physiological states or management strategies, but its future as a general indicator is still unknown.

Although TZD is a known ligand for PPARγ (Houseknecht et al., 2002), this is the first evidence that TZD administration causes upregulation of mRNA expression for PPARγ in dairy cattle (Figure 3-7). In addition to altering the expression of PPARγ mRNA, TZD had a significant effect on leptin mRNA (Figure 3-8). Although the reasons for the differential leptin expression based on level of TZD treatment are not apparent, it is very interesting to note that patterns of mRNA expression are in agreement with prepartum plasma leptin (Figure 3-1). Adipose tissue gene expression may have been affected by actual day relative to calving when biopsies were taken, as well as BCS. Because adipose tissue samples were taken at d -7 relative to expected calving date, there was quite a bit of variation in actual day relative to calving that adipose tissue was collected. Day of adipose tissue biopsy relative to actual calving ranged from 2 to 13 d. Especially for leptin, this is a period of dynamic change and in the span of one or two days the major regulatory signals could be very different. For PPARγ mRNA, BCS was determined to be a significant covariate ($P = 0.02$), and therefore included in the statistical model whereas day relative to actual calving date was not ($P = 0.79$). For leptin expression, although day prior to actual calving date was a factor initially ($P = 0.11$), it was not significant in the final model ($P = 0.16$), and so was eventually dropped. Body condition score was not a significant covariate ($P = 0.28$) for statistical analysis of leptin expression. So although it was hypothesized that body condition score and day relative to actual calving date may be clouding treatment effects, in almost all cases the influence did not reach levels that were statistically significant.

There were no effects of TZD administration on expression of either LPL or FAS in this study. Although TZD has been shown to increase expression of both of

these genes in nonruminants (Kageyama et al., 2003; Bogacka et al., 2004), its effects in ruminants are not yet known. Because both of these genes have been implicated in changes in lipid metabolism occurring during milk fat depression (Harvatine et al., 2009), it was hypothesized that they may play roles in changes in insulin resistance mediated by TZD administration. In this case, however, effects of treatment were not significant. For the case of LPL, it may not be a large factor in early lactation but instead may play a greater regulatory role in mid to late lactation (McNamara et al., 1987). Other factors besides LPL and FAS appear to be altering lipid metabolism in response to TZD administration. Other potential targets may include HSL or PEPCK. The cytosolic form of PEPCK has been shown to be increased with TZD, resulting in decreased fatty acid release (Tordjman et al., 2003b). This effect is likely a major factor in TZD's effect on insulin resistance and is a logical next step in exploring regulation of fatty acid metabolism during insulin resistance and potential mediation by TZD. There is also potential to explore how additional adipokines besides leptin and TNFα are affected by TZD administration in dairy cattle. One example would be adiponectin, which has been shown in humans to be increased by TZD (Tonelli et al., 2004), and may be an important regulator in the transition to early lactation (Lemor et al., 2009). Furthermore, TZD treatment has been shown to attenuate the prepartum decrease in plasma adiponectin (Kim et al., 2008).

Conclusions

Prepartum administration of TZD in transition dairy cattle has been shown to improve DMI and metabolic health (as measured by attenuated loss in BCS post-calving and reduced days to first ovulation) (Smith et al., 2007; Smith et al., 2009). Results shown here add to these previous bodies of work. Expression of PPARγ mRNA was upregulated by TZD administration. Additional results reported herein

illustrate that TZD directly or indirectly alters leptin mRNA expression in adipose tissue and its circulating peripartal concentrations. Plasma concentrations of TNFα were increased linearly by TZD administration, in patterns potentially opposite to results shown in nonruminants. Although there were no significant effects of TZD on RQUICKI, RQUICKI did vary based on day relative to parturition. The relationship of RQUICKI and day relative to calving is consistent with timing of insulin resistance and TNFα concentration around the time of calving, suggesting that RQUICKI might have potential as a qualitative measure of insulin resistance in dairy cattle. Given mixed results in this experiment and others reported, there is need to further evaluate RQUICKI before it is used regularly as a measure of insulin resistance in ruminants. There were no effects of TZD administration on expression of genes involved in lipid metabolism, LPL and FAS. This study suggests that additional factors than leptin, TNFα, LPL and FAS activity play a role in the effects of TZD administration on the metabolic health of transition dairy cows. It does, however, illustrate the importance of leptin, TNFα, and insulin sensitivity in temporal changes in the periparturient dairy cow.

ACKNOWLEDGEMENTS

The assistance of the following individuals was greatly appreciated for the completion of this study: K. L. Perfield, R. Ehrhardt, S. L. Giesy, and the staff at the Cornell University Dairy Teaching and Research Center.

CHAPTER 4: EFFECTS OF PLANE OF NUTRITION AND FEED DEPRIVATION ON INSULIN RESPONSES IN DAIRY CATTLE DURING LATE GESTATION[1]

Abstract

Nonlactating Holstein cows (n = 12) in late pregnancy were used to determine effects of plane of nutrition followed by feed deprivation on metabolic responses to insulin. Beginning 48 +/- 4.5 d prepartum, cows were fed either a High or Low diet to meet 143 and 88% of calculated energy requirements, respectively. Cows were subjected to an intravenous glucose tolerance test in the fed state [GTT; 0.25 g dextrose/kg of body weight (BW)] on d 14 of treatment and a hyperinsulinemic-euglycemic clamp in the fed state (HEC; 1µg/kg of BW/h) on d 15. Following 24 h of feed removal, cows were subjected to a second GTT on d 17 and a second HEC on d 18 after 48 h of feed removal. During the feeding period, plasma NEFA concentrations were higher for cows fed the Low diet versus those fed the High diet (163.6 vs. 73.1 µEq/L), whereas plasma insulin was higher for cows fed the High diet during the feeding period (11.1 vs. 5.2 µIU/mL). For cows assigned to both treatment groups, plasma glucose concentrations were lower after 48 versus 24 h of feed deprivation (54.6 vs. 67.6 mg/dL) but plasma NEFA concentrations were higher (792.6 vs. 219.3 µEq/L). Glucose area under the curve (AUC) during both GTT were higher for cows fed the Low diet than cows fed the High diet (4213 vs. 3750 mg/dL x 60 min) and was higher during the GTT in the feed deprived state (4878 vs. 3085

---

[1]Research material published in part as an abstract in 2009 (Schoenberg et al., 2009)

mg/dL x 60 min) than the fed-state GTT, suggesting slower clearance of glucose during negative energy balance either pre- or post-feed deprivation. This corresponded with a higher dextrose infusion rate during the fed-state HEC than the feed deprived-state HEC (203.3 vs. 90.1 ml/h; $P < 0.001$). Plasma NEFA decreased at a faster rate following GTT during feed deprivation compared to the fed state (8.7 vs. 2.9%/min). NEFA suppression was highest for cows fed the High diet during the feed deprived-state GTT, and lowest for cows fed the High diet during the fed-state GTT (68.6 vs. 50.3% decrease from basal). Cows fed the Low diet had greater NEFA AUC than those fed the High diet (-19044 vs. -12055 µEq/L x 60 min), as did cows during feed deprivation versus the fed state (-25238, -6258 µEq/L x 60 min). Plasma insulin responses to GTT were affected dramatically by feed deprivation such that cows had much lower insulin response to GTT by 24 h after feed removal (995 vs. 3957 µIU/mL x 60 min). During the fed-state HEC, circulating concentrations of NEFA were 21% below basal for cows fed the High diet and 62% below basal for cows fed the Low diet; during feed deprivation NEFA were 79 and 59% below basal for the High and Low diets, respectively (diet x HEC). These results suggest that cows fed below energy requirements or feed-deprived have slower clearance of glucose and larger NEFA responses to glucose challenge. Additionally, feed deprivation had a large effect on insulin secretion. Overall, effects of feed deprivation were larger than prior plane of nutrition.

Introduction

Cows during the early far-off dry period are experiencing relatively low metabolic demands as fetal growth is just beginning to accelerate and cows are no longer lactating. Fetal and uterine tissues are insulin-independent and so as energy demands increase with days of pregnancy, maternal peripheral tissues will become more insulin resistant in order to support fetal growth (Bell, 1995). These changes in the role of insulin have effects on energy metabolism in dry cows.

Much of the work in far-off dry cows has investigated the effect of dry cow diets on subsequent health and performance during the transition period and early lactation. Researchers have sought to determine why cows that are over-fed during the dry period have more severe decreases in peripartal DMI and higher postpartum concentrations of NEFA and β-hydroxybutyrate (BHBA) during the transition period (Agenas et al., 2003; Holtenius et al., 2003; Dann et al., 2005; Douglas et al., 2006; Dann et al., 2006). It is our hypothesis that increased body condition, or at least increased dietary energy level, makes cows more insulin resistant, leading to abnormal metabolic regulation. Cows fed 178% of calculated energy requirements during the entire dry period had greater insulin responses to glucose challenge, indicative of increased tissue resistance to insulin (Holtenius et al., 2003). Likewise, ewes that are over-conditioned have decreased insulin sensitivity as measured by the hyperinsulinemic-euglycemic clamp technique (Bergman et al., 1989).

Dann et al. (2006) reported that cows fed diets to achieve 150% of NRC requirement for energy during the far-off dry period had higher circulating concentrations of NEFA and BHBA and greater loss of BW during the postpartum period despite higher insulin levels when consuming the higher energy level diets. These results provide further evidence for effects of diet on insulin resistance in

adipose tissue. Overfeeding during the dry period resulted in increased esterification rates in adipose tissue prepartum (concurrent with higher circulating insulin) and greater lipolytic rates (and thus higher NEFA) postpartum (Rukkwamsuk et al., 1999b). The practical outcome is that cows overfed during the dry period lose more body condition postpartum (Agenas et al., 2003) and that these alterations in metabolic signaling have carryover effects that persist into early lactation.

The transition period is one of dynamic changes in metabolic regulation. It may be beneficial then to study changes in insulin resistance independent of additional adaptation occurring around the time of calving. In this case, it was our desire to measure the effects of plane of nutrition on metabolism during the dry period. In addition to changes in insulin resistance based on dietary energy level, it is of interest to determine how plane of nutrition interacts with subsequent negative energy balance. Rather than employ means such as infusion of fats in order to increase plasma NEFA (Pires et al., 2007b), a short-term feed deprivation was the approach selected to increase plasma NEFA to levels similar to that of early lactation.

Therefore, the overall objective of this experiment was to further characterize adaptation in response to various states of energy balance occurring during the dry period. Specifically, what effects would overfeeding have on glucose, insulin, and fatty acid metabolism and how might that be altered when the same cows are exposed to acute negative energy balance? The hypothesis was that plane of nutrition would impact insulin resistance as well as metabolic responses to negative energy balance.

A secondary objective for this experiment was to evaluate the use of HEC and GTT in measuring metabolic responses related to glucose, insulin, and fatty acid metabolism in dry cows. How might results obtained from HEC and GTT compare? Although the HEC is considered the “gold standard” for measuring changes in insulin sensitivity, it requires greater resources and the concurrent administration of large

amounts of glucose to maintain euglycemia (DeFronzo et al., 1979). Although the use of GTT during lactation is complicated due to the large insulin-independent glucose uptake by the mammary gland through GLUT1 and GLUT3 transporters (Bauman and Currie, 1980; Bell and Bauman, 1997), it can and has been used as a relative measure of changes in insulin resistance in calves and dry cows (Bauman and Currie, 1980; Pires et al., 2007b; Bossaert et al., 2009). Our hypothesis was that changes in insulin resistance would be reflected in both the HEC and GTT.

Materials and methods

Animals, treatments, and daily sampling

All procedures involving animals were approved prior to the onset of the experiment by the Cornell University Institutional Animal Care and Use Committee. The animal phase of this experiment took place from August through November 2008. Nonlactating Holstein cows (n = 12) entering second or greater lactation that had been dried off at approximately 50 d (48 +/- 4.5 d) before expected calving were selected from the Cornell Teaching and Research Center dairy herd and transported to the Large Animal Research and Teaching Unit (LARTU) and housed in individual tie stalls. Cows were assigned randomly to one of two treatments: those fed a high plane of nutrition (High) or those fed a low plane of nutrition (Low). Ingredient and nutrient content of the diets are described in Table 4-1. Samples of concentrate mixtures and TMR were obtained weekly throughout the experiment, and DM content was determined by drying at 55°C until a static weight was observed. Dry matter contents of the TMR were used in calculating DMI for the corresponding week. The weekly TMR samples were composited into 4-wk composite samples and submitted (3 each for both High and Low diets) to a commercial laboratory for wet chemistry analysis

Table 4-1. Experimental diet ingredients and chemical composition.

Ingredient (%, DM basis)	High	Low
Corn silage, processed	17.4	6.2
Wheat straw	20.3	51.0
Soybean hulls	18.75	12.88
Wheat mids	5.17	3.55
AminoPlus™ [1]	19.62	13.48
Soybean meal	8.03	5.52
Calcium Carbonate	2.88	1.98
Molasses	3.02	2.08
Salt	0.27	0.18
Magnesium Oxide	0.74	0.51
Trace mineral premix	0.07	0.05
Vitamin A, D, E premix[2]	0.07	0.05
Dicalcium phosphate	0.87	0.60
Calcium Sulfate	1.74	1.19
Vitamin E premix[3]	1.00	0.69

Chemical Composition[4] (DM basis, ± SD) of experimental diets.

Nutrient	High	Low
NE_L (Mcal/kg)[5]	1.35	1.21
CP (%)	23.3 (5.16)	16.2 (0.42)
Soluble protein, % of CP	21.3 (0.58)	21.0 (3.00)
ADF (%)	26.4 (0.57)	36.3 (0.81)
NDF (%)	44.0 (1.45)	56.7 (1.34)
Ca (%)	2.30 (0.33)	1.50 (0.28)
P (%)	0.50 (0.05)	0.40 (0.06)
K (%)	1.50 (0.11)	1.20 (0.24)
Mg (%)	0.70 (0.06)	0.40 (0.07)
Na (%)	0.20 (0.02)	0.10 (0.02)
S (%)	0.60 (0.02)	0.40 (0.05)

[1]Rumen undegradable protein supplement; AGP® Inc., Omaha, NE

[2]Contained 37, 113 IU/kg of vitamin A, 7, 216 IU/kg vitamin D, and 72, 165 IU/kg of vitamin E

[3]Contained 499,400 IU/kg of vitamin E

[4]Calculated by Dairy One Cooperative (Ithaca, NY) using NRC (2001) equations from three composite samples of each diet

[5]Estimated from CNCPS (v. 6.1)

(Dairy One Cooperative Inc., Ithaca, NY). Samples were analyzed for DM (method 930.15; (AOAC, 2000)), crude protein (CP) (method 990.03; AOAC, 2000), acid detergent fiber (ADF) and neutral detergent fiber (NDF) (Van Soest et al., 1991), and macro- and microminerals (Sirois et al., 1994). Both diets were fed for ad libitum intake, split between feedings that occurred at approximately at 1000 and 1600 h. On d 11-15 of the experiment, an additional feeding occurred at 1200 h in order to maintain more regular feeding intervals prior to the first GTT and HEC.

Body weights were determined for all cows before beginning the feeding period, on d 7 of the experiment, and on d 11, 12 and 13 of the experimental period. Two individuals recorded BCS for all cows prior to the start of the experiment, one week into the experiment, and again at the conclusion of the trial [(1.0 to 5.0 scale, (Wildman et al., 1982)]. The average BCS for both individuals was recorded as the BCS for each cow at a given time period.

Daily blood samples (10 ml) were collected via coccygeal venipuncture in order to determine baseline plasma glucose, insulin, and NEFA concentrations. The overall study design is depicted in Figure 4-1.

Glucose tolerance tests and hyperinsulinemic-euglycemic clamps

Cows were non-surgically fitted with two indwelling jugular catheters into each jugular vein one day prior to the GTT (on d 13). Catheters (Micro-Renathane® Implantation tubing, 2.03 mm o.d. x 1.02 mm i.d.; Braintree Scientific Inc., Braintree, MA) were inserted in conscious cows by percutaneous venipuncture.
Prior to insertion, the area on the neck was clipped to remove the hair, cleaned aseptically using Betadine scrub [Betadine Surgical Scrub (7.5% povidone-iodine); Purdue Frederick, Stamford, CT] and 70% ethanol in water. The jugular vein was

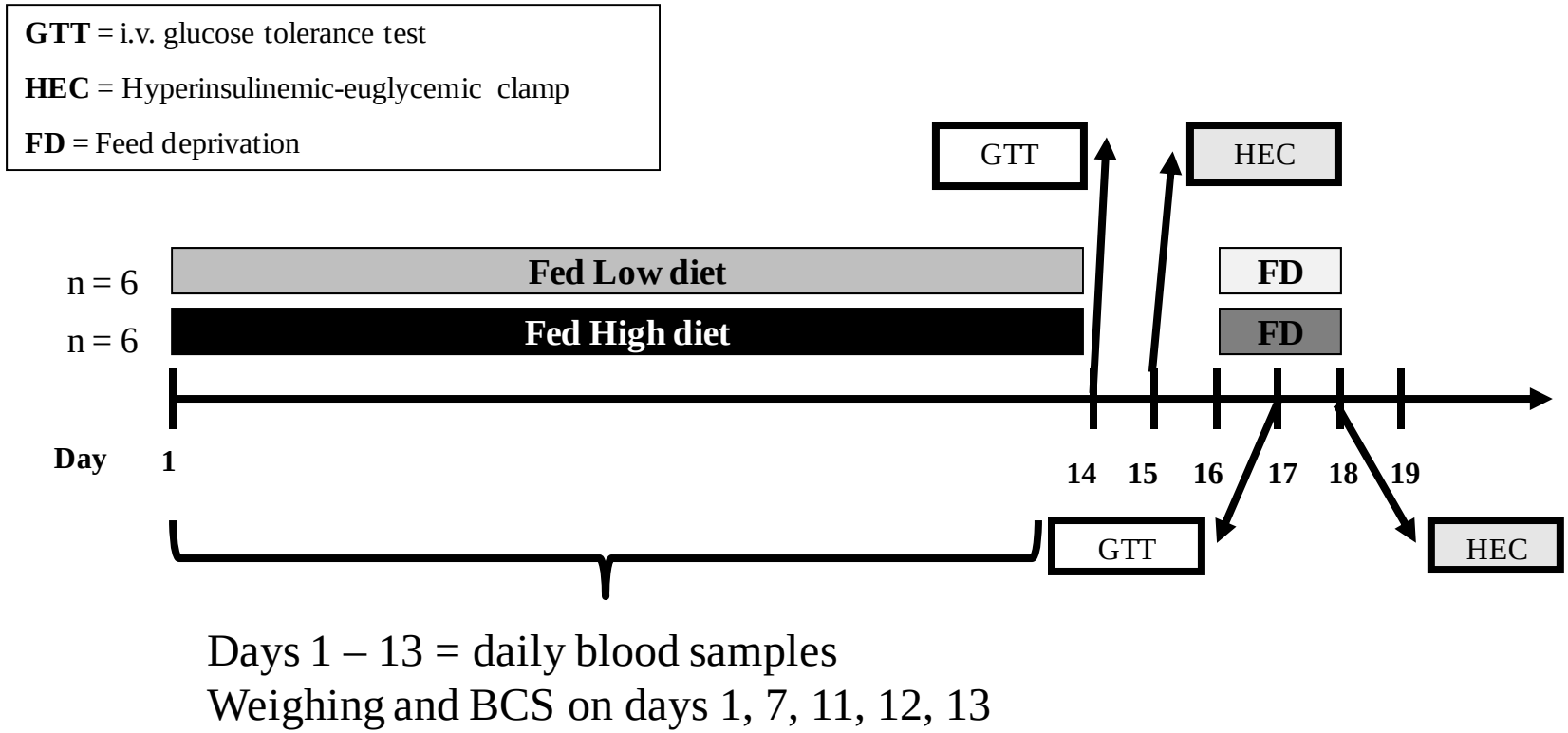

Figure 4-1. Study design. Figure represents overall study layout, by day.

punctured using a 12g Medicut^{TM} (12g, 2" Medicut intravenous cannula; Tyco Healthcare; Gosport, UK) needle and catheter insertion sleeve. Once the needle was removed, approximately 30 cm of sterile catheter was inserted through the Medicut sleeve into the jugular vein. The catheter was checked for proper function and the Medicut sleeve was removed. The catheter was flushed with sterile saline and left filled with sterile saline containing 200 U/mL heparin and secured to the skin of the neck using a butterfly and tag cement, placed within bandages, and the entire neck was wrapped with Optiplaste (Optiplaste 4"; BSN Medical Ltd.; Brierfield, England) and CoFlex (Coflex 4"; Andover Healthcare; Salisbury, MA) bandages to prevent the cow from dislodging the catheter. Patency of the catheters was maintained with regular flushing with heparinized saline (10 U/ml for frequent sampling; 100-500 U/ml otherwise).

On d 14 of the experimental period, all cows were subjected to an intravenous GTT similar to those described previously (Pires et al., 2007b). Feed was removed at approximately 0800 h. Briefly, 0.25 g/kg of body weight of glucose i.v. (dextrose 50% wt/vol) was administered over an average of 6.7 minutes from multiple 60 mL syringes approximately two hours after feed removal. Blood samples (10 ml) were collected -120, -15, -5, 0, 3, 6, 9, 12, 15, 20, 25, 30, 45, 60, 90, 120, 150, and 180 min relative to the glucose infusion from the catheter. The "-120", "-15", and "-5" min samples were taken relative to the beginning of the glucose infusion. The "0" sample was taken concurrently from the opposite jugular vein upon complete infusion from the final 60 mL syringe and the rest of the samples taken relative to the completion of the infusion.

On the following day (d 15), a HEC was performed on all cows. Procedures were similar to those described previously (Petterson et al., 1993; Blum et al., 1999). Briefly, insulin (1µg/kg of body weight; 27 IU/mg; at a rate of 9.0 ml/hr) was infused

into one jugular catheter. Insulin infusates were prepared using a 1 mg/mL bovine insulin (Insulin from bovine pancreas; ≥27 USP units/mg; Sigma Aldrich; St. Louis, MO) stock solution, sterile saline, and plasma harvested from each animal the day before at a final concentration of 2.5% of total infusate. The concentration of insulin required to maintain a 1 μg/kg of BW dose was based on the average of three weights taken on d 11, 12 and 13 of the experiment. Insulin solution was infused using the Macro XL Plum Pump (Abbott Laboratories; Bedford, MA) with a Lifeshield Primary IV Plum Set insert (#1642-48; Hospira; Lake Forest, IL). A priming dose of 1.5 mL of insulin infusate was delivered at the onset of the HEC. Blood samples were taken from the catheter contralateral to the infusion lines for frequent (every 5 min until steady-state concentrations of whole-blood glucose were reached; every 20 min thereafter) cow-side glucose measurements to enable glucose infusion adjustments to maintain euglycemia. Cow-side glucose measurements were conducted on whole blood using a handheld blood glucose meter (Precision Xtra; NDC 57599-8814-1; Abbott Laboratories; Bedford, MA). Euglycemia was determined as ±10% of baseline whole-blood glucose concentrations (the average of samples collected -15 and -5 minutes from the start of the HEC). Glucose (dextrose, 50% wt/vol) was infused i.v. from sterile bags via syringe pump (NE-300; New Era Syringe Pump Systems, Inc.; Farmingdale, NY) for at least 120 min until steady state was maintained. Parameters measured included plasma glucose, insulin, and NEFA concentrations determined as described below. Cows were considered “clamped”, or at steady-state plasma glucose for an average of 86 min. If two steady-states were reached during the duration of the HEC, the second steady-state was considered the clamped values for an individual cow.

Beginning on d 16 of the experimental period, feed for all cows was removed for a total of 56 h beginning at 0900 h. During feed deprivation, cows were

supplemented daily with NRC (NRC, 2001) requirements for vitamins and minerals with wheat middlings as a carrier (1 kg/d). Water was available to all animals for *ad libitum* consumption. During feed restriction, blood samples (10 ml) were collected via catheters twice daily at 0900 and 1600 h. After two days of feed restriction, all cows were subjected to a second GTT (d 18) followed 24 h later by another HEC as described above. At the conclusion of the second HEC, all cows were refed the Low diet for ad libitum intake until returning to the Dairy T&R within three days.

Plasma analyses

Blood samples, regardless of collection methods, were transferred into glass test tubes containing sodium heparin (100 IU/mL blood). Plasma was harvested following centrifugation (2,800 x g for 15 min at 4°C), snap-frozen in liquid N_2, and stored at -20°C until analyses for metabolites and insulin. Plasma concentrations of glucose were determined by enzymatic analysis (glucose oxidase) using a commercial kit (kit 510-A; Sigma Chemical). Intra- and interassay coefiecients of variation (CV) were 5.0% and 8.9% respectively. Plasma concentrations of NEFA were also analyzed by enzymatic analysis (NEFA-C; WAKO Pure Chemical Industries; Osaka, Japan). Intra- and interassay CVs were 3.8% and 12.8% respectively. All spectrophotometric measurements were conducted using a $Versa_{max}$ tunable microplate reader (Molecular Devices, Sunnyvale, CA). Plasma concentrations of insulin were determined by double-antibody radioimmunoassay (Ehrhardt RA et al., 2001). Intra- and interassay CVs were 14.8% and 16.5% respectively.

Calculations

Energy balance was calculated using Cornell Net Protein and Carbohydrate System v. 6.1 (Tylutki et al., 2008).

RQUICKI, the relative insulin sensitivity measure used in ruminants was calculated as the following (Holtenius and Holtenius, 2007):

$RQUICKI = 1 / [\log(G_b) + \log(I_b) + \log(NEFA_b)]$

Where
G_b = Basal glucose (mg/dL)
I_b = Basal insulin (μU/mL)
$NEFA_b$ = Basal NEFA (mmol/L)

such that a lower RQUICKI suggests greater insulin resistance.

Responses to GTT and HEC were measured as area under the curve (AUC), calculated using incremental change and trapezoidal rule via SAS (v. 9.2). Additional responses such as clearance rates, half-life, and time to reach basal concentrations were estimated using Proc NLIN of SAS (v. 9.2). The following represents an example of the SAS code used to generate estimated parameters:

```
Data GTTnlin8095fed;
INPUT Cow diet$ group time glc;
DATALINES;
8095   low    1      a      0      170
8095   low    1      a      3      158.4
8095   low    1      a      6      158.1
8095   low    1      a      9      153.3
8095   low    1      a      12     147.1
8095   low    1      a      15     145.7
8095   low    1      a      20     140.1
8095   low    1      a      25     125.3
8095   low    1      a      30     123.8
8095   low    1      a      45     113.6
8095   low    1      a      60     98.6
run;
proc nlin data = GTTnlin8095fed;
parms A =      170
      k = -1 to 1
        ;
        model glc = A * exp(-k*time);
        output out=p p=predict;
        run;
```

Parameter A represents a starting value for maximum plasma concentration, as entered from analyzed results. Clearance rate (CR) was estimated using the slope of the line (for the first 30 min for responses in plasma glucose, the first 60 min for plasma NEFA) generated by estimated values from SAS.

Time to reach half maximal concentration was calculated as:
{[ln(2)]/CR}x100;

Time to reach basal concentration was calculated as:
{(ln[ta] – ln[tb])/CR} x100;
where ta and tb are the concentration in plasma at times a and b respectively.

Statistical analysis

Statistical analysis was performed as analysis of variance (ANOVA) on measures conducted over time (plasma concentrations) using the MIXED procedure of SAS (2001) for a completely randomized design with repeated measures where appropriate. The statistical model included fixed effects of covariate, treatment, time and the interaction of treatment and time. The random effect was cow nested within treatment. For each variable, six covariance structures were evaluated (first-order autoregressive, heterogeneous first-order autoregressive, compound symmetry, heterogeneous compound symmetry, first-order ante-dependence, and unstructured) and the structure with the smallest Akaike's information criterion was used for further analysis. The method of Kenward-Rogers was used for calculation of denominator degrees of freedom. Covariates were dropped from the statistical model if $P > 0.15$ and the data were reanalyzed. Significance was declared as P values ≤ 0.05; trends as $0.15 \geq P \geq 0.05$.

Results

Daily intake and plasma

Figure 4-2 illustrates the patterns of DMI throughout the trial. There was a significant diet by day interaction ($P = 0.03$) such that cows fed the low diet consumed less DM than cows fed the high diet (10.9 vs. 18.5 kg DMI/d). Table 4-2 shows the average plasma metabolite concentrations for each treatment during the first 13 d of the experimental period and after 24 and 48 h of feed deprivation. There was no effect of treatment on plasma glucose for the first 13 d of the trial (66.8 for low vs. 66.4 mg/dL for high; $P = 0.84$). During the first 13 d of the experiment, cows fed the Low diet had higher plasma NEFA (164 vs. 73 μEq/L; $P < 0.01$) and lower plasma insulin (5.2 vs. 11.1 μIU/mL; $P < 0.01$) concentrations. There was no effect of treatment on calculated RQUICKI for the first 13 d ($P = 0.57$). Cows fed the Low diet had higher circulating NEFA after 24 (929 vs. 656 μEq/L; $P = 0.03$) and 48 h (1361 vs. 923 μEq/L; $P = 0.01$) of feed deprivation. There were no significant differences in basal plasma glucose levels throughout the trial ($P > 0.20$), and no significant differences in plasma insulin after 24 or 48 h of feed deprivation ($P > 0.10$).

Glucose tolerance tests and hyperinsulinemic-euglycemic clamps

Figure 4-3 illustrates the change in plasma glucose levels following glucose infusion; glucose clearance was slower in cows fed the Low diet and during feed deprivation. Responses are quantified in Table 4-3. The most apparent differences, as detailed in Table 4-3, were found between cows in either the fed versus feed-deprived state. After 24 h of feed deprivation, cows had lower basal plasma glucose (55 vs. 68 mg/dL; $P < 0.001$), lower minimum glucose during GTT (50 vs. 62 mg/dL; $P < 0.001$), slower glucose clearance rate (1.5 vs. 2.3 %/min; $P = 0.003$), greater glucose

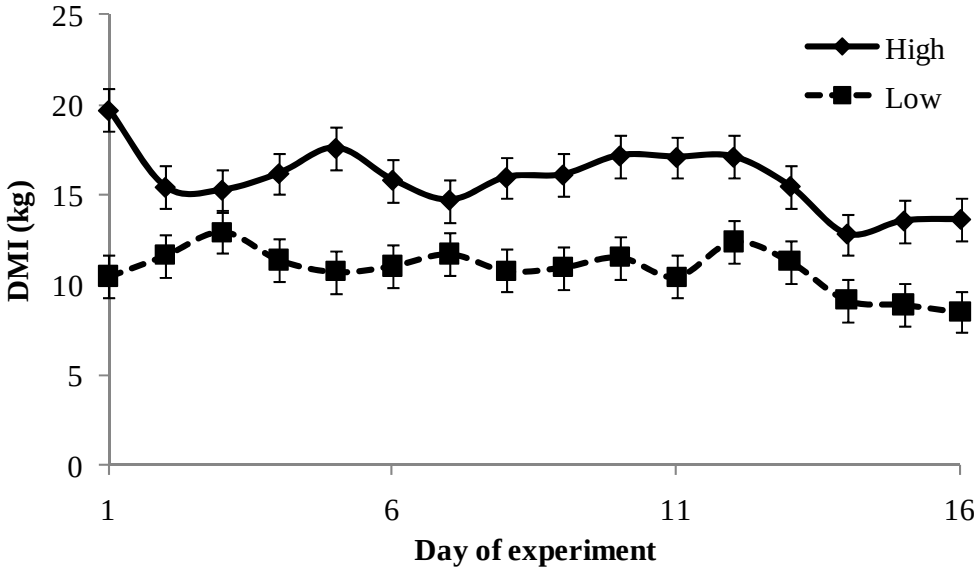

Figure 4-2. Daily dry matter intake. Cows were fed one of two dietary treatments (High vs. Low plane of nutrition) for two weeks as represented by day of experiment. Values represent least squares means with error bars representing SEM; n = 6 for both High and Low. The *P* value for effect of dietary treatment was *P* = < 0.0001; for day was *P* = 0.01; for diet by day interaction was P = 0.03.

Table 4-2. Daily metabolites. For cows fed two dietary energy levels (High and Low) for 14 days and subjected to a 24 and 48 hour feed deprivation.

	d 1 to 13				After 24 hr feed deprivation				After 48 hr feed deprivation			
	Treatment				Treatment				Treatment			
	Low	High	SEM	*P*	Low	High	SEM	*P*	Low	High	SEM	*P*
NEFA (µEq/L)	**163.6**	**73.1**	11.5	**<0.01**	**928.9**	**656.4**	74.9	**0.03**	**1361.1**	**923.0**	98.6	**0.01**
Glucose (mg/dL)	66.4	66.8	1.3	0.84	53.1	56.1	1.8	0.25	45.0	50.3	2.7	0.20
Insulin (µIU/mL)	**5.2**	**11.1**	1.2	**<0.01**	2.6	5.4	1.1	0.10	1.7	2.1	0.40	0.60

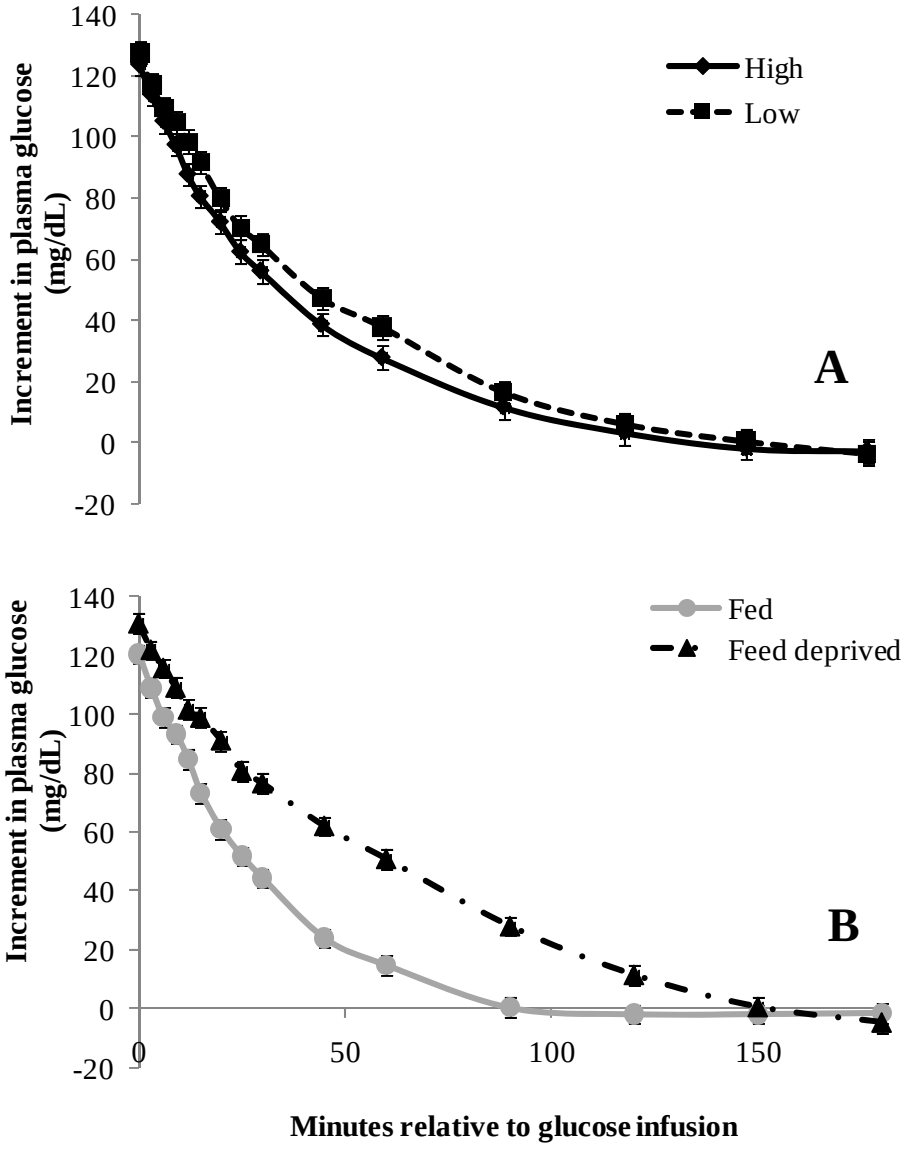

Figure 4-3. Plasma glucose responses to glucose tolerance test. Cows were fed one of two dietary treatments for two weeks (High vs. Low; A) and subjected to an intravenous glucose tolerance test in fed state or after 24 h of feed deprivation (Fed vs. Feed deprived; B). Values represent least squares means, incremental change from basal plasma levels by minute relative to glucose infusion. Error bars represent SEM. Results detailed in Table 4-3.

Table 4-3. Effects of diet and feed deprivation on glucose response to i.v. glucose tolerance test.

	Diet				Fed vs. Feed Deprived (FD)				Diet*Fed/Feed Deprived (FD) Interaction					
Measure[a]	High	Low	SEM	*P*	Fed	FD	SEM	*P*	High Fed	High FD	Low Fed	Low FD	SEM	*P*
Basal glucose (mg/dL)	62.5	59.7	1.6	0.25	67.6	54.6	1.2	<**0.001**	68.9	56.1	66.4	53.1	1.7	0.67
Minimum glucose (mg/dL)	57.9	53.7	1.8	0.15	62.0	49.6	1.4	<**0.001**	65.3	50.5	58.7	48.8	2.0	**0.07**
Maximum glucose (mg/dL)	188.3	185.5	3.8	0.61	186.3	187.6	3.0	0.66	187.9	188.7	184.6	186.4	4.3	0.87
CR_{30} (%/min)	2.0	1.8	0.15	0.39	2.3	1.5	0.15	**0.003**	2.4	1.6	2.1	1.5	0.20	0.64
$T_{1/2}$ (min)	39.3	42.6	3.7	0.54	33.0	49.0	3.7	**0.007**	29.5	49.1	36.4	48.8	5.3	0.51
T_{basal} (min)	90.2	105.3	6.6	**0.12**	68.8	126.7	6.6	<**0.001**	59.1	121.4	78.5	132.1	9.4	0.65
AUC_{30}	2537	2732	11	0.25	2280	2989	85	<**0.001**	2148	2926	2412	3052	121	0.32
AUC_{60}	3750	4213	168	**0.07**	3085	4878	168	<**0.001**	2779	4721	3392	5034	237	0.53
AUC_{90}	4344	5032	260	**0.09**	3313	6063	243	<**0.001**	2009	5780	3718	6346	343	0.71

[a] Basal glucose = mean glucose concentration at -15 and -5 min prior to GTT; Minimum glucose = minimum glucose during GTT; Maximum glucose = maximum glucose during GTT; CR_{30} = clearance rate during the first 30 min of GTT; $T_{1/2}$ = Time to reach half maximal glucose concentration; T_{basal} = time to reach basal glucose concentration; AUC_{30} = area under the curve during the first 30 min of GTT [mg/dL x 30 min]; AUC_{60} = area under the curve during the first 60 min of GTT [mg/dL x 60 min]; AUC_{90} = area under the curve during the first 90 min of GTT [mg/dL x 90 min]

half-life (49 vs. 33 min; $P = 0.007$), longer time to return to basal (127 vs. 69 min; $P < 0.001$), and greater AUC (4878 vs. 3085 mg/dL x 60 min; $P < 0.001$). There were no differences among treatments in maximum glucose during GTT, as would be expected ($P < 0.61$). There were no significant differences observed based on dietary treatment ($P > 0.07$), although AUC tended to be higher for cows fed the Low diet (4213 vs. 3750 mg/dL x 60 min; $P = 0.07$) and the interaction of both dietary treatment and fed versus feed-deprived state was also insignificant ($P > 0.07$).

Similar to plasma responses in glucose, the most apparent differences in plasma insulin during GTTs were between the fed versus feed-deprived state (Figure 4-4 and Table 4-4). As depicted in Figure 4-4, insulin secretion in response to glucose infusion was dramatically lower for cows in the feed-deprived versus fed state. Cows after 24 h of feed deprivation had lower basal insulin (4 vs. 8 μIU/mL; $P = 0.08$), had lower maximum insulin (37 vs. 160 μIU/mL; $P < 0.001$), and lower insulin AUC (995 vs. 3967 μIU/mL x 60 min; $P < 0.001$; Table 4-4). There were no differences in plasma insulin response during GTT based on dietary treatment ($P > 0.21$), nor interactions of dietary treatment and whether or not cows were in the Fed state ($P > 0.14$).

There were significant differences based on dietary treatment as measured by changes in plasma levels of NEFA during the GTTs. Illustrated in Figure 4-5, cows fed the Low diet had a greater overall reduction in plasma NEFA during GTT and cows after 24 h of feed deprivation had greater responses in plasma NEFA following glucose infusion. These responses are quantified in Table 4-5. Based on dietary treatment, cows that were fed the Low diet had higher basal NEFA prior to glucose infusion (636 vs. 386 μEq/L; $P = 0.02$), had higher minimum NEFA during GTT (272 vs. 134 μEq/L; $P = 0.04$), greater NEFA clearance rate (7.1 vs. 4.5 %/min), and more

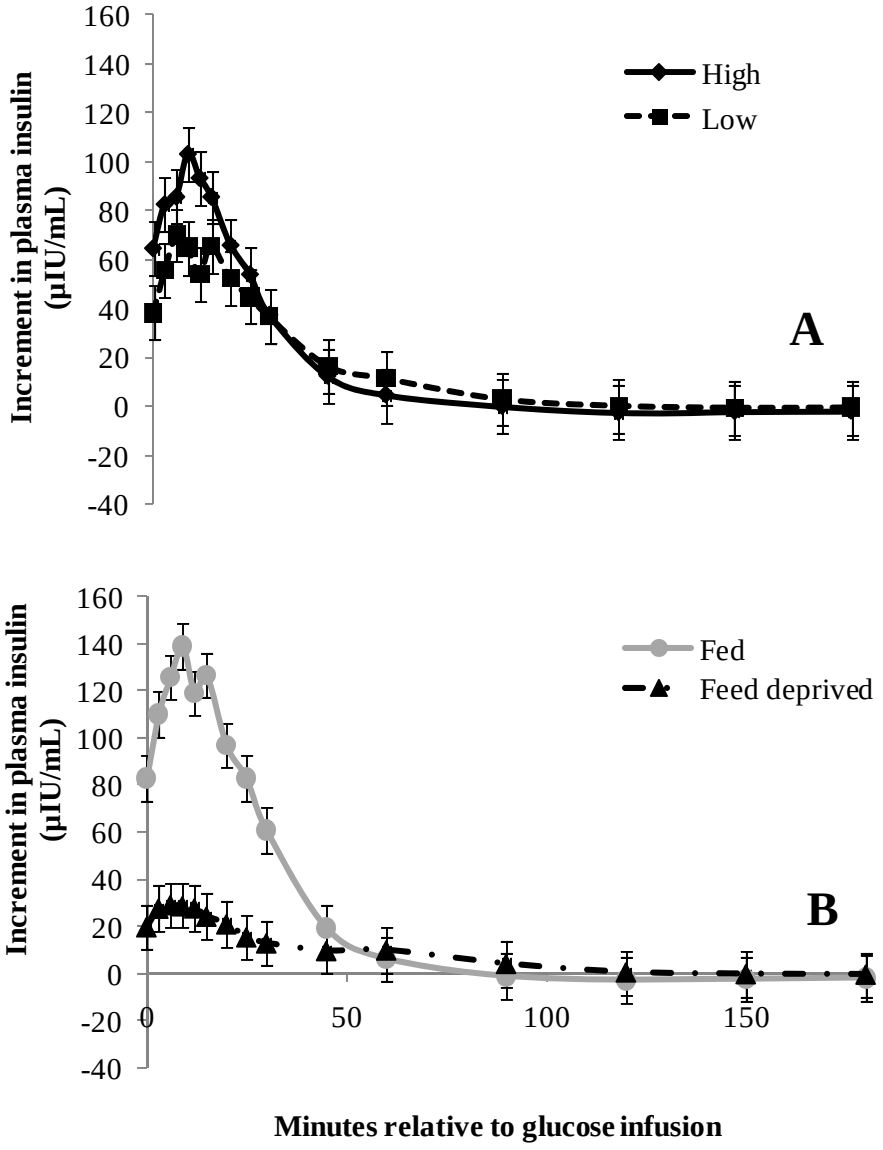

Figure 4-4. Plasma insulin responses to glucose tolerance test. Cows were fed one of two dietary treatments for two weeks (High vs. Low; A) and subjected to an intravenous glucose tolerance test in fed state or after 24 hours of feed deprivation (Fed vs. Feed deprived; B). Values represent least squares means, incremental change from basal plasma levels by minute relative to glucose infusion. Error bars represent SEM. Results detailed in Table 4-4.

Table 4-4. Effects of diet and feed deprivation on plasma insulin response to i.v. glucose tolerance test.

	Diet				Fed vs. Feed Deprived (FD)				Diet*Fed/Feed Deprived (FD) Interaction					
Measure[a]	High	Low	SEM	*P*	Fed	FD	SEM	*P*	High Fed	High FD	Low Fed	Low FD	SEM	*P*
Basal insulin (µIU/mL)	7.8	4.4	1.2	**0.08**	8.1	4.1	1.8	**0.08**	10.2	5.5	6.1	2.6	2.5	0.78
Maximum insulin (µIU/mL)	113.7	83.3	20.1	0.31	159.6	37.4	22.7	**<0.001**	186.2	41.3	133.0	33.6	32.1	0.25
AUC_{30}	2220	1629	320	0.21	3166	682	320	**<0.001**	3756	683	2576	681	453	0.21
AUC_{60}	2714	2237	507	0.53	3957	995	408	**<0.001**	4485	943	3428	1047	577	0.17
AUC_{90}	2779	2453	533	0.68	4030	1202	415	**<0.001**	4468	1089	3591	1315	587	**0.14**

[a] Basal insulin = mean insulin concentration at -15 and -5 minutes prior to IVGTT; Maximum insulin = minimum insulin during IVGTT; AUC_{30} = area under the curve during the first 30 min of IVGTT [µIU/mL x 30 min]; AUC_{60} = area under the curve during the first 60 min of IVGTT [µIU/mL x 60 min]; AUC_{90} = area under the curve during the first 90 min of IVGTT [µIU/mL x 90 min]

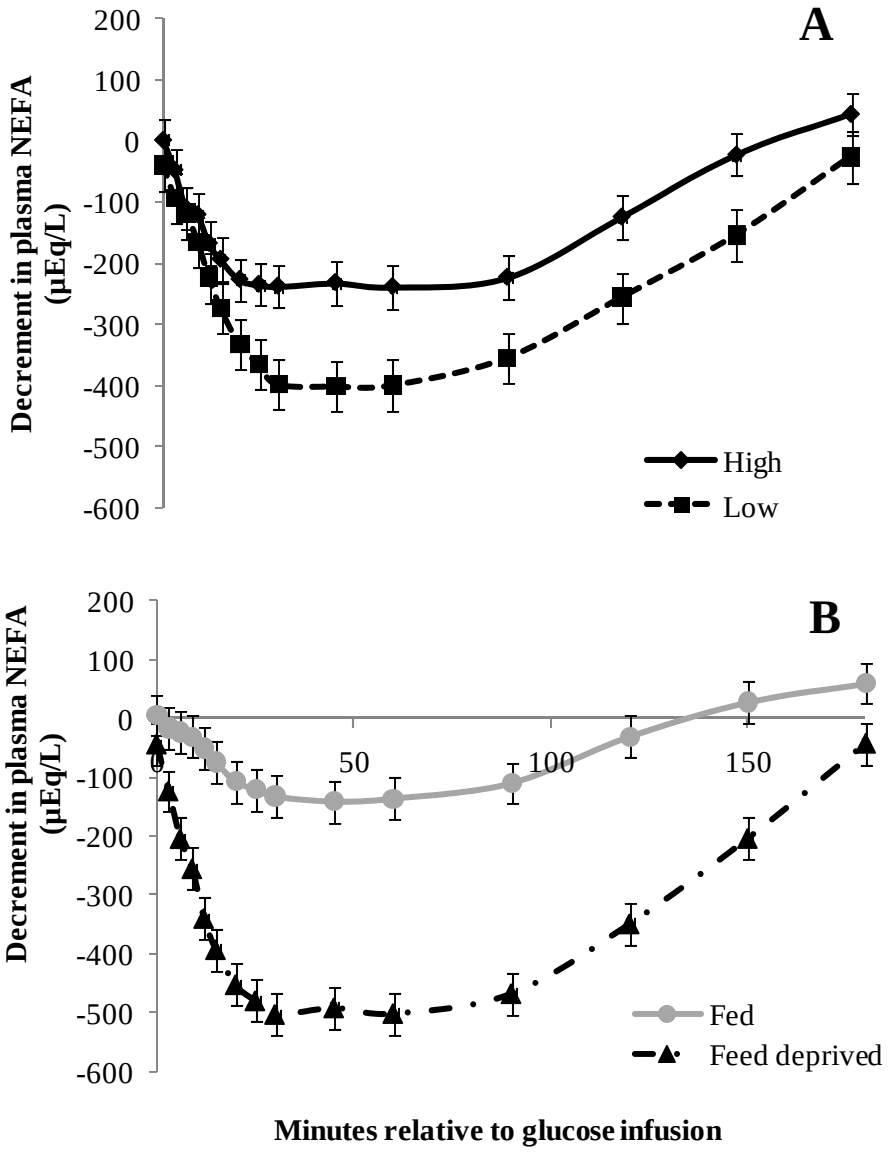

Figure 4-5. Plasma non-esterified fatty acid responses to glucose tolerance test. Cows were fed one of two dietary treatments for two weeks (High vs. Low; A) and subjected to an intravenous glucose tolerance test in fed state or after 24 h of feed deprivation (Fed vs. Feed deprived; B). Values represent least squares means, incremental change from basal plasma levels by minute relative to glucose infusion. Error bars represent SEM. Results detailed in Table 4-5.

Table 4-5. Effects of diet and feed deprivation on plasma NEFA response to i.v. glucose tolerance test.

	Diet				Fed vs. Feed Deprived (FD)				Diet*Fed/Feed Deprived (FD) Interaction					
Measure[a]	High	Low	SEM	*P*	Fed	FD	SEM	*P*	High Fed	High FD	Low Fed	Low FD	SEM	*P*
Basal NEFA (µEq/L)	386.4	625.5	54.8	**0.02**	219.3	792.6	43.4	<**0.001**	116.3	656.4	322.2	928.9	61.4	0.42
Minimum NEFA (µEq/L)	133.5	271.8	40.6	**0.04**	76.1	329.3	54.5	<**0.001**	60.3	206.8	91.9	451.7	14.9	**0.07**
CR_{60} (%/min)	4.5	7.1	0.77	**0.04**	2.9	8.7	0.62	<**0.001**	1.6	7.4	4.2	10.0	0.89	0.95
$T_{1/2}$ (min)	45.2	17.4	10.6	**0.09**	53.9	8.7	14.6	**0.01**	80.7	9.7	27.1	7.8	20.6	**0.10**
GSRN	59.5	67.4	3.8	**0.18**	58.4	68.4	4.4	**0.03**	50.3	68.6	66.4	68.3	6.2	**0.07**
AUC_{30}	-5009	-7447	777	**0.04**	-2129	-10326	777	<**0.001**	-664	-9354	-3595	-11298	1099	0.66
AUC_{60}	-12055	-19044	1789	**0.01**	-6258	-25238	1789	<**0.001**	-2326	-21785	-10189	-28690	2530	0.85
AUC_{90}	-18978	-30739	2865	**0.01**	-9944	-39773	2865	<**0.001**	-3697	-34260	-16191	-45287	4052	0.86

[a] Basal NEFA = mean NEFA concentration at -15 and -5 minutes prior to IVGTT; Minimum NEFA = minimumNEFA during IVGTT; $CR6_0$ = clearance rate during the first 60 min of IVGTT; GSRN = glucose-stimulated reduction of NEFA (% reduction from basal); $T_{1/2}$ = Time to reach half maximal NEFA concentration; AUC_{30} = area under the curve during the first 30 min of IVGTT [µEq/L x 30 min]; AUC_{60} = area under the curve during the first 60 min of IVGTT [µEq/L x 60 min]; AUC_{90} = area under the curve during the first 90 min of IVGTT [µEq/L x 90 min]

substantial AUC (-19044 vs. -12055 μEq/L x 60 min; $P = 0.01$). There was also a tendency for cows fed the Low diet to have a faster NEFA half-life (17 vs. 45 min; $P = 0.09$). All measured responses for cows in the fed versus feed-deprived state were significant such that feed-deprived cows had higher basal NEFA (793 vs. 219 μEq/L; $P < 0.001$), higher minimum NEFA (329 vs. 76; $P < 0.001$), greater NEFA clearance rate (8.7 vs. 2.9 %/min; $P < 0.001$), faster NEFA half-life (8.7 vs. 53.9 min; $P = 0.01$), greater glucose-stimulated reduction in NEFA (68.4 vs. 58.4 % reduction from basal; $P = 0.03$), and more substantial AUC (-25238 vs. -6258 μEq/L x 60 min; $P < 0.001$). There were no significant interactions of dietary treatment and whether or not cows were in the Fed state in plasma NEFA response to GTT ($P < 0.07$).

The responses measured during HEC take place during the steady-state, or "clamped" phase. Metabolic responses to HEC are detailed in Table 4-6 and reflect significant differences in response values during steady-state for HEC performed during the fed versus feed-deprived state. Feed-deprived cows required less glucose to maintain euglycemia (90 vs. 203 mL/hr; $P < 0.001$), had higher plasma NEFA during the clamp (330 vs. 63 μEq/L; $P < 0.001$), and greater NEFA suppression (69 vs. 41%; $P < 0.001$). As expected, there was no difference in insulin concentration during the clamp across all treatments ($P > 0.18$). In terms of dietary effects, there were no differences in glucose required to maintain euglycemia based on dietary treatment ($P = 0.81$), but there was a tendency for cows fed the Low diet to have greater NEFA suppression (60 vs. 50 %; $P = 0.11$). There was a significant ($P = 0.004$) dietary and fed versus feed-deprived state interaction observed in plasma NEFA during the HEC such that cows fed the High diet and in the Fed state had the lowest plasma NEFA (55 μEq/L), cows fed the Low diet and in the feed-deprived state had the highest plasma NEFA (456 μEq/L), and cows in the feed-deprived state previously receiving the High diet had intermediary NEFA levels (205 μEq/L), as did cows receiving the Low

Table 4-6. Variables measured or calculated during steady-state hyperinsulinemic-euglycemic clamp.

	Diet				Fed vs. Feed Deprived (FD)				Diet*Fed/Feed Deprived (FD) Interaction					
Measure[a]	High	Low	SEM	*P*	Fed	FD	SEM	*P*	High Fed	High FD	Low Fed	Low FD	SEM	*P*
Dextrose infusion rate (mL/hr)	149.0	144.5	12.1	0.81	203.2	90.1	17.0	**<0.001**	195.0	102.9	211.6	77.4	25.3	0.25
Insulin at clamp (µIU/mL)	34.9	55.1	9.7	0.18	46.9	43.1	10.7	0.66	40.6	29.1	53.2	53.2	15.1	0.40
Plasma NEFA (µEq/L)	130.0	262.9	22.7	**0.001**	62.6	330.4	30.4	**<0.001**	55.1	204.9	70.1	455.8	42.8	**0.004**
NEFA Suppression (%)	49.9	60.1	4.3	**0.11**	41.1	68.9	4.3	**<0.001**	20.7	79.1	61.5	58.6	6.1	**<0.001**

[a] NEFA Suppression calculated as: (1-((NEFA at clamp (µEq/L) / basal NEFA)) x 100

diet and in the Fed state (70 μEq/L). Suppression of NEFA during the clamp also had an interaction ($P < 0.001$) of diet and Fed state such that cows fed the High diet in the Fed state had 21% NEFA suppression, cows previously fed the High diet in the feed-deprived state had 79% NEFA suppression, cows previously fed the Low diet in the feed-deprived state had 62% NEFA suppression and cows previously fed the Low diet in the feed-deprived state had 59% NEFA suppression. Finally, there were no significant dietary and Fed versus feed-deprived state interactions related to either glucose required to maintain euglycemia or insulin concentration during the clamp ($P > 0.25$).

Discussion

The original intent of this experiment was to determine the effects of dietary energy level on insulin sensitivity and responsiveness and the potential interaction of dietary energy (maintenance versus high) when cows are in negative energy balance. Due to a feed mixing error, the dietary energy level of the low energy level diet was much lower than originally anticipated. Furthermore, the high forage level of the Low diet limited intake as shown in Figure 4-2. The result was that cows fed the High diet were meeting 143% of calculated energy requirement and cows fed the Low diet were meeting 88% of calculated energy requirement based on CNCPS v. 6.1 (Tylutki et al., 2008). Though there were no significant differences in daily plasma glucose for cows fed both diets, the cows fed the High diet had two-times greater circulating insulin levels (Table 4-2), corresponding with previous results for cows on high planes of nutrition (Holtenius et al., 2003). The result of the low dietary energy level (Table 4-1) in conjunction with lower intake resulted in higher plasma NEFA for cows fed the Low diet (Table 4-2).

Based on prior work (Holtenius et al., 2003), and because cows in the current study fed the High diet had higher circulating levels of insulin but no difference in plasma glucose (versus cows fed the Low diet), we expected that cows fed the High diet would be more insulin resistant. The RQUICKI calculation has been used to describe differences in apparent insulin resistance in ruminants (Holtenius and Holtenius, 2007). Additionally, as seen in Chapter 3, RQUICKI may detect differences in insulin resistance based on day relative to calving. However, despite this, there were no significant differences in calculated RQUICKI for the first two weeks in this experiment. This may correlate with the lack of dietary differences as measured by responses to GTT in the Fed state (Table 4-3). As discussed below, responses to GTT as measured by changes in fatty acid metabolism may be more meaningful in this experimental design.

In addition, the results using RQUICKI as a measure of insulin resistance in ruminants have been mixed. Although Holtenius and Holtenius (2007) drew conclusions about insulin sensitivity using RQUICKI in healthy obese cows, strong correlations with insulin sensitivity measures in cows with metabolic disorders are lacking (Kerestes et al., 2009). Bossaert et al. (2009) were able to correlate RQUICKI and GTT measures in calves, further illustrating the need for future evaluation of RQUICKI as a measure of insulin sensitivity. It appears that RQUICKI may be an appropriate measure in some metabolic circumstances but may lack the ability to detect differences in others. In this case, RQUICKI was unable to detect treatment differences. It is worth repeating that treatment differences based on diet were less dramatic in the current study and may be the reason why differences in RQUICKI were not significant.

The high NEFA likely was a factor in the slower glucose clearance during GTTs (Figure 4-3; (Pires et al., 2007a)). This shift in glucose clearance to the right (as

observed in this trial as decreased glucose clearance rates, increased half-life, increased time to basal, and increased AUC) is assumed to involve decreased insulin sensitivity (Kahn, 1978). Because a single glucose dose was used in this instance there is little that can be ultimately said about changes in responsiveness, although it is noted that there was no difference in peak plasma glucose levels for any of the treatment combinations. It could be argued that this would implicate more changes in insulin sensitivity than responsiveness in these cows (Kahn, 1978). Additionally, responses to GTT do involve coordinated responses related to both insulin as well as direct glucose effectiveness (Kahn, 1978; Muniyappa et al., 2008).

Compared to the results seen here, Pires et al. (2007b) reported similar changes in glucose clearance for cows that were treated with a tallow infusion for 11 h in order to increase plasma NEFA. The authors were attempting to mimic the increase in plasma NEFA that occurs in early lactation in a similar manner to our feed deprivation situation, and admit that different physiological conditions exist in the experimentally induced negative energy balance versus early lactation. However, elevation of NEFA is only one aspect that affects insulin resistance during late pregnancy and early lactation. The effect of elevated NEFA on glucose clearance is illustrated in the dramatic differences in clearance of glucose in fed versus feed-deprived cows (Figure 4-3). In this experiment, the effect of a fed versus feed-deprived state was much larger than plane of nutrition prior to feed deprivation.

Interestingly, the plasma concentrations of NEFA that Pires et al. (2007b) reported in their study were nearly identical to those cows in the current study on the Low diet in the fed state. This suggests that in the current study there was likely an element of insulin resistance due to low energy balance even during the fed state, which may account for the slightly slower glucose clearance in cows fed the Low diet as measured by AUC (Table 4-3). Additionally, Pires et al. (2007a) also completed

GTT on cows following 48-h of feed deprivation that were treated or not with nicotinic acid in order to reduce NEFA. Cows that were feed-deprived for 48 h and not treated with nicotinic acid had similar plasma NEFA as the cows in the current study on the High diet after feed deprivation. Like this study, cows with higher NEFA had slower glucose clearance. The difference, however, was that cows in the current study had much lower secretion of insulin.

Slower glucose clearance would suggest reduced insulin secretion, decreased sensitivity or responsiveness of tissues to insulin, or a combination of these. Certainly for cows in the feed-deprived state, there was attenuated insulin secretion during the GTT (Figure 4-5). Cows in the feed-deprived state had a much lower insulin AUC as well as lower maximum insulin secretion in response to glucose infusion. Hove (1978) deprived lactating cows of feed for 48 h and subjected them to a GTT. He noted a much lower insulin response to GTT, and that unlike non-ketotic cows, there was lack of a biphasic insulin response. A similar biphasic response was seen in fed cows in this study (Figure 4-5). Likewise, cows with metabolic acidosis are shown to have much lower insulin response to glucose challenge (Bigner et al., 1996). In GTT performed in pregnant or nonpregant ewes, Regnault et al. (2004), determined that pregnant ewes had much lower insulin secretion compared to non-pregnant nonlactating ewes. This corresponds with increasing rates of lipolysis in ewes as gestation progresses, which allows circulating NEFA to act as an alternate fuel source and promote insulin resistance of peripheral tissues in order to promote substrate use by the gravid uterus (Regnault et al., 2004). Insulin deficiency that was produced artificially through the use of a somatostatin infusion in dogs showed evidence of the direct effects of hyperglycemia on fatty acid metabolism (Park et al., 1990).

There were additional differences in fatty acid metabolism in response to GTT in this experiment. Despite having the lowest secretion of insulin during GTT in this

experiment, cows previously fed the Low diet and in a feed-deprived state had the greatest clearance rates of NEFA following glucose infusion (Table 4-4). Previous reports of NEFA responses during GTT in cows that were supplemented with chromium in an effort to improve insulin sensitivity showed no significant differences in NEFA clearance rates (Hayirli et al., 2001; Sumner et al., 2007). Given that cows fed the Low diet or cows in the feed-deprived state experienced greater reduction in plasma NEFA during GTT, had greater NEFA clearance rate during GTT, and greater AUC, this may suggest that these cows had lower insulin resistance related to lipid metabolism despite having slower glucose clearance. In a study performed in hypertensive adult sheep, there were no significant differences in glucose-associated insulin resistance but there was increased insulin sensitivity of the inhibition of lipolysis (Gatford et al., 2000).

Similar to Gatford et al. (2000), there were differences in plasma NEFA during HEC steady-state observed in this experiment. Not only did cows on the Low diet and those in a feed-deprived state have faster NEFA CR during GTT, but they also had a greater suppression of NEFA during HEC (Tables 4-4 and 4-6). These differences were also noted without having significant differences in circulating insulin during the clamp. One of the only reported prior instances of comparing both HEC and GTT in ruminant animals was done in calves (Hostettler-Allen et al., 1994). In the case of calves, the authors did little to compare data gleaned from the HEC and GTT, but did note changes in insulin resistance as measured by multiple methods. For the current experiment, an overall average glucose infusion rate of 2.1 mg dextrose/min^{-1} kg BW^{-1}, is slightly lower than doses required to maintain euglycemia in calves (Hostettler-Allen et al., 1994). The authors concluded that calves are more sensitive to insulin than adult ruminants, as would be supported by comparing the results of this experiment. When comparing results in unfed calves, there also seems

to be attenuated insulin secretion in response to glucose challenge (Hostettler-Allen et al., 1994). Unfortunately, unlike in this trial, HEC and GTT were not preformed on the same animals. The study described here may be the only trial completed in ruminants where HEC and GTT were performed on the same animals in both a fed and feed-deprived state.

An additional outcome of this experiment was the ability to compare the overall results obtained from the GTT and HEC. Although the HEC is considered the "gold standard" for measuring insulin sensitivity (DeFronzo et al., 1979), a great deal of information can be obtained from the GTT at a much lower input of resources. The benefit to using the HEC is that it allows one to measure glucose disposal rate without having the added effect of increased insulin section (DeFronzo et al., 1979). One downfall to the HEC is that it requires steady-state to be reached at supraphysiological levels and therefore may not accurately reflect dynamic or acute changes that may be biologically relevant (i.e. in terms of responses to meals, feed deprivation, or acute stress; (Trout et al., 2007). In this case, measures of insulin resistance between the GTT and HEC corresponded such that cows that were without feed for 48 h required a lower dextrose infusion rate to maintain euglycemia during the HEC (Table 4-6). The corresponding measure during the GTT would be the slower glucose clearance for feed-deprived cows. Neither the GTT nor HEC detected significant differences based on dietary treatment in insulin resistance (as measured by clearance rates and AUC during GTT and glucose infusion rates during HEC).

Both the GTT and HEC detected potential differences in fatty acid metabolism based on dietary treatment and whether or not cows were in the fed versus feed-deprived state. In the case of GTT, as described above, cows fed the Low diet had greater NEFA clearance and AUC in response to glucose infusion. These observations correspond with greater NFEA suppression in cows fed the Low diet at steady-state

during the HEC. Differences in fatty acid metabolism for cows that were either in the Fed versus feed-deprived state were also depicted in the HEC such that the cows in the feed-deprived state had greater NEFA suppression during HEC, corresponding with greater NEFA clearance rates and AUC during GTT. The significant interaction of diet and Fed versus feed-deprived state as measured during the HEC by NEFA suppression had very similar relationships as corresponding measurements during GTT. For glucose-stimulated reduction in NEFA during GTT, cows in the feed-deprived state fed the High diet had the greatest NEFA suppression while cows in the Fed state fed the High diet had the lowest NEFA suppression. Similarities in these responses between the HEC and GTT suggest that measures from GTT and HEC may be comparable.

Conclusions

Although the original intent of this experiment was to determine the effect of plane of nutrition and feed deprivation on insulin and glucose metabolism in far-off dry cows, the overall effects of feed deprivation proved to be much greater than that of prior plane of nutrition. Cows not consuming feed for 24 h had slower glucose clearance during GTT than those in the fed state. In addition, cows without feed for 48 h required less glucose to maintain euglycemia during HEC. Furthermore, plasma NEFA responses were more intriguing, the most notable being the dramatic response in plasma NEFA (greater NEFA clearance rate and AUC) during GTT despite attenuated insulin secretion (as measured by AUC) in cows not consuming feed for 24 h. These responses correspond with greater NEFA suppression for cows in the feed-deprived state during HEC. While the effect of diet on circulating NEFA had an effect on metabolic responses (as seen in reduced glucose clearance and greater reduction in NEFA during GTT and HEC), there was less of an effect on glucose metabolism

directly. For example, there was only a slight reduction in plasma glucose clearance during GTT for cows fed the Low versus High diet as measured by glucose AUC. Additionally, responses were for the most part void of interaction between dietary treatments and fed versus feed-deprived measures.

These results have implications from the standpoint of far-off dry cattle nutrition as well as methodological approaches. In terms of dry cow nutrition, it is important to note that extreme negative energy balance will have an effect on glucose, insulin, and fatty acid metabolism. Cows in this trial in negative energy balance had reduced glucose tolerance but potentially heightened response to glucose and insulin in terms of fatty acid metabolism. After 24 h of feed removal, cows had much lower secretion of insulin, adding evidence that prolonged negative energy balance and increased NEFA may reduce the pancreas' ability to secrete insulin as has been seen in ketotic and feed-deprived animals. It further proves that prolonged negative energy balance should be avoided, as is often seen in early lactation. The experiment also provided evidence for the use of GTT and HEC in experimental design since many variables measured between the two corresponded with each other. While comparison of results in both situations may not be appropriate in some instances, it appears as though GTT might be a suitable candidate to answer experimental questions related to glucose, insulin, and fatty acid metabolism.

ACKNOWLEDGEMENTS

The assistance of the following individuals was greatly appreciated for the completion of this study: R. Ehrhardt, J.M. Huzzey, L.A. Winkelman, C. Hammont, M. Cummings, P.Molesky, J. Schoenberg, M. Ramos, L. Furman, D.Parr, and the staff at the Cornell University Dairy Teaching and Research Center.

`

CHAPTER 5: EFFECTS OF PLANE OF NUTRITION AND 2,4-THIAZOLIDINEDIONE ON INSULIN RESPONSES AND ADIPOSE TISSUE GENE EXPRESSION IN DAIRY CATTLE DURING LATE GESTATION[1]

Abstract

Specific mechanisms by which dry period dietary energy level and thiazolidinedione (TZD) administration affect transition cow metabolism are not known, but we hypothesize effects are mediated via changes in insulin, glucose, or fatty acid metabolism. The objective of this experiment was to determine the effects of the insulin-sensitizing agent TZD and dietary energy level on glucose and fatty acid metabolism during late gestation in dairy cows. Multiparous Holstein cows (n = 32) approximately 50 d prior to expected calving date were dried-off and assigned to one of two dietary energy levels for three weeks (High, 1.52 Mcal/kg NE_L; or Low, 1.34, Mcal/kg NE_L) and treated daily during the final two weeks with 4.0 mg TZD/kg BW (TZD) or saline (Saline) in a completely randomized design. Cows fed the Low diet had lower DMI (12.8 vs. 16.1 kg/d) and higher plasma NEFA (103.3 vs. 82.4μEq/L) than cows fed the High diet. Cows administered TZD had higher plasma glucose (62.5 vs. 59.6 mg/dL) than saline controls and cows fed the High diet had higher plasma insulin (35.1 vs. 25.3 μIU/mL) compared to those fed the Low diet. After two weeks of TZD treatment, all cows were subjected to an intravenous glucose tolerance test (GTT; 0.25 g dextrose/kg BW) followed 110 min later by an insulin challenge (IC; 1.0

---

[1]Research material published in part as conference proceedings in 2010 (Schoenberg and Overton, 2010)

μg/kg BW). There was a trend for cows fed the High diet to have lower area under the curve (AUC) for plasma glucose during GTT (1895 vs. 2410 mg/dLx90 min) than those fed the Low diet; however, cows fed the Low diet had more negative NEFA AUC (-4838 vs -2137 μEq/Lx90 min) and greater NEFA clearance rates (1.35 vs. 0.63 %/min) during GTT, suggesting differential responses of tissue glucose and fatty acid metabolism in response to dietary energy level. During IC, TZD-treated cows tended to have more negative glucose AUC (-45.0 vs. -12.1 mg/dL x 15 min) than controls, suggesting that TZD-treated cows had greater responses to insulin. Interactions of diet and TZD were only significant for NEFA responses to IC such that cows fed the Low diet receiving TZD had a negative AUC (-80 μEq/L x 15 min), cows fed the High diet and treated with either saline or TZD had slightly positive AUC (65 and 67 μEq/L x 15 min, respectively), and cows fed the Low diet receiving saline had the most positive AUC (517 μEq/L x 15 min). These results indicate that energy level and insulin-sensitizing agents affect glucose and lipid metabolism during the dry period, which may have implications for the transition period.

`

Introduction

The transition period requires complex coordination of tissues in order to support the onset of lactation (Bauman and Currie, 1980). Although the transition period was first defined by Grummer (1995) as the three weeks prior to and following calving, much research has focused on the role of nutrition during the far-off as well as the close-up dry periods. Maximizing prepartum dry matter or energy intake had previously been recommended (Curtis et al., 1984, 1985), however, many research groups have reported potentially negative consequences of overfeeding cows during the dry period (Grummer, 1995; Holtenius et al., 1996; Dann et al., 1999; Agenas et al., 2003; Holtenius et al., 2003; Dann et al., 2005; Dann et al., 2006). Many of the more recent experiments reported sharper decreases in DMI and more dramatic increases in NEFA prior to and post-calving for cows that were overfed during the dry period. The consequences of excessive postpartum mobilization of adipose tissue are increased incidences of metabolic disorders such as ketosis and fatty liver due to prolonged negative energy balance (Grummer, 1993; Douglas et al., 2006).

Feeding a controlled energy diet throughout the dry period has been shown to limit body condition losses post-calving, especially if gain in body condition is limited (Richards et al., 2010). Furthermore, recent data suggests that changes in energy metabolism may occur with overfeeding without observable changes in BCS. In a group of non-pregnant dry cows that were fed either a low (NE_L = 1.37 Mcal/kg) or moderate (NE_L = 1.61 Mcal/kg) energy level, the cows that were fed the moderate energy level deposited greater than 70% more internal fat despite no significant differences in assigned BCS (Nikkhah et al., 2008). Therefore, changes in BCS alone may not be enough to determine the effects of overfeeding on transition cow

metabolism. An understanding of coordinated changes in whole-body metabolism during situations of overfeeding is necessary.

Insulin, in particular changes in tissue responses to insulin, may be a key factor in these alterations in energy metabolism in overfed cows. Cows fed at 178% of calculated energy requirements during the entire dry period had greater insulin responses to glucose challenge, indicating insulin resistance (Holtenius et al., 2003). Dann et al. (2006) reported that cows fed at 150% of NRC-predicted energy requirements in the far-off dry period had higher NEFA and BHBA and lost more body weight post-calving despite having higher insulin levels during the dry period. The potential role for insulin resistance in overfed cows and how it could be mediated is not known.

The use of an insulin-sensitizing agent, TZD, in transition dairy cattle resulted in increased DMI and decreased NEFA during the transition period (Smith et al., 2007; Smith et al., 2009). This PPARγ ligand has insulin sensitizing-effects targeted to adipose tissue (Sundvold et al., 1997; Hammarstedt et al., 2005). The first use of TZD in ruminants reversed TNFα-induced insulin resistance, further supporting its potential application during the transition period (Kushibiki et al., 2001).

Much of the research conducted on plane of nutrition in dry cows has focused on responses in BCS loss and corresponding metabolites; therefore, there is need for further study of adipose tissue metabolism. Overfeeding during the dry period resulted in increased esterification rates in adipose tissue prepartum (concurrent with higher circulating insulin) and greater lipolytic rates (and thus higher NEFA) post-partum (Rukkwamsuk et al., 1999b). These changes in regulation partially explain the greater post-partum loss of body condition observed in cows overfed during the dry period. Given these observations, there is a need to clarify the potential mechanisms

by which controlled plane of nutrition and insulin-sensitizing agents can positively affect the metabolic health of transition dairy cows.

Therefore, the objectives of this experiment were to determine the effects of dietary energy level and administration of an insulin-sensitizing agent (TZD) on glucose and fatty acid metabolism during the dry period. Specifically, what effect would excessive energy balance have on plasma concentrations of glucose, insulin, and fatty acids, and what interactions would exist between dietary energy level and TZD administration? In order to determine these effects, responses to glucose and insulin challenges and responses in gene expression in adipose tissue were measured. The hypothesis was that plane of nutrition would impact energy metabolism related to insulin resistance, with potential interactions of TZD treatment.

Materials and methods

Animals, treatments, and daily sampling

The animal phase of this experiment took place from November 2009 through February 2010. All procedures involving animals were approved prior to the onset of the experiment by the Cornell University Institutional Animal Care and Use Committee. The overall project design is depicted in Figure 5-1. Holstein cows (n = 32) entering second or greater lactation were selected from the Cornell Teaching and Research Center dairy herd and dried off at approximately 50 d before expected calving date and moved to individual tie stalls to begin the experiment within one week. Based on actual calving dates, cows began the experiment an average of 48 (+/- 8) d prior to calving. Cows were assigned randomly to one of two dietary treatments: those fed a high plane of nutrition (High) or those fed a low plane of nutrition (Low), based on dietary energy level and the resulting DMI. Table 5-1 details diet ingredients and chemical composition. Diets were sampled and analyzed by Dairy One

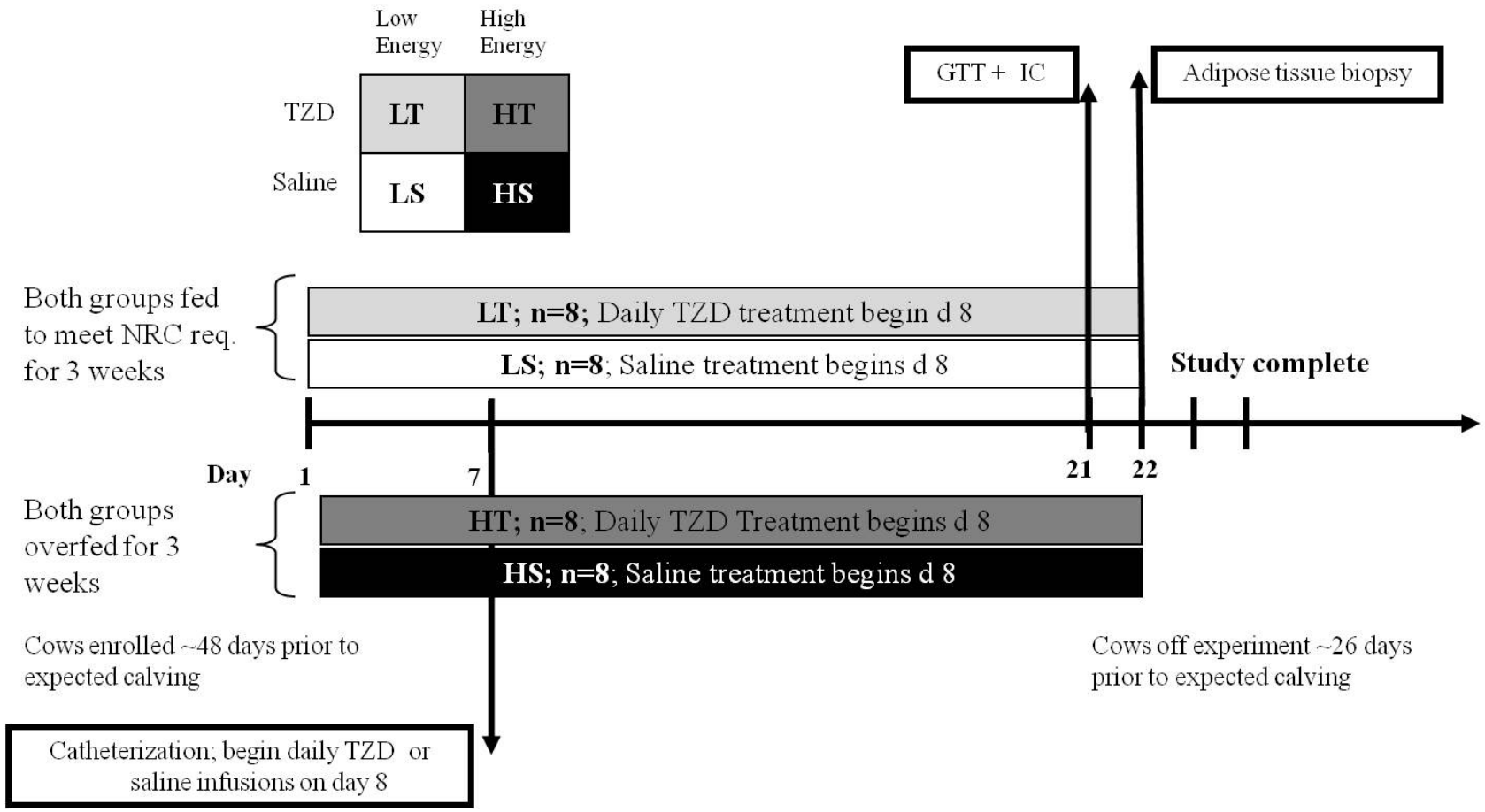

Figure 5-1. Study design. Figure represents overall study layout, by day.

Table 5-1. Experimental diet ingredients and chemical composition.

Ingredient (%, DM basis)	High	Low
Corn silage, processed	62.7	32.3
Wheat straw	7.0	33.4
Soybean hulls	9.53	10.77
Distillers grains (with solubles)	9.09	10.29
AminoPlus™ [1]	6.21	7.03
Soybean meal	3.12	3.53
Calcium Carbonate	1.23	1.39
Molasses	0.68	0.86
Salt	0.27	0.31
Magnesium Oxide	0.05	0.06
Selenium 0.06%	0.03	0.03
Vitamin A, D, E premix[2]	0.03	0.03
1100 Dairy™ [3]	0.03	0.03
Zinc Sulfate	0.0003	0.0003
Vitamin E premix[4]	0.0003	0.0003

Chemical Composition[5] (DM basis, ± SD) of experimental diets.

Nutrient	High	Low
NE_L (Mcal/kg)	1.52 (0.02)	1.34 (0.05)
CP (%)	13.5 (0.51)	14.0 (0.40)
Soluble protein, % of CP	33.7 (0.58)	30.3 (1.53)
Acid detergent insoluble CP (%)	0.70 (0.06)	0.80 (0.06)
Neutral detergent insoluble CP (%)	2.40 (0.21)	2.90 (0.06
ADF (%)	25.6 (1.55)	35.0 (1.87)
NDF (%)	41.4 (0.45)	52.3 (0.75)
Starch (%)	25.9 (0.66)	15.2 (0.70)
NFC, [6] (%)	39.3 (0.99)	28.1 (0.68)
Ether extract (%)	2.10 (0.25)	2.20 (0.30)
Ash (%)	6.10 (0.19)	6.40 (0.19)
Ca (%)	0.60 (0.08)	0.70 (0.04)
P (%)	0.30 (0.01)	0.30 (0.01)
K (%)	1.20 (0.08)	1.10 (0.03)
Mg (%)	0.20 (0.01)	0.20 (0.00)
Na (%)	0.20 (0.03)	0.20 (0.03)
Cl (%)	0.30 (0.01)	0.30 (0.02)
S (%)	0.20 (0.01)	0.20 (0.01)
DCAD[7], mEq/100 g DM	17.7 (0.58)	15.0 (0.00)

[1]Rumen undegradable protein supplement; AGP® Inc., Omaha, NE

[2]Contained 37, 113 IU/kg of vitamin A, 7, 216 IU/kg vitamin D, and 72, 165 IU/kg of vitamin E

[3]Cargill Animal Nutrition propietary blend, Elk River, MN

[4]Contained 499,400 IU/kg of vitamin E

[5]Calculated by Dairy One Cooperative (Ithaca, NY) using NRC (2001) equations from three composite samples of each diet

[6]Calculated as 100-[(NDF- NDFCP) + CP + ash + ether extract] (NRC, 2001)

[7]Calculated as mEq [(Na + K) - (Cl + S)]/100 g DM (NRC, 2001)

Cooperative (Ithaca, NY) as described in Chapter 4 in addition to neutral detergent insoluble CP and acid detergent insoluble CP (Licitra et al., 1996), ether extract (EE; method 2003.05;(AOAC, 2000)), ash (method 942.05; AOAC, 2000), and starch (Application note 319, TSI Inc., Yellow Springs, OH). Both diets were fed for ad libitum intake once per day at approximately 1000 h for a total of three weeks.

On d 7 of the experiment, cows were fitted with indwelling jugular catheters as described in Chapter 4. Patency of the catheters (Micro-Renathane® Implantation tubing, 2.03 mm o.d. x 1.02 mm i.d.; Braintree Scientific Inc., Braintree, MA) was maintained with regular flushing with heparinized saline (10 units/ml for frequent sampling; 100-500 U/ml otherwise). Heparinized saline with an antibiotic was used for overnight storage of catheters to prevent bacterial growth (Naxcel®; 4 mg/mL of saline; Pfizer Inc., New York, NY). There were no observed infections or complications in cows with catheters installed for two weeks.

Body weights were determined for all cows before beginning the feeding period, and again once per week until the end of the trial. Two individuals recorded BCS (Wildman et al., 1982) for all cows prior to the start of the experiment, and weekly until the conclusion of the trial. The average BCS for both individuals was recorded as the BCS for each cow at a given time period. Daily blood samples (10 ml) were collected at approximately 1200 h each day via coccygeal venipuncture prior to catheter insertion and via jugular catheter beginning on d eight. Additionally, a blood sample was drawn prior to the start of the experiment to serve as a covariate for each animal.

Beginning on d 8 of the experiment, cows were infused with a TZD solution at a dose of 4 mg/kg body weight. The BW used to calculate dose was an average of the two BW measurements collected on d 1 and 7 of the experimental period. The TZD was solubilized into solution as follows. All glassware and weighing tools were

sterilized via autoclave prior to preparation of the solution. The crystalline TZD (2,4-Thiazolidinedione, Technical grade, 90%; Sigma-Aldrich Corp.; St. Louis, MO) was weighed into sterilized glass bottles for each cow and 60 mL of sterile saline (0.9% wt:vol) was added to the bottle. The bottles were then capped with rubber Wheaton stoppers and aluminum seals (AluminumSeal 20mm FlipCap; Wheaton Science Products; Millville, NJ), placed into a hot water bath (37°C) for 20 min, and vortexed until TZD remained in solution. Storage of bottles overnight for the next day was devoid from light and at room temperature. On the morning of dose administration, the aluminum seal on each bottle was opened and the TZD solution was removed through the rubber stopper via sterile needle and 60 mL syringe. If syringes were exposed to extreme cold for a long period of time, TZD had a tendency to come out of solution at which time a warm water bath and shaking was sufficient to resolubilize the TZD. Doses were administered to each cow at approximately 1300 h each day via jugular catheter.

Glucose tolerance tests and insulin challenges

At the end of the experiment (d 22), all cows were subjected to an intravenous GTT similar to those conducted previously (Pires et al., 2007a; Pires et al., 2007b) and described in Chapter 4. Feed was removed at approximately 0700 h. Briefly, 0.25 g of glucose (dextrose 50% wt/vol) per kilogram of BW was administered in under seven min from multiple 60 mL syringes approximately two h after feed removal. Blood samples (10 ml) were collected -15, -5, 0, 5, 10, 15, 20, 25, 30, 45, 60, and 90 min relative to the glucose infusion into the catheter. The “-15”, and “-5” min samples were time relative to the beginning of the glucose infusion. The “0” sample was collected immediately following the conclusion of the glucose infusion and a 20 mL

saline flush. The rest of the samples were timed and collected relative to the completion of the infusion.

At 110 min following glucose infusion, an insulin challenge (IC) was administered to each cow. For the IC, cows received 1µg of insulin (insulin from bovine pancreas; ≥27 USP units/mg; Sigma Aldrich; St. Louis, MO) per kilogram of BW at a solution concentration of 0.2 mg/mL. The total infusion volume averaged 2.45 mL. The insulin dose was followed immediately by 20 mL of saline flush. Blood samples were then collected at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, and 180 min following the insulin infusion.

Plasma analyses

Blood samples, regardless of collection methods, were transferred into glass test tubes containing sodium heparin (100 IU/mL blood). Plasma was harvested following centrifugation (2,800 x g for 15 min at 4°C), snap-frozen in liquid N_2, and stored at -20°C until analyses for metabolites and insulin. Plasma concentrations of glucose were determined by enzymatic analysis (glucose oxidase) using a commercial kit (kit 510-A; Sigma Chemical). Intra- and interassay coefficients of variation were 4.9 and 8.0% respectively. Plasma concentrations of NEFA were also analyzed by enzymatic analysis (NEFA-C; WAKO Pure Chemical Industries; Osaka, Japan). Intra- and interassay coefficients of variation were 4.1 and 9.7% respectively. All spectrophotometric measurements were conducted using a $Versa_{max}$ tunable microplate reader (Molecular Devices, Sunnyvale, CA). Plasma concentrations of insulin were determined by double-antibody radioimmunoassay (Porcine Insulin RIA Cat. #PI-12K; LINCO Research; Millipore; St. Charles, MO). The methodology of the insulin kit is very similar to that of the RIA used to analyze plasma insulin in Chapter 4 (Ehrhardt RA et al., 2001) and has a reported specificity to bovine insulin of

90%. Intra- and interassay coefficients of variation for the insulin assay were 1.7 and 7.5%, respectively.

Adipose tissue biopsies

On d 23 of the experiment, adipose biopsies were collected from all cows. The procedure used is similar to that described by Houseknecht and Bauman (1997). The area below the spinal processes between the hips and the pins of the cow was palpated to locate an area with sufficient subcutaneous fat. The area was brushed and clipped to remove excess hair and prepared with betadine surgical scrub (Betadine Surgical Scrub (7.5% povidone-iodine; Purdue Frederick; Stamford, CT) and 70% ethanol in water. Cows were administered 20 mg of the sedative zylazine hydrochloride (Rompun 2%, Bayer Inc., Sarnia, Ontario, Canada). A local anesthetic (Lidocaine HCL, 2%, 18cc) was injected subcutaneously around the biopsy site. A 4-8 cm incision was made and a small biopsy (0.5-1.5 g) was removed using sterile forceps and scalpel. The incision was closed with 6-12 surgical skin staples using a surgical staple gun (Precise Vista Skin Stapler 35W; 3M; St. Paul, MN). The wound was topically treated with a topical antiseptic (BluKote^{TM} aerosol spray; H.W. Naylor Co., Morris, NY). Adipose tissue samples were rinsed with sterile saline, cleaned of connective tissue, cut into three aliquots, placed into plastic vials, and quenched in liquid N_2. Cows were monitored for 7 d for signs of infection or fever. No cows experienced complications from the biopsies and the staples were removed after 7 days.

Quantitative RT-PCR

Adipose tissue gene expression was conducted as described in Chapter 3. Instead of using 18S as a reference gene, the quantitative gene expression data shown in the present chapter are in proportion to the geometric mean of the expression of

both 18S and β2-microglobulin. In addition to measuring expression of PPARγ, leptin, LPL, and FAS, gene expression of TNFα and adiponectin also were analyzed. The primers used were previously noted in Chapter 3 and in Table 5-2. Expression analysis for four cows (two on the Low TZD treatment combination, two on the Low Saline treatment combination) was not included due to poor quality of isolated RNA.

Calculations

Energy balance was calculated using NRC requirements during gestation (NRC, 2001) and NE_L of feedstuffs as analyzed.

RQUICKI, the relative insulin sensitivity measure used in ruminants was calculated as the following (Holtenius and Holtenius, 2007):

$$RQUICKI = 1 / [\log(G_b) + \log(I_b) + \log(NEFA_b)]$$

Where
G_b = Basal glucose (mg/dL)
I_b = Basal insulin (μU/mL)
$NEFA_b$ = Basal NEFA (mmol/L)

such that a lower RQUICKI suggests greater insulin resistance.

Responses to GTT and HEC were measured as area under the curve (AUC), calculated using incremental change and trapezoidal rule via SAS (v. 9.2). Additional responses such as clearance rates, half-life, and time to reach basal concentrations were estimated using ProcNLIN of SAS (v. 9.2) as described in Chapter 4.

Statistical analysis

Statistical analysis was performed as analysis of variance (ANOVA) on measures conducted over time (plasma concentrations) using the MIXED procedure of SAS (2001) for a completely randomized design with repeated measures where appropriate. The statistical model included fixed effects of covariate, treatment, time

Table 5-2. Primer sequences for adipose tissue mRNA expression.

Target	Forward Primer Sequence	Reverse Primer Sequence	Source
TNFα	CCCATCTACCAGGGAGGAGT	GGCGATGATCCCAAAGTAGA	(Komatsu et al., 2005)
Adiponectin	GTGGCTCTGATTCCACACCT	GCCATGACTGGGTAAGGCTA	(Boisclair, Y.R., personal communication)
β-2-microglobulin	CATCCAGCGTCCTCCAAAGAT	CCCCATTCTTCAGCAAATCG	(Harvatine and Bauman, 2006)

and the interaction of treatment and time. The random effect was cow nested within treatment. For each variable, six covariance structures were evaluated (first-order autoregressive, heterogeneous first-order autoregressive, compound symmetry, heterogeneous compound symmetry, first-order ante-dependence, and unstructured) and the structure with the smallest Akaike's information criterion was used for further analysis. The method of Kenward-Rogers was used for calculation of denominator degrees of freedom. Covariates were dropped from the statistical model if $P > 0.15$ and the data were reanalyzed. Significance was declared as P values ≤ 0.05; trends as $0.15 \geq P \geq 0.05$.

Results

Daily intake and plasma

There was a significant effect of diet on DMI during the last two weeks of the experiment such that cows fed the High diet consumed 16.1 (± 0.4) kg/d of DM and cows fed the Low diet consumed 12.8 (± 0.4) kg/d of DM ($P = < 0.0001$). Figure 5-2 illustrates the diet effects on DMI throughout the entire trial. There was trend for a diet by day interaction ($P = 0.07$) such that cows fed the High diet consistently ate more than cows fed the Low diet (16.1 vs. 12.8 kg/d) throughout the experiment, and cows fed the Low diet decreased intake slightly during week two of the experiment. There were no significant effects of TZD administration or interactions of TZD with dietary treatment, though the four treatment combinations are illustrated in Figure 5-2. The resulting energy balance for cows on each diet was 172 (± 3.5) % calculated energy balance for cows on the High diet and 119 (± 3.5) % for cows on the Low diet during the last two weeks of the trial ($P < 0.0001$; (NRC, 2001). Consistent with the

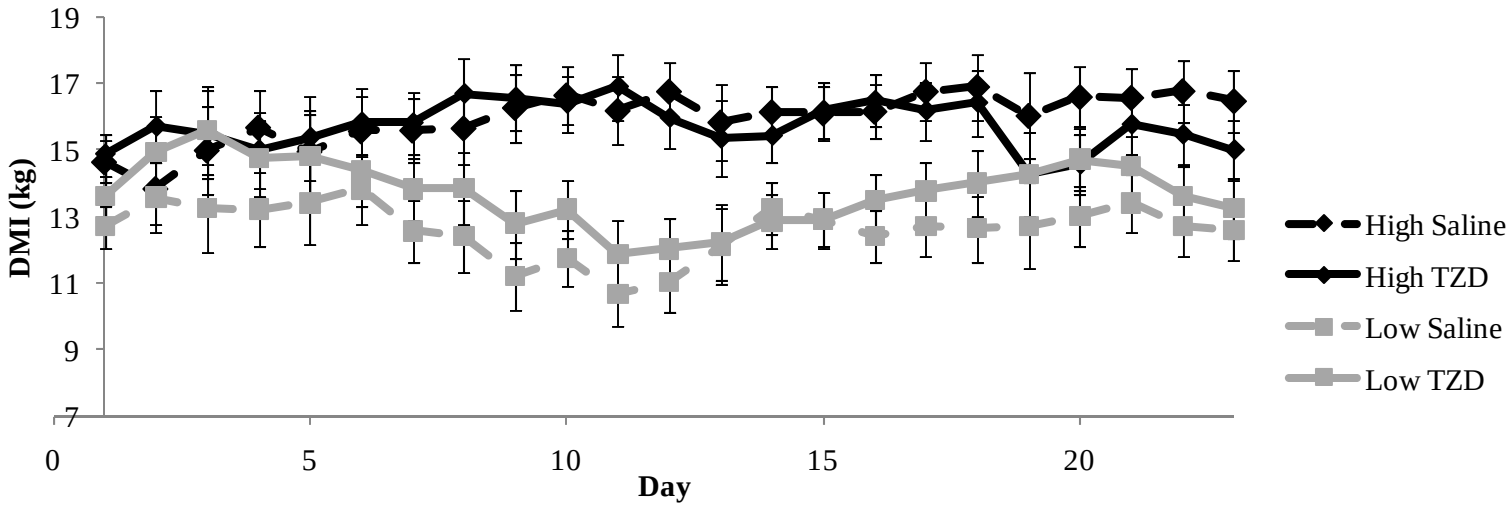

Figure 5-2. Daily dry matter intake. Cows were fed one of two dietary treatments (High vs. Low dietary energy level) for three weeks, and treated daily with either TZD or Saline for the final two weeks of the experiment. Values represent least squares means with error bars representing SEM; n = 8 for each of the four treatment combinations. The *P* value for the final two weeks for effect of diet was $P < 0.0001$; for TZD treatment was $P = 0.71$; for diet by TZD interaction was $P = 0.24$.

lack of a direct effect of TZD administration on DMI, there were no effects of TZD on calculated energy balance (P = 0.69), nor interaction of diet and TZD (P = 0.48). Cows fed the High diet gained 0.30 (± 0.07) units of BCS throughout the study and cows fed the Low diet gained 0.14 (± 0.07) (P = 0.11). There was no effect of TZD (P = 0.43), nor a TZD by diet interaction (P = 0.95) on BCS change throughout the trial.

Figure 5-3 depicts data for plasma metabolites, insulin, and calculated RQUICKI based upon daily plasma samples collected during the experiment. Statistics for plasma glucose, NEFA, and insulin concentrations were completed using results from the last two weeks of the trial as that was the time period that cows were receiving both dietary as well as TZD or Saline treatment. Administration of TZD increased plasma glucose (62.5 vs. 59.6 mg/dL; P = 0.03) compared to controls. There was no effect of diet on daily plasma glucose (P = 0.80). There was no effect of TZD on plasma insulin concentrations (P = 0.47), but cows fed the High diet had greater plasma concentrations of insulin (35 vs. 25 μIU/mL; P = 0.03) than those fed the Low diet. Cows fed the High diet also had lower plasma NEFA concentration (82 vs. 103 μEq/L; P = <0.0001) than those fed the Low diet. For the length of the trial, cows fed the High diet had lower RQUICKI (0.45 vs. 0.48; P = 0.03), but there was no effect of TZD (P = 0.16), nor interaction between diet and TZD (P = 0.89).

Glucose tolerance tests and insulin challenges

Figure 5-4 illustrates the change in plasma glucose levels following glucose infusion. Slightly slower clearance of glucose is visually apparent for cows fed the Low diet and for cows administered Saline control compared to their corresponding treatments. These differences are quantified in Table 5-3. There was a trend (P = 0.13) for a greater AUC for cows fed the Low diet versus the High diet, suggesting

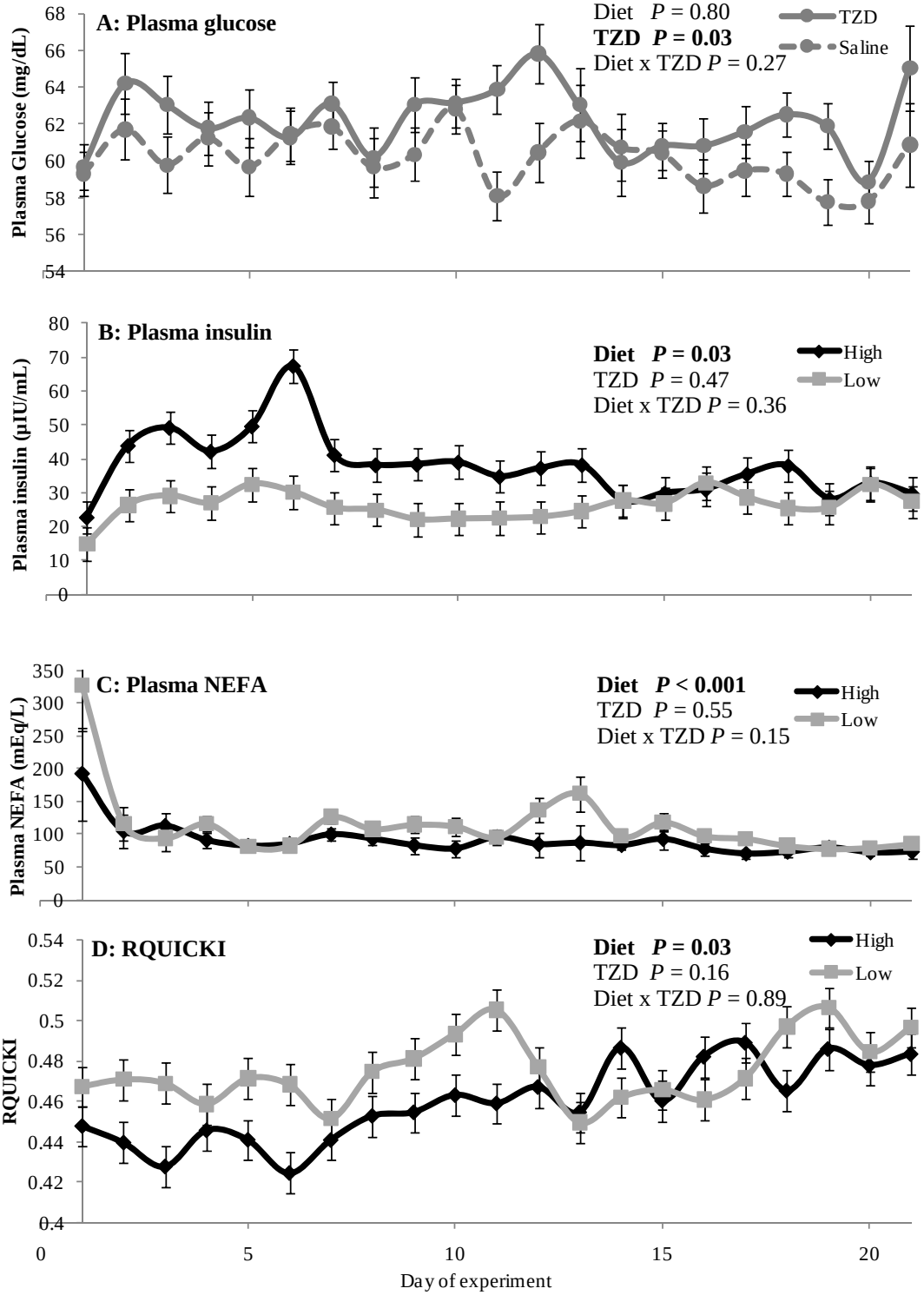

Figure 5-3. Daily plasma metabolites. Cows were fed one of two dietary treatments (High vs. Low dietary energy level) for three weeks, and treated daily with either TZD or Saline for the final two weeks of the experiment. RQUICKI = 1 / [log(glucose) + log(insulin) + log(NEFA)]. Values represent least squares means with error bars representing SEM; n = 16 for each treatment depicted. The *P* values for each are shown on the graphs.

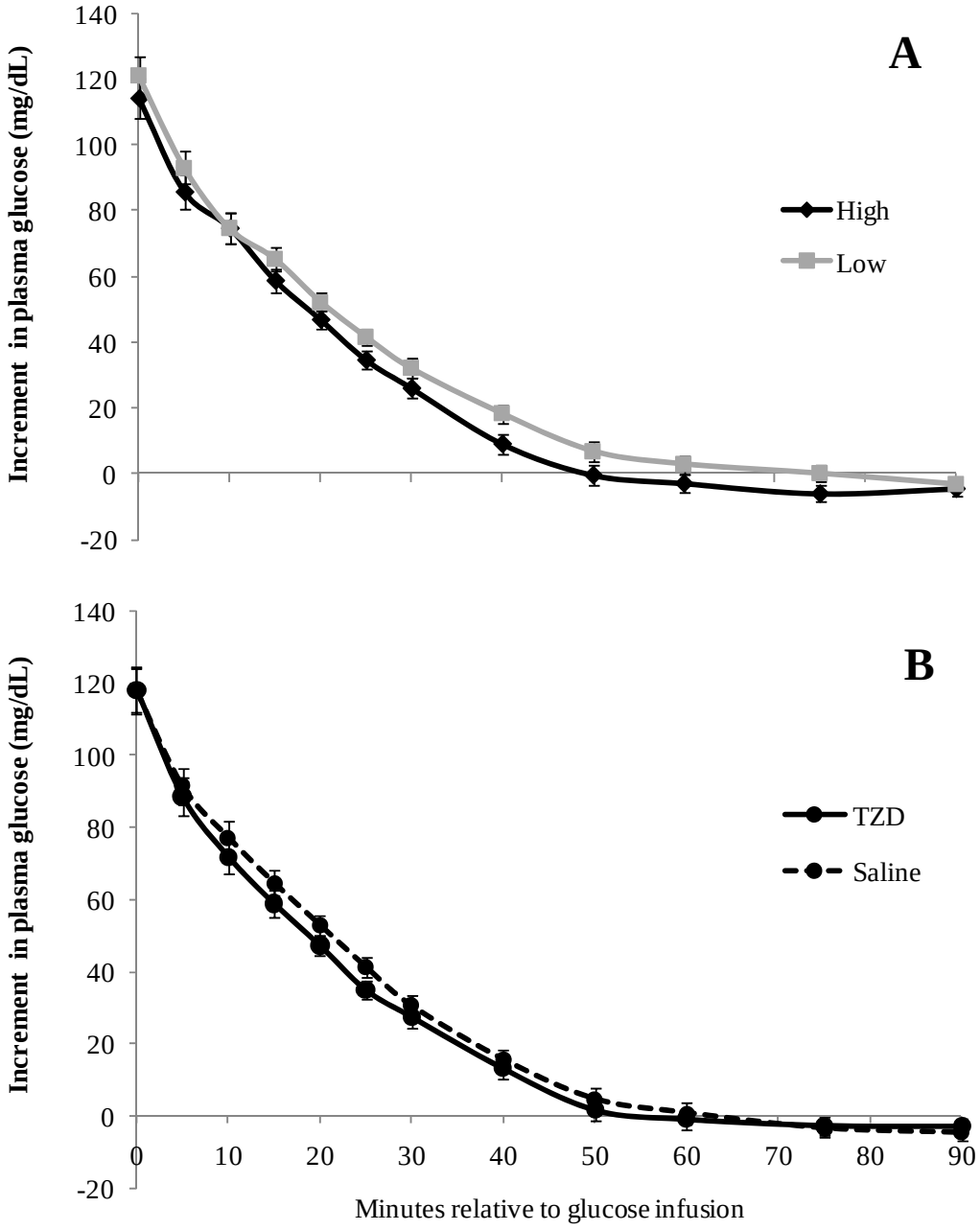

Figure 5-4. Plasma glucose responses to glucose tolerance test. Cows were fed one of two dietary treatments (High vs. Low dietary energy level; A) for three weeks, and treated daily with either TZD or Saline (B) for the final two weeks of the experiment. Values represent least squares means with error bars representing SEM; n = 16 for each treatment depicted. Results are detailed in Table 5-3.

Table 5-3. Effects of diet and TZD-administration on glucose responses to i.v. glucose tolerance test.

	Diet				TZD				TZD*Diet Interaction					
Measure[a]	High	Low	SEM	*P*	Saline	TZD	SEM	*P*	High Saline	High TZD	Low Saline	Low TZD	SEM	*P*
Basal glucose (mg/dL)	67.8	61.2	1.6	**0.01**	65.0	64.0	1.6	0.64	70.0	65.6	60.1	62.4	2.2	**0.14**
Minimum glucose (mg/dL)	57.3	54.7	2.0	0.38	55.6	56.4	2.0	0.79	57.9	56.7	53.4	56.0	2.8	0.50
Maximum glucose (mg/dL)	182.2	180.6	4.4	0.81	182.2	180.6	4.3	0.79	187.6	176.7	176.8	184.4	6.2	**0.14**
CR_{30} (%/min)	2.61	2.5	0.13	0.53	2.51	2.59	0.13	0.67	2.66	2.56	2.37	2.62	0.18	0.35
$T_{1/2}$ (min)	27.4	29.6	1.5	0.32	29.1	27.9	1.5	0.57	27.2	27.6	31.0	28.2	2.1	0.45
T_{basal} (min)	49.3	55.1	2.8	0.16	53.8	50.6	2.8	0.43	49.9	48.8	57.8	52.4	3.9	0.59
AUC_{30}	1826	1979	97	0.28	1998	1808	97	0.18	1946	1706	2049	1911	137	0.71
AUC_{60}	2029	2418	178	**0.13**	2367	2080	178	0.26	2164	1893	2571	2266	252	0.95
AUC_{90}	1895	2410	231	**0.13**	2289	2017	231	0.41	2021	1771	2558	2263	327	0.95

[a] Basal glucose = mean glucose concentration at -15 and -5 min prior to GTT; Minimum glucose = minimum glucose during GTT; Maximum glucose = maximum glucose during GTT; CR_{30} = clearance rate during the first 30 min of GTT; $T_{1/2}$ = Time to reach half maximal glucose concentration; T_{basal} = time to reach basal glucose concentration; AUC_{30} = area under the curve during the first 30 min of GTT [mg/dL x 30 min]; AUC_{60} = area under the curve during the first 60 min of GTT [mg/dL x 60 min]; AUC_{90} = area under the curve during the first 90 min of GTT [mg/dL x 90 min]

slower glucose clearance. Additional measures during GTT (minimum glucose, maximum glucose, clearance rate, half-life, and time to return to basal) were not significant based on dietary treatment. Furthermore, there were no significant differences in any of the GTT measures in response to TZD administration.

Plasma insulin responses to GTT were nearly identical based upon dietary treatment, but there was potentially lower secretion of insulin in response to GTT in cows that were administered TZD (Figure 5-5). These responses are quantified in Table 5-4. The AUC for plasma insulin responses to glucose challenge tended to be lower for cows treated with TZD versus saline (4381 vs. 5711 μIU/mL x 60 min; P = 0.15). Additional measures of plasma insulin during GTT were not different between cows administered TZD or saline. Basal insulin concentration was higher for cows fed the High diet (19.3 vs. 14.3; P = 0.02), as was also reflected in daily plasma levels in Figure 5-3, but this was the only plasma insulin response measure during GTT that was different based on dietary treatment. In addition, there were no interactions of diet and TZD administration on plasma insulin responses measured during GTT.

Differences in plasma NEFA responses during GTT appeared to be more dramatic in cows fed the Low diet versus those fed the High diet, as observed in Figure 5-6, while there were no obvious differences in plasma NEFA response to GTT due to TZD administration. These responses are quantified in Table 5-5. Responses in plasma NEFA during GTT were statistically different for several measures in response to dietary treatment. Basal and minimum NEFA were lower for cows fed the High diet than cows fed the Low diet (92 vs. 142 μEq/L; P = 0.03 and 44 vs. 54 μEq/L; P = 0.11, respectively). Clearance rate of NEFA during GTT was faster for cows on the Low diet as seen in Figure 5-6. (1.35 vs. 0.63 %/min; P = 0.01). Glucose-stimulated reduction in NEFA also was greater in cows fed the Low diet

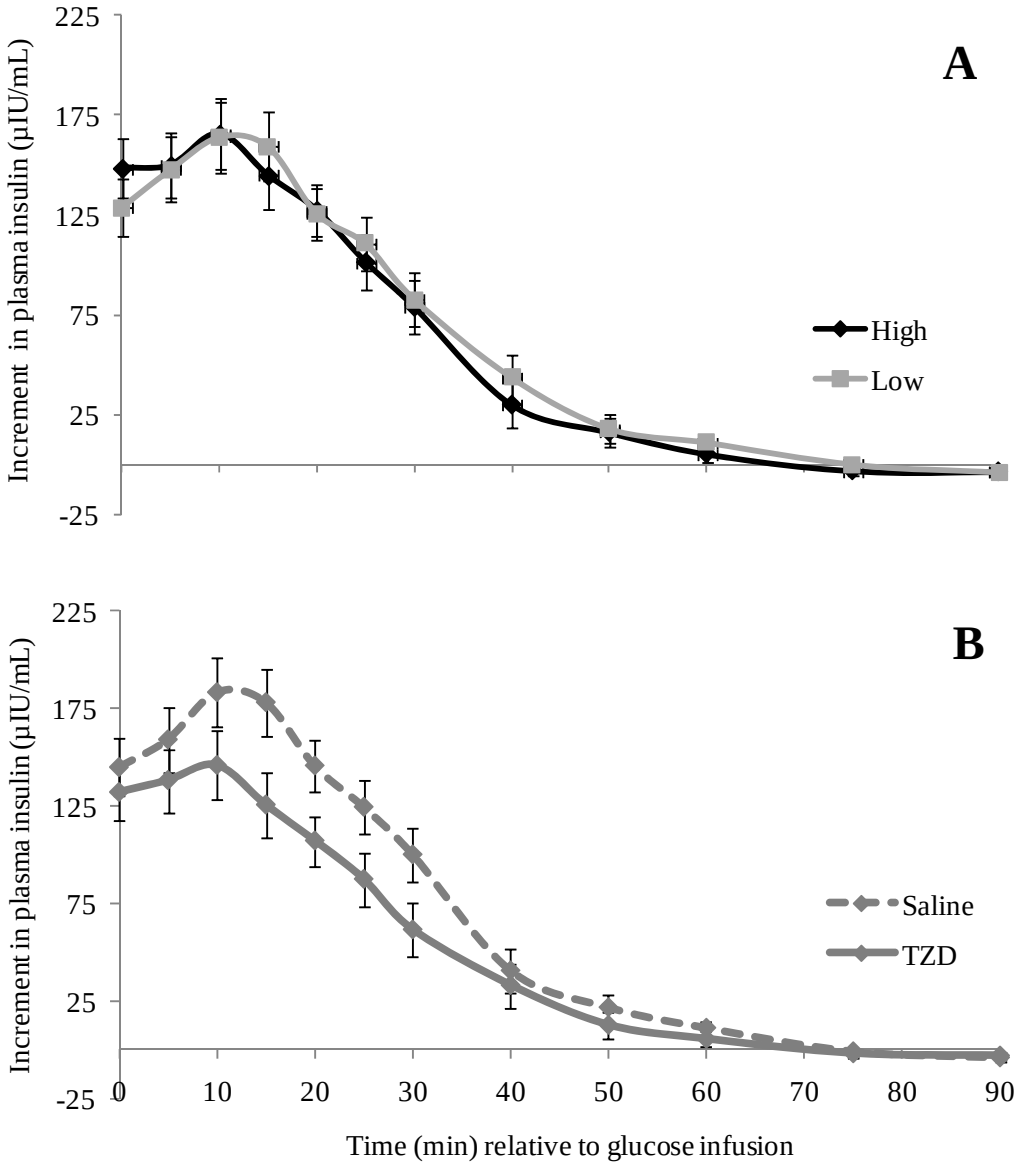

Figure 5-5. Plasma insulin responses to glucose tolerance test. Cows were fed one of two dietary treatments (High vs. Low dietary energy level; A) for three weeks, and treated daily with either TZD or Saline (B) for the final two weeks of the experiment. Values represent least squares means with error bars representing SEM; n = 16 for each treatment depicted. Results are detailed in Table 5-4.

Table 5-4. Effects of diet and TZD-administration on plasma insulin response to i.v. glucose tolerance test.

	Diet				TZD				TZD*Diet Interaction					
Measure[1]	High	Low	SEM	*P*	Saline	TZD	SEM	*P*	High Saline	High TZD	Low Saline	Low TZD	SEM	*P*
Basal insulin (µIU/mL)	19.3	14.3	1.4	**0.02**	17.7	15.9	1.4	0.37	21.1	17.5	14.3	14.4	1.9	0.36
Maximum insulin (µIU/mL)	214.3	249.3	29.1	0.40	241.3	186.8	29.1	0.65	241.9	186.8	240.7	257.9	41.2	0.39
AUC_{30}	4138	3933	487	0.77	4509	3562	483	0.18	5110	3166	3907	3958	688	0.16
AUC_{60}	5124	4967	643	0.87	5711	4381	637	**0.15**	6407	3842	5016	4919	901	0.18
AUC_{90}	5071	5018	657	0.96	5723	4367	656	**0.15**	6374	3768	5071	4965	921	0.19

[1] Basal insulin = mean insulin concentration at -15 and -5 minutes prior to GTT; Maximum insulin = minimum insulin during GTT; AUC_{30} = area under the curve during the first 30 min of GTT [µIU/mL x 30 min]; AUC_{60} = area under the curve during the first 60 min of GTT [µIU/mL x 60 min]; AUC_{90} = area under the curve during the first 90 min of GTT [µIU/mL x 90 min]

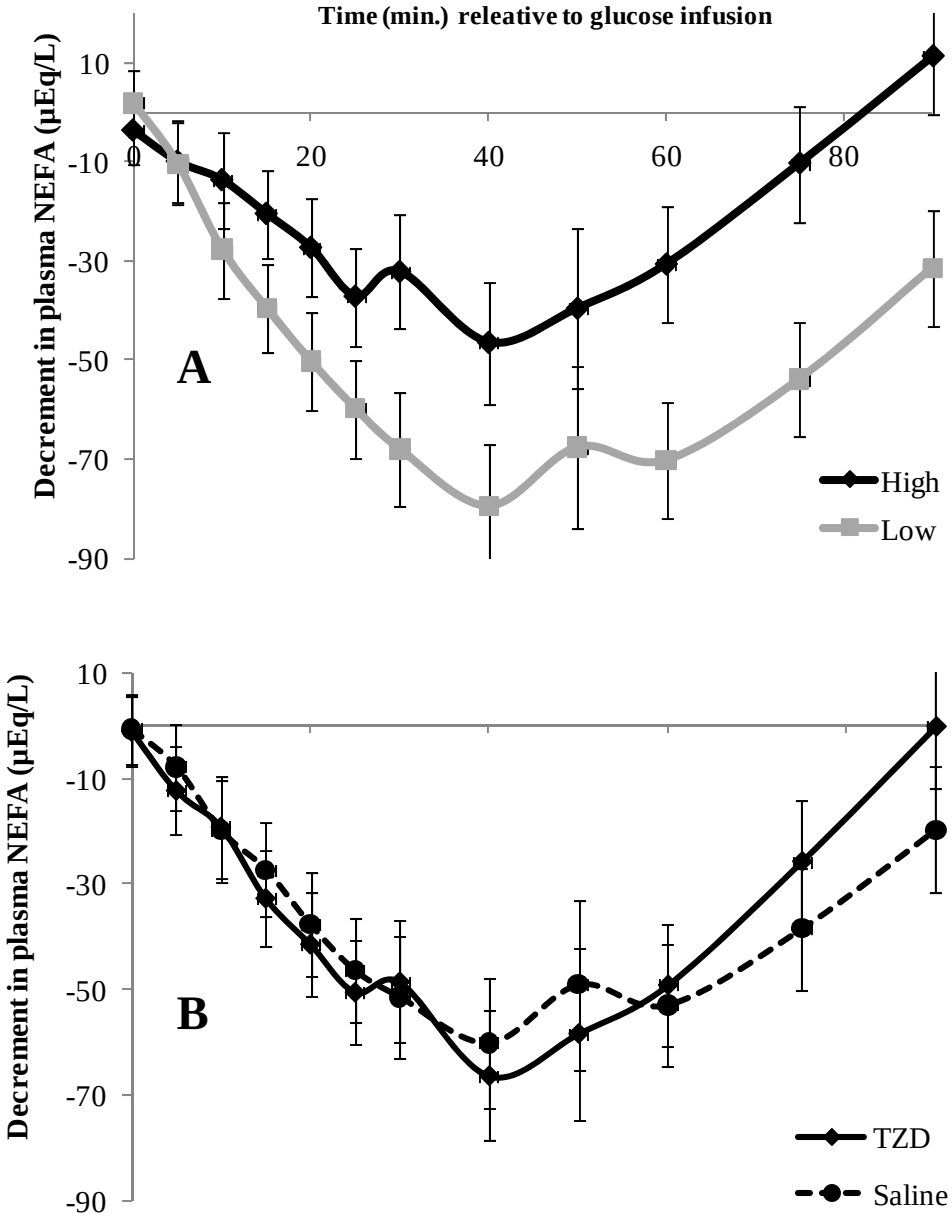

Figure 5-6. Plasma NEFA responses to glucose tolerance test. Cows were fed one of two dietary treatments (High vs. Low dietary energy level; A) for three weeks, and treated daily with either TZD or Saline (B) for the final two weeks of the experiment. Values represent least squares means with error bars representing SEM; n = 16 for each treatment depicted. Results are detailed in Table 5-5.

Table 5-5. Effects of diet and TZD-administration on plasma NEFA response to i.v. glucose tolerance test.

	Diet				TZD				TZD*Diet Interaction					
Measure[1]	High	Low	SEM	*P*	Saline	TZD	SEM	*P*	High Saline	High TZD	Low Saline	Low TZD	SEM	*P*
Basal NEFA (µEq/L)	92.2	142.4	14.8	**0.03**	119.2	115.4	14.7	0.86	87.0	97.5	151.4	133.3	21.0	0.50
Minimum NEFA (µEq/L)	44.0	54.0	4.2	**0.11**	50.6	47.4	4.2	0.59	43.7	44.2	57.4	50.5	5.9	0.54
CR_{60} (%/min)	0.63	1.35	0.18	**0.01**	0.98	1.0	0.18	0.94	0.60	0.66	1.36	1.34	0.26	0.86
$T_{1/2}$ (min)	136.3	92.2	31.4	0.33	145.6	83.0	31.4	0.17	203.4	69.1	87.7	96.8	44.5	**0.12**
GSRN	49.6	40.6	3.1	**0.05**	48.1	42.2	3.1	0.19	51.7	47.5	44.5	36.9	4.4	0.71
AUC_{30}	-641	-1086	246	0.21	-819	-906	246	0.81	-501	-780	-1138	-1032	348	0.58
AUC_{60}	-1827	-3247	586	**0.09**	-2432	-2642	586	0.80	-1534	-2120	-3331	-3165	829	0.95
AUC_{90}	-2137	-4838	880	**0.04**	-3413	-3562	880	0.91	-1774	-2599	-5349	-4326	1244	0.49

[1] Basal NEFA = mean NEFA concentration at -15 and -5 minutes prior to GTT; Minimum NEFA = minimum NEFA during GTT; CR60 = clearance rate during the first 60 min of GTT; GSRN = glucose-stimulated reduction of NEFA (% of basal); $T_{1/2}$ = Time to reach half maximal NEFA concentration; AUC_{30} = area under the curve during the first 30 min of GTT [µEq/L x 30 min]; AUC_{60} = area under the curve during the first 60 min of GTT [µEq/L x 60 min]; AUC_{90} = area under the curve during the first 90 min of GTT [µEq/L x 90 min]

(40.6 vs. 49.6% of basal NEFA; $P = 0.05$). Plasma NEFA response as measured by AUC was more significant for cows fed the Low diet (-4838 vs. -2137 μEq/L x 90 min; $P = 0.04$). There were no significant interactions of diet and TZD administration on plasma NEFA responses to GTT.

Figure 5-7 represents the changes in plasma glucose in response to IC. The only differences in plasma glucose response to insulin challenge were tendencies in AUC for plasma glucose within 15 min post-insulin infusion ($P = 0.08$; Table 5-6). Within 15 min of insulin infusion, the AUC for plasma glucose for cows administered TZD was -45 mg/dL x 15 min while cows receiving saline had AUC of -12 mg/dL x 15 min. There were no additional differences in plasma glucose responses to insulin challenge based on either dietary or TZD administration; nor were there any significant interactions of the two.

There were no treatment differences in plasma insulin response to insulin challenge (Figure 5-8, Table 5-7). As visually depicted in Figure 5-9, there were differences in plasma NEFA response during IC. As detailed in Table 5-8, cows administered TZD had more negative AUC during the minutes immediately following insulin infusion than cows administered Saline control (-6.4 vs. 291.1 μEq/L x 15 min; $P = 0.04$). Additionally, there was significant interaction of TZD and dietary treatments for the same measurement ($P = 0.04$) such that cows fed the Low diet and receiving TZD had the most negative AUC (-228 μEq/L x 15 min) and cows on the Low diet not receiving TZD had the most positive AUC (979 μEq/L x 15 min) while cows fed the High diet that were receiving TZD or saline control had intermediary responses in AUC (26 and 149 μEq/L x 15 min, respectively). No additional differences in plasma NEFA response to IC were observed, nor were additional interactions of dietary and TZD treatment.

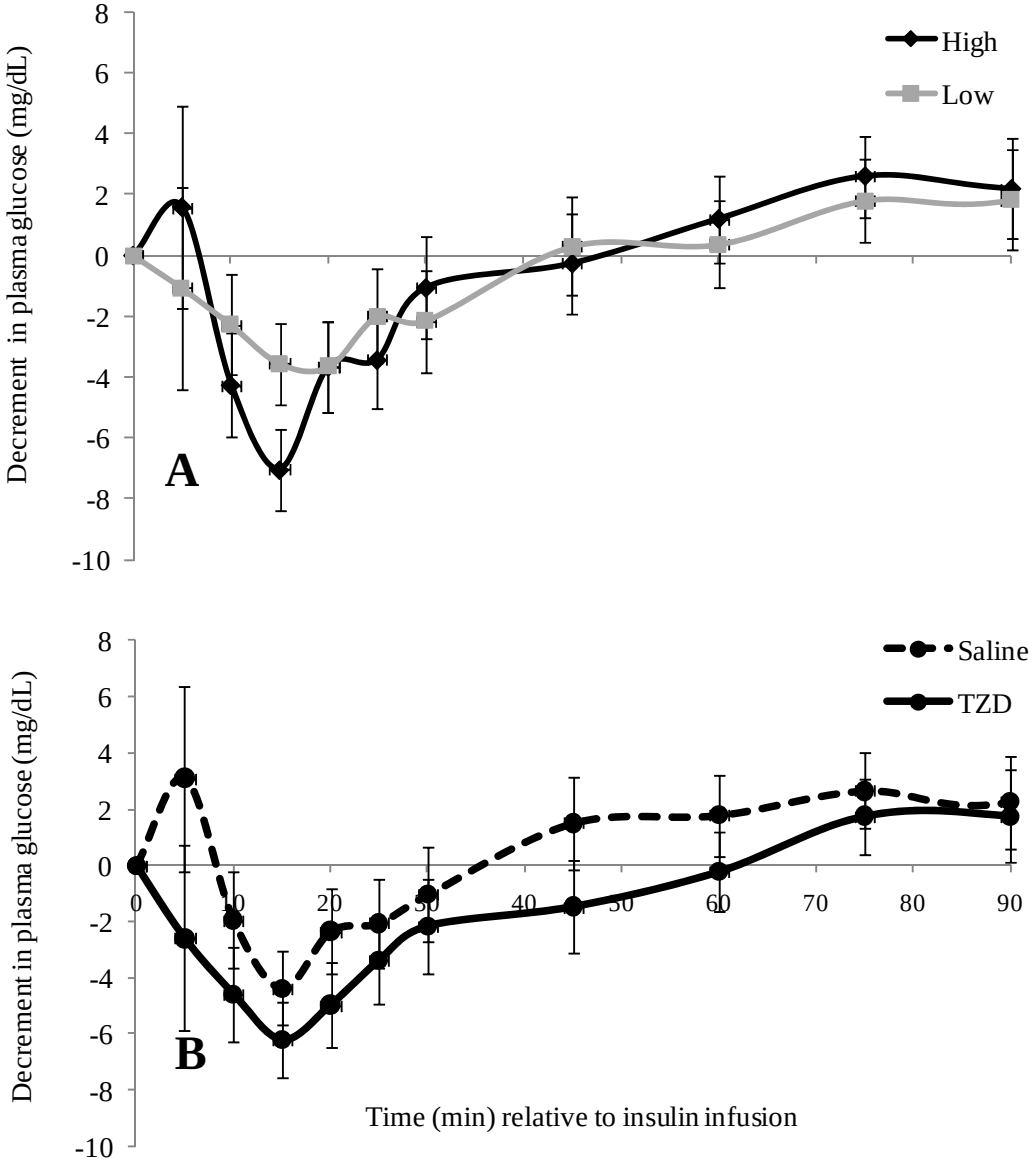

Figure 5-7. Plasma glucose responses to insulin challenge. Cows were fed one of two dietary treatments (High vs. Low dietary energy level; A) for three weeks, and treated daily with either TZD or Saline (B) for the final two weeks of the experiment. Values represent least squares means with error bars representing SEM; n = 16 for each treatment depicted. Results are detailed in Table 5-6.

Table 5-6. Effects of diet and TZD-administration on plasma glucose response to i.v. insulin challenge.

	Diet				TZD				TZD*Diet Interaction					
Measure[1]	High	Low	SEM	*P*	Saline	TZD	SEM	*P*	High Saline	High TZD	Low Saline	Low TZD	SEM	*P*
Basal glucose (mg/dL)	64.5	59.1	1.5	**0.02**	60.9	62.7	1.5	0.43	64.6	64.3	57.2	61.1	2.2	0.33
Minimum glucose (mg/dL)	54.7	52.0	1.1	0.12	54.0	52.7	1.2	0.43	55.8	53.6	52.3	51.7	1.7	0.64
CR (%/min)	0.46	0.24	0.12	0.22	0.34	0.37	0.12	0.86	0.44	0.49	0.24	0.25	0.35	0.91
ISRG (% of basal)	81.2	85.1	2.1	0.21	83.8	82.6	2.1	0.68	80.4	82.0	87.1	83.1	2.9	0.34
AUC_{15}	-34.2	-22.9	12.9	0.54	-12.1	-45.0	12.9	**0.08**	-19.0	-49.4	-5.3	-40.6	18.3	0.89
AUC_{30}	-89.9	-65.4	28.4	0.55	-47.7	-107.6	28.4	**0.15**	-67.8	-111.9	-27.5	-103.3	40.1	0.70
AUC_{180}	106.8	265.8	234.4	0.64	414.4	-41.8	234.4	0.18	153.3	60.4	675.5	-144.0	331.4	0.28

[1] Basal glucose = mean glucose concentration at -15 and -5 minutes prior to IC; Minimum glucose = minimum glucose during IC; CR = clearance rate of glucose during IC; ISRG = reduction of plasma glucose from basal levels; AUC15 = area under the curve during the first 15 min of IC [mg/dL x 15 min]; AUC_{30} = area under the curve during the first 30 min of IC [mg/dL x 30 min]; AUC180 = area under the curve during the first 180 min of IC [mg/dL x 180 min]

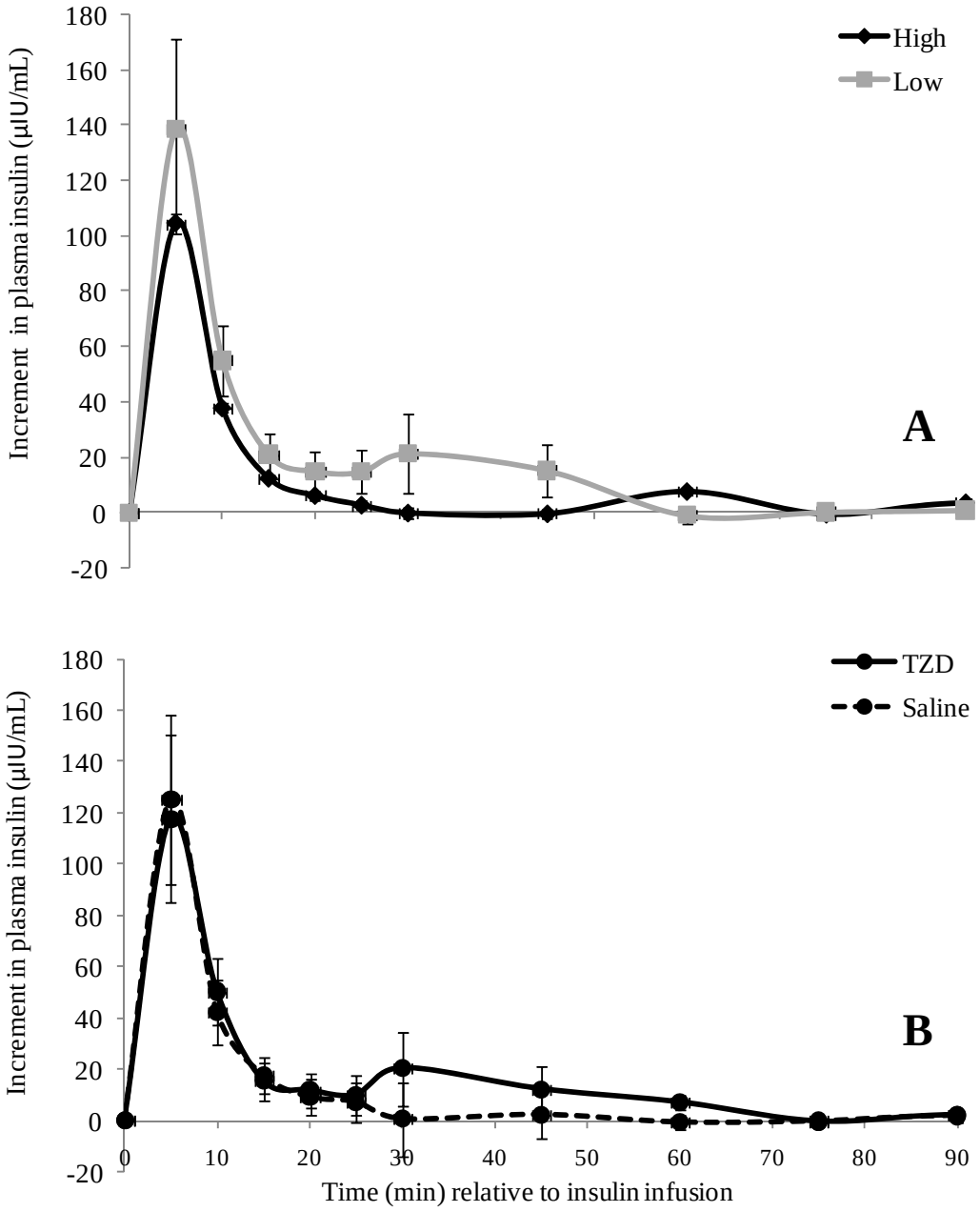

Figure 5-8. Plasma insulin responses to insulin challenge. Cows were fed one of two dietary treatments (High vs. Low dietary energy level; A) for three weeks, and treated daily with either TZD or Saline (B) for the final two weeks of the experiment. Values represent least squares means with error bars representing SEM; n = 16 for each treatment depicted. Results are detailed in Table 5-7.

Table 5-7. Effects of diet and TZD-administration on plasma insulin response to i.v. insulin challenge.

	Diet				TZD				TZD*Diet Interaction					
Measure[1]	High	Low	SEM	*P*	Saline	TZD	SEM	*P*	High Saline	High TZD	Low Saline	Low TZD	SEM	*P*
Basal insulin (µIU/mL)	15.3	11.9	1.5	**0.12**	13.4	13.8	1.5	0.85	16.4	14.2	10.4	13.4	2.1	0.21
Minimum insulin (µIU/mL)	10.3	8.4	0.9	0.17	9.3	9.5	0.9	0.88	14.1	9.5	7.4	9.4	1.3	0.17
Maximum insulin (µIU/mL)	121.5	170.3	33.4	0.31	138.7	153.1	33.4	0.76	111.2	131.9	166.3	174.3	47.2	0.89
AUC_{15}	490.4	673.7	162.3	0.43	579.3	584.8	162.3	0.98	450.3	530.5	708.2	639.1	229.5	0.98
AUC_{30}	565.1	928.1	221.6	0.26	708.8	784.3	221.6	0.81	496.9	633.3	920.8	935.4	313.4	0.85
AUC_{180}	1134	1312	485	0.80	859.1	1587	481.3	0.30	566.5	1702	1152	1472	685.8	0.55

[1] Basal insulin = mean insulin concentration at -15 and -5 minutes prior to IC; Minimum insulin = minimum insulin during IC; Maximum insulin = maximum insulin during IC; AUC15 = area under the curve during the first 15 min of IC [µIU/mL x 15 min]; AUC_{30} = area under the curve during the first 30 min of IC [µIU/mL x 30 min]; AUC180 = area under the curve during the first 180 min of IC [µIU/mL x 180 min]

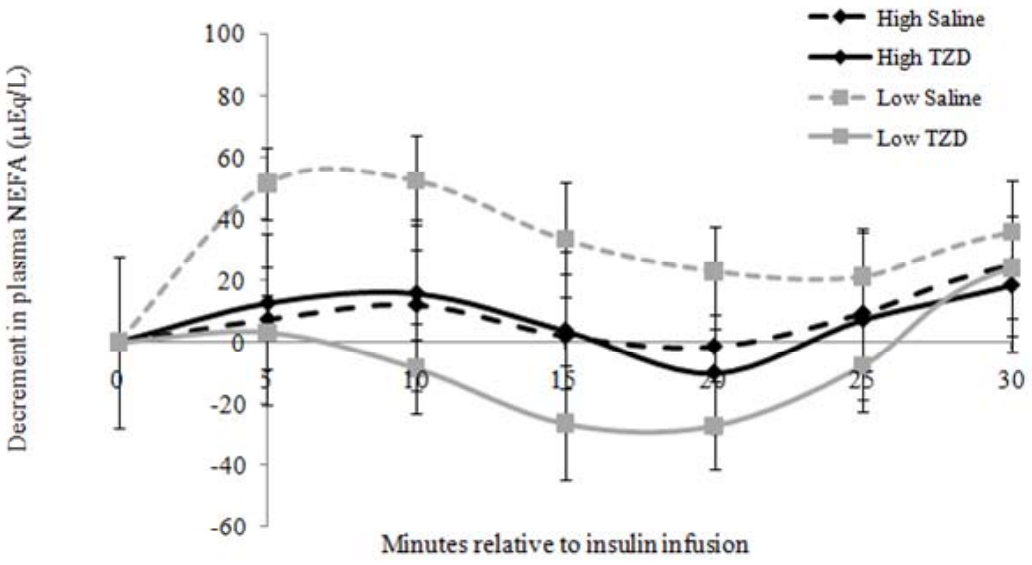

Figure 5-9. Plasma NEFA responses to insulin challenge. Cows were fed one of two dietary treatments (High vs. Low dietary energy level) for three weeks, and treated daily with either TZD or Saline for the final two weeks of the experiment. Values represent least squares means with error bars representing SEM; n = 8 for each treatment combination depicted. Results are detailed in Table 5-8.

Table 5-8. Effects of diet and TZD-administration on plasma NEFA response to i.v. insulin challenge.

	Diet				TZD				TZD*Diet Interaction					
Measure[1]	High	Low	SEM	*P*	Saline	TZD	SEM	*P*	High Saline	High TZD	Low Saline	Low TZD	SEM	*P*
Basal NEFA (µEq/L)	114.0	126.2	13.5	0.53	110.3	130.0	13.5	0.31	102.8	125.2	117.7	134.8	19.1	0.89
Minimum NEFA (µEq/L)	97.3	114.6	10.6	0.26	104.4	107.5	10.6	0.84	87.2	107.5	121.7	107.6	14.9	0.26
CR_{20} (%/min)	1.8	2.6	0.45	0.21	2.0	2.4	0.45	0.56	1.0	2.4	3.0	2.2	0.63	**0.08**
$T_{1/2}$ (min)	76.2	71.3	36.3	0.92	88.2	59.3	34.0	0.56	80.0	72.5	96.5	46.1	47.5	0.67
ISRN (% of basal)	85.9	93.6	5.2	0.30	92.4	87.2	5.2	0.48	80.6	91.4	104.3	83.0	7.3	0.04
AUC_{15}	66.2	218.6	95.5	0.28	291.1	-6.4	95.1	**0.04**	65.2	67.3	517.1	-80.0	134.5	**0.04**
AUC_{30}	87.5	375.6	241.6	0.41	563.9	-100.8	239.6	**0.06**	149.0	26.0	978.7	-227.5	338.8	**0.12**
AUC_{180}	11634	17447	2046	**0.06**	15704	13378	2029	0.43	12483	10785	18924	15970	2870	0.83

[1] Basal NEFA = mean NEFA concentration at -15 and -5 minutes prior to IC; Minimum NEFA = Minimum NEFA during IC; CR20 = Clearance rate for NEFA for 20 minutes post-IC; $T_{1/2}$ = Half-life for NEFA during IC; ISRN = Insulin-stimulated reduction of NEFA during IC; AUC15 = area under the curve during the first 15 min of IC [µEq/L x 15 min]; AUC_{30} = area under the curve during the first 30 min of IC [µEq/L x 30 min]; AUC180 = area under the curve during the first 180 min of IC [µEq/L x 180 min]

Adipose tissue gene expression

Table 5-9 details the effects of dietary and TZD-treatment on adipose tissue mRNA expression. There was a trend for cows fed the High diet to have greater expression of LPL (2.2 vs. 1.6 relative units; $P = 0.10$) and to have significantly greater expression of PPARγ (2.4 vs. 1.3 relative units; $P = 0.02$). Additional differences based on dietary treatment or TZD administration or interactions of the two were not significant.

Discussion

The diets in this experiment were designed to achieve different planes of nutrition despite both diets being fed for *ad libitum* intake. As reported in Table 5-1 and Figure 5-2, the combination of lower dietary energy concentration and higher forage content of the Low diet resulted in a 53% lower calculated energy balance for cows fed the Low diet. The difference in calculated energy balance for cows fed the two dietary treatments were similar to past experimental conditions during the dry period (Dann et al., 2006). Additionally, changes in BCS for cows fed the High diet were similar to those described in previous studies focused on changes in plane of nutrition during the dry period despite a dietary treatment period of only three weeks (Dann et al., 2006; Richards et al., 2010).

In previous studies with dairy cattle, prepartum TZD administration did not affect prepartum DMI (Smith et al., 2007; Smith et al., 2009). In those studies, however, prepartum diets were of moderate energy level (1.60 Mcal NEL/kg), and in one case were limit-fed to meet only 130% of energy requirements in the prepartum period (Smith et al., 2007). Additionally, cows were much closer to calving (3 weeks)

Table 5-9. Effects of diet and TZD-administration on adipose tissue mRNA expression.

	Diet				TZD				TZD*Diet Interaction					
Gene[1]	High	Low	SEM	*P*	Saline	TZD	SEM	*P*	High Saline	High TZD	Low Saline	Low TZD	SEM	*P*
Adiponectin	0.26	0.23	0.02	0.34	0.24	0.26	0.02	0.66	0.26	0.27	0.23	0.24	0.03	0.99
Fatty acid synthase	24.3	18.0	3.2	0.21	23.2	19.1	3.3	0.40	26.3	22.3	20.2	15.9	5.0	0.97
Leptin	6.4	4.3	1.2	0.20	4.8	5.9	1.1	0.48	5.4	7.4	4.3	4.4	1.6	0.55
Lipoprotein Lipase	2.2	1.6	0.29	**0.10**	1.9	1.9	0.27	0.87	2.1	2.3	1.6	1.5	0.40	0.70
PPARγ	2.4	1.3	0.31	**0.02**	1.9	1.8	0.30	0.86	2.6	2.2	1.2	1.4	0.40	0.50
TNFα	4.4	4.4	0.60	0.98	4.4	4.4	0.60	0.99	3.8	5.0	5.0	3.8	0.90	0.18

[1] Values expressed are relative mRNA abundance, in relationship to the geometric mean of two housekeeping genes (18S and β2-microglobulin)

in those studies. In the case of the current experiment, however, TZD administration might have had an effect on DMI in a very desirable manner. Since TZD administration increased DMI by 1 kg DM/day for cows fed the Low diet, and decreased DMI by 0.5 kg DM/day for cows fed the High diet, this could have implications for cows overfed during the dry period, though the magnitude of change in DMI may not be biologically relevant, nor did it reach significance in this experiment. Control of energy intake during the dry period has been shown to improve performance and metabolic health of dairy cattle postpartum (Dann et al., 2006)

Because overfeeding energy to nonlactating cows has been shown to increase internal fat deposition independent of observable increases in body condition (Nikkhah et al., 2008), it could be hypothesized that cows on this experiment fed the High diet may have deposited more visceral adipose as well as increased body condition score. Unfortunately, dissection of internal adipose stores was not completed in this experiment, so results can only be hypothesized. Loss of BCS post-calving was also not recorded in this experiment as cows were removed from the controlled experiment at approximately three weeks prior to calving. Given the similarities with BCS changes from other experiments, had dietary treatments continued through early lactation, there might have been more dramatic loss of BCS for cows fed the High diet and for cows not treated with TZD (Dann et al., 2006; Smith et al., 2007; Smith et al., 2009). In a previous study that administered TZD to cows prepartum, there were no significant differences in BCS prepartum, but cows treated with TZD lost less BCS postpartum which may parallel current results (Smith et al., 2009).

Administration of TZD increased plasma glucose (Figure 5-3A). Results of TZD treatment on plasma glucose in ruminants have been mixed such that it either

tended to increase or did not increase plasma glucose during TZD administration (Smith et al., 2007; Smith et al., 2009). In steers that were treated with TNFα and had elevated plasma glucose over control cows, there were decreases in plasma glucose with TZD administration (Kushibiki et al., 2001). However, in that experiment, there was not a control group of cows that were administered TZD and so direct effects of TZD on circulating glucose concentrations is not possible. It is possible that the effects of TZD administration on plasma glucose are dependent upon the degree or form of insulin resistance in treated animals. In agreement with previous results, TZD administration did not have an effect on plasma insulin (Figure 5-3B). Also consistent with previous results, cows fed the High diet had increased plasma insulin throughout the experiment (Holtenius et al., 2003; Dann et al., 2006).

The differences in plasma levels of insulin and NEFA resulted in cows fed the High diet having a lower overall RQUICKI, which would suggest greater insulin resistance in those cows (Holtenius and Holtenius, 2007). Greater insulin resistance in cows overfed during the dry period has been measured directly in other studies and is suggested as a mechanism by which overfed cows have altered metabolic regulation during the transition period (Holtenius et al., 2003). Similar to the cows evaluated via RQUICKI by Holtenius and Holtenius (2007), the cows in this experiment were relatively healthy and not under metabolic stress. Effective and repeatable evaluation of insulin resistance using RQUICKI in cows with metabolic disorders is lacking (Kerestes et al., 2009). Bossaert et al. (2009) were able to correlate RQUICKI and GTT measures in calves, further illustrating the need for future evaluation of RQUICKI as a measure of insulin sensitivity. It appears that RQUICKI may be an appropriate measure in some metabolic circumstances but may lack the ability to detect differences in others. In this case, RQUICKI was able to detect dietary

treatment differences consistent with previous results, and results shown here in response to GTT and IC.

Unlike in Chapter 4, there were fewer differences in clearance of glucose during GTT in the current experiment (Figure 5-4, Table 5-3). The trend for smaller glucose AUC for cows fed the Low diet may be explained by the slightly higher NEFA in these cows. More interesting, though not statistically significant, is the trend for cows administered TZD to have less insulin secretion in response to GTT (Table 5-4). Since differences were not observed in the rate of glucose clearance based on TZD-treatment, yet the TZD-treated cows had to secrete less insulin to clear the glucose, this suggests differences in glucose metabolism. Cows that were receiving chromium-methionine as a means to improve glucose tolerance, had reduced secretion of insulin in response to a GTT similar to the one performed here (Hayirli et al., 2001).

Plasma insulin responses to GTT are nearly identical to those illustrated in the fed state in Chapter 4. The lack of altered response in plasma insulin secretion due to dietary treatment is a reflection of the energy balance met by each of the dietary treatments. In Chapter 4, the Low diet and feed-deprived state may have reduced normal insulin responses due to prolonged negative energy balance while in the current experiment cows fed the Low diet were predicted to be in positive energy balance. As such, there did not appear to be affects on actual insulin secretion in the current study. As discussed, the heightened negative energy balance in cows fed the Low diet and those undergoing feed removal in Chapter 4 may have resulted in reduced capability of the pancreas to secrete insulin. Similar reduction of insulin responses have been seen in other instances of feed removal and acidotic cows (Hove, 1978; Bigner et al., 1996). Additional evidence for normal insulin secretion and metabolism in animals fed both the High and Low diets in the current study are the lack of differences in plasma insulin responses to insulin challenge (Table 5-7). Given

that there were no differences in insulin responses as measured by AUC, one can assume that there were no differences in insulin clearance based on dietary or TZD treatment. Differential responses to GTT and IC as measured by changes in plasma glucose and NEFA responses are more likely due then to tissue responses to insulin rather than changes in insulin secretion or clearance.

Additional differences were seen in plasma NEFA responses to GTT (Figure 5-6, Table 5-5). Based on differences in clearance rate, AUC, and glucose-stimulated-reduction-of-NEFA, it appears as though cows fed the Low diet had more dramatic changes in fatty acid metabolism in response to a glucose infusion. Bines and Morant (1983) hypothesized that thin cows had a more rapid rate of fatty acid synthesis, resulting in lower blood fatty acid precursors and more rapid absorption from the rumen as measured by responses to restricted and *ad libitum* feeding. The authors also suggested that this may explain why thin cows have a greater ability to increase intake than fat cows. A similar response was quantified by Rukkwamsuk, et al. (1999b) when they showed that despite having greater rates of esterification in adipose tissue prepartum, cows overfed during the dry period have higher rates of lipolysis postpartum (Rukkwamsuk et al., 1998).

In one of the few studies to report NEFA responses to GTT, cows were receiving chromium-methionine and there were no reported differences in NEFA responses to GTT (Hayirli et al., 2001). Any reported differences by the authors were due to differences in basal levels of NEFA prior to administration of the GTT. Results were similar for cows administered chromium propionate and then subjected to a similar GTT where no significant differences in plasma NEFA response to GTT were observed (Sumner et al., 2007). This could suggest that unlike during the use of chromium supplements as an insulin sensitizer, we are seeing additional differences in NEFA responses to GTT with TZD administration. Therefore, TZD might be

additionally beneficial due to its effects on fatty acid metabolism independent of glucose metabolism. This is consistent with the actions of TZD acting most directly on PPARγ specific to adipose tissue (Sundvold et al., 1997).

Despite having similar responses in plasma insulin to IC, there was a tendency for cows administered TZD to have more dramatic decreases in plasma glucose following insulin infusion (Figure 5-7, Table 5-6, and Figure 5-8). Previous insulin challenges completed on cows administered TZD did not show significant differences in plasma glucose or insulin responses at two insulin challenge doses (Smith et al., 2007). The authors stated this as evidence that TZD does not alter the important adaptation of reduced glucose utilization by skeletal muscle in preparation for lactation. Because TZD is a PPARγ ligand and expression is low in skeletal muscle (Sundvold et al., 1997), this likely a valid conclusion. Additionally, the cows in Smith et al. (2007) experiment were administered IC at 9 or 10 d prior to expected calving date, a time when insulin resistance of skeletal muscle is likely to be relatively high (Bauman and Currie, 1980). In contrast, the cows in this experiment were three weeks prior to calving at the time of the IC, a period of time when effects on whole-body glucose metabolism might be more measurable.

While the most differences in plasma NEFA responses to GTT were attributed to dietary (vs. TZD) treatment, there were a greater differences in plasma NEFA response to IC attributed to TZD administration (vs. dietary treatment) (Figure 5-9, Table 5-8). There were also instances of significant interactions of TZD and diet. The result is that cows administered TZD appeared to have greater response in plasma NEFA to insulin, a response that may be further amplified in cows fed the Low diet. Furthermore, these differences were observed despite no significant differences in plasma insulin response during IC (Figure 5-8, Table 5-7). This would suggest that differences in plasma NEFA responses to IC are not due to differences in insulin

secretion nor clearance and are more likely due to adipose tissue response to insulin. Therefore, cows treated with TZD, and especially cows also fed the Low diet, had greater ability of insulin to alter rates of either lipolysis or lipogenesis, or a combination. Treatment of human adipocytes *in vitro* with TZDs has been shown to enhance the effects of insulin on lipid metabolism (McTernan et al., 2002). However, the ability of TZD to enhance these effects is lost under situations of chronic hyperinulinemia (McTernan et al., 2002). This helps to explain how hyperinsulinemic individuals (both obese humans and overfed ruminants) can experience greater rates of lipolysis and/or body weight gain (McTernan et al., 2002; Dann et al., 2006)

Similar to results shown in Chapter 3, there were few effects of treatment on mRNA expression within subcutaneous adipose tissue. As discussed previously, although TZD has been shown to increase expression of both LPL and FAS in nonruminants, the effects in ruminants are not yet known (Kageyama et al., 2003; Bogacka et al., 2004). Because both of these genes have been implicated in changes in lipid metabolism occurring during milk fat depression (Harvatine et al., 2009), it was hypothesized that they may play roles in changes in insulin resistance mediated by TZD administration. In this case, however, there did not appear to be significant effects. In the case of LPL, it may not be a large factor in early lactation but instead may play a greater regulatory role in mid to late lactation (McNamara et al., 1987). There was a tendency for cows fed the High diet to have increased mRNA expression of LPL, which may be related to increased insulin concentration caused by dietary treatment (Faulconnier et al., 1996).

It is unknown why there was an effect of diet on PPARγ expression; however, it has been shown in human adipose tissue to be upregulated by insulin and downregulated by a low calorie diet (Vidal-Puig et al., 1997). Although TZD administration was shown in Chapter 3 to upregulate PPARγ expression, the reason

for a lack of an effect in the current study is unknown. Unlike cows in this experiment, cows investigated in Chapter 3 were much closer to calving and fed a single, moderate dietary energy level. It is possible that timing relative to calving and/or dietary energy level affect the potential for TZD to upregulate PPARγ in ruminants. There are additional factors beyond those investigated here that regulate changes in fatty acid metabolism due to TZD administration in dairy cattle.

Conclusions

The results shown here indicate that changes in insulin resistance may be different in relation to glucose and fatty acid metabolism based on dietary treatment as well as the use of an insulin sensitizing agent. While plasma glucose responses to GTT were more pronounced in cows fed a high plane of nutrition, cows fed a lower plane of nutrition had more dramatic responses in plasma NEFA. These results may be similar to the growing body of evidence which suggests that overfed cows experience changes in energy metabolism that ultimately results in greater loss of body condition and lower DMI postcalving.

The author expected to see more significant interaction between dietary treatment and TZD-administration. The reasons for the lack of interaction may have to do with the experimental design. Rather than complicate measures in energy metabolism by completing the study close to calving, the experiment was conducted in the early dry period in the hopes that responses due to diet and TZD might be more easily characterized. This may have resulted in a lack of response interaction. In the future, it might be beneficial to carry a similar experimental design through the transition into early lactation, or to subject cows to a period of negative energy balance similar to the one completed in Chapter 4.

The responses related to glucose and fatty acid metabolism observed in overfed dry cows needs to be further characterized. From this study, it is clear that evaluation of dietary treatments and metabolic intervention needs to include measures of both glucose and fatty acid metabolism. Responses may be differential for adipose tissue, and other insulin-dependent tissues in the body. As suggested, differences between independent adipose tissue depots needs further exploration as well.

ACKNOWLEDGEMENTS

The assistance of the following individuals was greatly appreciated for the completion of this study: R. Ehrhardt, J. Huzzey, L. Winkelman, C. Ryan, J.Ropke, E. Susskind, M. Hurley, B. Pomeroy, R. Watters, R. Higgs, M. Ramos-Nieves, S. Giesy, J. Schoenberg, V. Kim, and the staff at the Cornell University Dairy Teaching and Research Center.

CHAPTER 6: INTEGRATED SUMMARY AND CONCLUSIONS

The metabolic adaptations that support the onset of lactation include increased mobilization of fatty acids from adipose tissue and increased hepatic gluconeogenesis (Bell, 1995). Orchestration of nutrient partitioning is necessary to meet new metabolic demands coinciding with a simultaneous decrease in dry matter intake around calving, which requires mobilization of body fat reserves (Bauman and Currie, 1980). Insulin resistance during pregnancy and early lactation is a normal homeorhetic adaptation, the purpose of which is to spare glucose for the gravid uterus and mammary gland (Bauman and Currie, 1980). However, extreme insulin resistance may contribute to excess mobilization of adipose tissue in early lactation which is detrimental to the metabolic health of dairy cattle (Drackley, 1999).

Recent evidence shows that cows that are overfed and gain excessive body condition during the dry period often experience prolonged negative energy balance in early lactation (Douglas et al., 2006; Dann et al., 2006). Additionally, overfed cows were also determined to have increased insulin resistance as indicated by decrease clearance of glucose following glucose infusion (Holtenius et al., 2003). Given these findings, there is potential to investigate the roles of insulin resistance in dry cows and what influence plane of nutrition and adipose tissue metabolism might have on metabolic health of these animals.

Previous work demonstrated that administration of insulin sensitizing agents to prepartum dairy cattle can have positive effects on transition cow metabolism (Smith et al., 2007; Smith et al., 2009). One objective of this dissertation was to begin to explain the mechansism by which TZD administration may increase DMI and reduce NEFA in transition dairy cows. In Chapter 3, plasma leptin was found to be linearly increased postpartum by prepartum TZD administration of 2.0 and 4.0 mg TZD/kg of

BW. Plasma concentrations of TNFα were also shown to be linearly increased by increasing dose of TZD administration. A calculated measure of insulin resistance, RQUICKI, suggested that cows administered TZD had increased insulin sensitivity. Also reported in Chapter 3 was the first evidence that TZD administration in ruminants upregulates PPARγ, as measured by mRNA levels. There was no effect of TZD administration on adipose tissue mRNA expression for either LPL or FAS. These results implicate altered expression and plasma concentrations of leptin, plasma TNFα, but not LPL or FAS as potential mechanisms for the effects of TZD administration on transition dairy cattle.

An additional objective of this work was to further elucidate the role of insulin resistance in dry cows, and its potential influence on transition cow metabolism, especially during instances of altered plane of nutrition. In Chapter 4, cows subjected to high and low planes of energy and then subjected to feed deprivation had different responses in glucose and fatty acid metabolism as measured by both GTT and HEC. For example, cows fed the diet with lower energy had slower clearance of glucose during GTT but more dramatic decreases in NEFA during GTT. Overall, the effects of feed deprivation were much greater than prior plane of nutrition. Cows that were deprived of feed for 24h had much slower clearance of glucose during GTT, suggesting greater insulin resistance. In addition, following feed deprivation, cows had greatly attenuated insulin response to glucose infusion. This highlights that not only plane of nutrition but also acute periods of negative energy balance, such as that which occurs around the time of calving, can have profound effects on insulin responses in dairy cattle. The results also highlight that variables measured during GTT and HEC can be compared such as clearance rate during GTT and glucose infusion rate during HEC, and NEFA clearance rate during GTT and NEFA suppression during HEC.

Finally, in Chapter 5, the effects of both plane of nutrition and the use of the insulin-sensitizing agent TZD were investigated. This was the first experiment where TZD administration was combined with different dietary treatments. Responses to treatment were measured using a combination GTT and IC approach, as well as measures of adipose tissue mRNA expression of various genes. There were differential effects of plane of nutrition on glucose and fatty acid metabolism such that cows fed a lower energy level diet had tendency for smaller glucose AUC following GTT but more negative NEFA AUC and greater NEFA clearance. Administration of TZD tended to decrease the amount of insulin secreted during GTT, suggesting greater insulin sensitivity. The only significant interactions of plane of nutrition and TZD administration were that plasma NEFA responses happened more quickly and were greatest for cows treated with TZD and fed the lower plane of energy. While TZD administration or diet did not affect FAS, leptin, TNFα, or adiponectin mRNA expression, the higher energy level diet increased mRNA of PPARγ and LPL. It is possible that these effects and potential interactions of diet and TZD would be more dramatic closer to the time of calving. Results from GTT and IC indicate that energy level and insulin-sensitizing agents affect glucose and lipid metabolism during the dry period, which may have implications for the transition period.

These experiments were the first to analyze plasma concentration of leptin and TNFα from dairy cattle treated with TZD. This work began to explain potential metabolic outcomes of TZD administration in transition dairy cows. Additionally, results in Chapter 4 illustrated the potential effects of negative energy balance on insulin responses and secretion. It also provided comparison of measurements taken during GTT and HEC, illustrating that both can be used to determine changes in glucose and fatty acid metabolism in dairy cattle. It appears that plane of energy may

not affect responses in cattle to TZD administration, at least during the far-off dry period.

In conclusion, the transition period continues to be a dynamic area of research in which additional work is required. Future work should include additional characterization of insulin resistance during the dry period, including responses in additional adipokines. There is opportunity to use GTT and IC in order to assess insulin responses in dairy cattle, and future use should involve accurate reporting of not only glucose and insulin responses to these challenges, but also NEFA in order to characterize changes in fatty acid metabolism. There may also be opportunity to use RQUICKI as a relative measure of insulin resistance in dairy cattle, but additional testing is required to see if it is an appropriate measure in all circumstances. In the future, potential application of TZD in transition dairy cattle may not depend on plane of nutrition, but further work should be completed during the transition period in order to solidify this conclusion. Overall this work added to the body of evidence that suggests that insulin resistance in dry cows can potentially have large effects on transition cow metabolism.

REFERENCES

Agenas, S., E. Burstedt, and K. Holtenius. 2003. Effects of feeding intensity during the dry period. 1. Feed intake, body weight, and milk production. J. Dairy Sci. 86:870-882.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598-1624.

Allen, M. S., B. J. Bradford, and K. J. Harvatine. 2005. The cow as a model to study food intake regulation. Annu. Rev. Nutr. 25:523-547.

Andersen, J. B., D. G. Mashek, T. Larsen, M. O. Nielsen, and K. L. Ingvartsen. 2002. Effects of hyperinsulinaemia under euglycaemic condition on liver fat metabolism in dairy cows in early and mid-lactation. J. Vet. Med. A Physiol Pathol. Clin. Med. 49:65-71.

AOAC. 2000. Official Methods of Analysis. 17th edition. Association of Official Analytical Chemists, Arlington, VA.

Arner, P. 2001. Free fatty acids--do they play a central role in type 2 diabetes? Diabetes Obes. Metab 3 Suppl 1:S11-S19.

Arner, P. 2003. The adipocyte in insulin resistance: key molecules and the impact of the thiazolidinediones. Trends Endocrinol. Metab 14:137-145.

Barthel, A. and D. Schmoll. 2003. Novel concepts in insulin regulation of hepatic gluconeogenesis. Am. J. Physiol Endocrinol. Metab 285:E685-E692.

Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Bell, A. W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim Sci. 73:2804-2819.

Bell, A. W. and D. E. Bauman. 1997. Adaptations of glucose metabolism during pregnancy and lactation. J. Mammary. Gland. Biol. Neoplasia. 2:265-278.

Benson, J. A. and C. K. Reynolds. 2001. Effects of abomasal infusion of long-chain fatty acids on splanchnic metabolism of pancreatic and gut hormones in lactating dairy cows. J. Dairy Sci. 84:1488-1500.

Bergman, E. N., R. P. Brockman, and C. F. Kaufman. 1974. Glucose metabolism in ruminants: comparison of whole-body turnover with production by gut, liver, and kidneys. Fed. Proc. 33:1849-1854.

Bergman, E. N., S. S. Reulein, and R. E. Corlett. 1989. Effects of obesity on insulin sensitivity and responsiveness in sheep. Am. J. Physiol 257:E772-E781.

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

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

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy Sci. 88:2017-2026.

Bertics, S. J., R. R. Grummer, C. Cadorniga-Valino, and E. E. Stoddard. 1992. Effect of prepartum dry matter intake on liver triglyceride concentration and early lactation. J. Dairy Sci. 75:1914-1922.

Bigner, D. R., J. P. Goff, M. A. Faust, J. L. Burton, H. D. Tyler, and R. L. Horst. 1996. Acidosis effects on insulin response during glucose tolerance tests in Jersey cows. J. Dairy Sci. 79:2182-2188.

Bines, J. A. and S. V. Morant. 1983. The effect of body condition on metabolic changes associated with intake of food by the cow. Br. J. Nutr. 50:81-89.

Block, S. S., W. R. Butler, R. A. Ehrhardt, A. W. Bell, M. E. Van Amburgh, and Y. R. Boisclair. 2001. Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. J. Endocrinol. 171:339-348.

Block, S. S., R. P. Rhoads, D. E. Bauman, R. A. Ehrhardt, M. A. McGuire, B. A. Crooker, J. M. Griinari, T. R. Mackle, W. J. Weber, M. E. Van Amburgh, and Y. R. Boisclair. 2003. Demonstration of a role for insulin in the regulation of leptin in lactating dairy cows. J. Dairy Sci. 86:3508-3515.

Blum, J. W., R. M. Bruckmaier, and P. Y. Vacher. 1999. Insulin-dependent whole-body glucose utilization and insulin-responses to glucose in week 9 and week 19 of lactation in dairy cows fed rumen-protected crystalline fat or free fatty acids. Domest. Anim Endocrinol. 16:123-134.

Blum, J. W., H. Dosogne, D. Hoeben, F. Vangroenweghe, H. M. Hammon, R. M. Bruckmaier, and C. Burvenich. 2000. Tumor necrosis factor-[alpha] and nitrite/nitrate responses during acute mastitis induced by Escherichia coli infection and endotoxin in dairy cows. Domestic. Animal Endocrinology 19:223-235.

Bobe, G., J. W. Young, and D. C. Beitz. 2004. Invited review: pathology, etiology, prevention, and treatment of fatty liver in dairy cows. J. Dairy Sci. 87:3105-3124.

Bogacka, I., H. Xie, G. A. Bray, and S. R. Smith. 2004. The effect of pioglitazone on peroxisome proliferator-activated receptor-gamma target genes related to lipid storage in vivo. Diabetes Care 27:1660-1667.

Bonnet, M., Y. Faulconnier, J. Flechet, J. F. Hocquette, C. Leroux, D. Langin, P. Martin, and Y. Chilliard. 1998. Messenger RNAs encoding lipoprotein lipase, fatty acid synthase and hormone-sensitive lipase in the adipose tissue of underfed-refed ewes and cows. Reprod. Nutr. Dev. 38:297-307.

Bossaert, P., J. L. Leroy, S. De Campeneere, S. De Vliegher, and G. Opsomer. 2009. Differences in the glucose-induced insulin response and the peripheral insulin responsiveness between neonatal calves of the Belgian Blue, Holstein-Friesian, and East Flemish breeds. J. Dairy Sci. 92:4404-4411.

Boston, R. C., J. R. Roche, G. M. Ward, and P. J. Moate. 2008. A novel minimal model to describe non-esterified fatty acid kinetics in Holstein dairy cows. J. Dairy Res. 75:13-18.

Carswell, E. A., L. J. Old, R. L. Kassel, S. Green, N. Fiore, and B. Williamson. 1975. An endotoxin-induced serum factor that causes necrosis of tumors. Proceedings of the National Academy of Sciences of the United States of America 72:3666-3670.

Catalano, P. M., S. E. Nizielski, J. Shao, L. Preston, L. Qiao, and J. E. Friedman. 2002. Downregulated IRS-1 and PPARgamma in obese women with gestational diabetes: relationship to FFA during pregnancy. Am. J. Physiol Endocrinol. Metab 282:E522-E533.

Chelikani, P. K., J. D. Ambrose, D. H. Keisler, and J. J. Kennelly. 2004. Effect of short-term fasting on plasma concentrations of leptin and other hormones and metabolites in dairy cattle. Domest. Anim Endocrinol. 26:33-48.

Chilliard, Y., M. Bonnet, C. Delavaud, Y. Faulconnier, C. Leroux, J. Djiane, and F. Bocquier. 2001. Leptin in ruminants. Gene expression in adipose tissue and mammary gland, and regulation of plasma concentration. Domest. Anim Endocrinol. 21:271-295.

Chilliard, Y., C. Delavaud, and M. Bonnet. 2005. Leptin expression in ruminants: nutritional and physiological regulations in relation with energy metabolism. Domest. Anim Endocrinol. 29:3-22.

Choi, B. R., D. L. Palmquist, and M. S. Allen. 2000. Cholecystokinin mediates depression of feed intake in dairy cattle fed high fat diets. Domest. Anim Endocrinol. 19:159-175.

Cooke, R. F., D. R. Silva, D. Z. Caraviello, S. J. Bertics, M. H. Ramos, and R. R. Grummer. 2007. Supplemental choline for prevention and alleviation of fatty liver in dairy cattle. J Dairy Sci 90:2413-2418.

Curtis, C. R., H. N. Erb, C. J. Sniffen, and R. D. Smith. 1984. Epidemiology of parturient paresis: predisposing factors with emphasis on dry cow feeding and management. J. Dairy Sci. 67:817-825.

Curtis, C. R., N. E. Hollis, J. S. Charles, R. D. Smith, and David S.Kronfeld. 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. J. Dairy Sci. 68:2347-2360.

Dann, H. M., N. B. Litherland, J. P. Underwood, M. Bionaz, A. D'Angelo, J. W. McFadden, and J. K. Drackley. 2006. Diets during far-off and close-up dry periods affect periparturient metabolism and lactation in multiparous cows. J. Dairy Sci. 89:3563-3577.

Dann, H. M., D. E. Morin, G. A. Bollero, M. R. Murphy, and J. K. Drackley. 2005. Prepartum intake, postpartum induction of ketosis, and periparturient disorders affect the metabolic status of dairy cows. J. Dairy Sci. 88:3249-3264.

Dann, H. M., G. A. Varga, and D. E. Putnam. 1999. Improving energy supply to late gestation and early postpartum dairy cows. J. Dairy Sci. 82:1765-1778.

DeFronzo, R. A., J. D. Tobin, and R. Andres. 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am. J. Physiol 237:E214-E223.

Delarue, J. and C. Magnan. 2007. Free fatty acids and insulin resistance. Curr. Opin. Clin. Nutr. Metab Care 10:142-148.

Douglas, G. N., T. R. Overton, H. G. Bateman, H. M. Dann, and J. K. Drackley. 2006. Prepartal plane of nutrition, regardless of dietary energy source, affects periparturient metabolism and dry matter intake in Holstein cows. J. Dairy Sci. 89:2141-2157.

Drackley, J. K. 1999. ADSA foundation scholar award. Biology of dairy cows during the transition period: the final frontier? J. Dairy Sci. 82:2259-2273.

Drackley, J. K., S. S. Donkin, and C. K. Reynolds. 2006. Major advances in fundamental dairy cattle nutrition. J. Dairy Sci. 89:1324-1336.

Drackley, J. K., T. R. Overton, and G. N. Douglas. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. J. Dairy Sci. 84:E100-E112.

Ehrhardt RA, F. A. U., F. A. U. Slepetis RM, F. A. U. Bell AW, and Y. R. Boisclair. 2001. Maternal leptin is elevated during pregnancy in sheep. Domest. Anim Endocrinol. 21:85-96.

Ehrhardt, R. A., R. M. Slepetis, J. Siegal-Willott, M. E. Van Amburgh, A. W. Bell, and Y. R. Boisclair. 2000. Development of a specific radioimmunoassay to measure physiological changes of circulating leptin in cattle and sheep. J. Endocrinol. 166:519-528.

Elkins, D. A. and D. M. Spurlock. 2009. Phosphorylation of perilipin is associated with indicators of lipolysis in Holstein cows. Horm. Metab Res. 41:736-740.

Emery, R. S., J. S. Liesman, and T. H. Herdt. 1992. Metabolism of long chain fatty acids by ruminant liver. J. Nutr. 122:832-837.

Farney, J. K., L. K. Mamedova, and B. J. Bradford. 2010. Modification and validation of a bovine TNFalpha enzyme-linked immunosorbent assay with improved sensitivity. J. Dairy Sci. 93(E-Suppl.):299(Abstr)-

Faulconnier, Y., L. Guillon, and Y. Chilliard. 1996. Lipoprotein lipase and glucose-6-phosphate dehydrogenase activities in bovine and ovine adipose tissue incubated for 7 days: effects of insulin and/or dexamethasone. Comp Biochem. Physiol B Biochem. Mol. Biol. 113:421-426.

Faulkner, A. and H. T. Pollock. 1990. Metabolic responses to euglycaemic hyperinsulinaemia in lactating and non-lactating sheep in vivo. J. Endocrinol. 124:59-66.

Garnsworthy, P. C. 2006. Body condition score in dairy cows: targets for production and fertility. Recent Advances in Animal Nutrition Eds. P. C. Garnsworthy and J. Wiseman 61-86.

Gastaldelli, A., E. Ferrannini, Y. Miyazaki, M. Matsuda, A. Mari, and R. A. DeFronzo. 2007. Thiazolidinediones improve beta-cell function in type 2 diabetic patients. Am. J Physiol Endocrinol. Metab 292:E871-E883.

Gatford, K. L., E. M. Wintour, M. J. De Blasio, J. A. Owens, and M. Dodic. 2000. Differential timing for programming of glucose homoeostasis, sensitivity to insulin and blood pressure by in utero exposure to dexamethasone in sheep. Clin. Sci. (Lond) 98:553-560.

Goff, J. P. and R. L. Horst. 1997. Physiological changes at parturition and their relationship to metabolic disorders. J. Dairy Sci. 80:1260-1268.

Greenberg, A. S. and M. L. McDaniel. 2002. Identifying the links between obesity, insulin resistance and beta-cell function: potential role of adipocyte-derived cytokines in the pathogenesis of type 2 diabetes. Eur. J. Clin. Invest 32 Suppl 3:24-34.

Griinari, J. M., M. A. McGuire, D. A. Dwyer, D. E. Bauman, and D. L. Palmquist. 1997. Role of insulin in the regulation of milk fat synthesis in dairy cows. J. Dairy Sci. 80:1076-1084.

Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882-3896.

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. J. Anim Sci. 73:2820-2833.

Guo, L. and R. Tabrizchi. 2006. Peroxisome proliferator-activated receptor gamma as a drug target in the pathogenesis of insulin resistance. Pharmacol. Ther. 111:145-173.

Hammarstedt, A., C. X. Andersson, S. Rotter, V, and U. Smith. 2005. The effect of PPARgamma ligands on the adipose tissue in insulin resistance. Prostaglandins Leukot. Essent. Fatty Acids 73:65-75.

Hartwell, J. R., M. J. Cecava, and S. S. Donkin. 2000. Impact of dietary rumen undegradable protein and rumen-protected choline on intake, peripartum liver triacylglyceride, plasma metabolites and milk production in transition dairy cows. J Dairy Sci 83:2907-2917.

Harvatine, K. J. and D. E. Bauman. 2007. Expression of PPAR and LXR nuclear hormone receptor families are not modified during milk fat depression induced by diet or treatment with *trans*-10, *cis*-12 conjugated linoleic acid (CLA). J. Dairy Sci. 90(Suppl.1):59 (Abstr).

Harvatine, K. J., J. W. Perfield, and D. E. Bauman. 2009. Expression of enzymes and key regulators of lipid synthesis is upregulated in adipose tissue during CLA-induced milk fat depression in dairy cows. J. Nutr. 139:849-854.

Hayirli, A., D. R. Bremmer, S. J. Bertics, M. T. Socha, and R. R. Grummer. 2001. Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. J. Dairy Sci. 84:1218-1230.

Hayirli, A., R. R. Grummer, E. V. Nordheim, and P. M. Crump. 2002. Animal and Dietary Factors Affecting Feed Intake During the Prefresh Transition Period in Holsteins. J. Dairy Sci. 85:3430-3443.

Hocquette, J. F., B. Graulet, and T. Olivecrona. 1998. Lipoprotein lipase activity and mRNA levels in bovine tissues. Comp Biochem. Physiol B Biochem. Mol. Biol. 121:201-212.

Holtenius, K., S. Agenas, C. Delavaud, and Y. Chilliard. 2003. Effects of feeding intensity during the dry period. 2. Metabolic and hormonal responses. J. Dairy Sci. 86:883-891.

Holtenius, P. and K. Holtenius. 2007. A model to estimate insulin sensitivity in dairy cows. Acta Vet. Scand. 49:29-31.

Holtenius, P., G. Olsson, M. Emanuelson, and H. Wiktorsson. 1996. Effects of different energy levels, concentrate/forage ratios and lipid supplementation to the diet on the adaptation of the energy metabolism at calving in dairy cows. Zentralbl. Veterinarmed. A 43:427-435.

Hostettler-Allen, Robyn Leigh, L. Tappy, and J. W. Blum. 1994. Insulin resistance, hyperglycemia, and glucosuria in intensively milk-fed calves. Journal of Animal Science 72:160-173.

Houseknecht, K. L. and D. E. Bauman. 1997. Regulation of lipolysis by somatotropin: functional alteration of adrenergic and adenosine signaling in bovine adipose tissue. J. Encrinol. 152:465-475.

Houseknecht, K. L., B. M. Cole, and P. J. Steele. 2002. Peroxisome proliferator-activated receptor gamma (PPARgamma) and its ligands: a review. Domestic Animal Endocrinology 22:1-23.

Hove, Knut. 1978. Insulin Secretion in Lactating Cows: Responses to Glucose Infused Intravenously in Normal, Ketonemic, and Starved Animals. J. Dairy Sci. 61:1407-1413.

Ingvartsen, K. L. and J. B. Andersen. 2000. Integration of metabolism and intake regulation: a review focusing on periparturient animals. J. Dairy Sci. 83:1573-1597.

Ingvartsen, K. L. and Y. R. Boisclair. 2001. Leptin and the regulation of food intake, energy homeostasis and immunity with special focus on periparturient ruminants. Domest. Anim Endocrinol. 21:215-250.

Jesse, B. W., R. S. Emery, and J. W. Thomas. 1986a. Aspects of the Regulation of Long-Chain Fatty Acid Oxidation in Bovine Liver1. J. Dairy Sci. 69:2298-2303.

Jesse, B. W., R. S. Emery, and J. W. Thomas. 1986b. Control of Bovine Hepatic Fatty Acid Oxidation1. J. Dairy Sci. 69:2290-2297.

Ji, P., J. S. Osorio, J. K. Drackley, and J. J. Loor. 2010. Insulin signal transduction in adipose tissue of peripartal dairy cows fed two levels of dietary energy prepartum. J. Dairy Sci. 93(E-Suppl.):870 (Abstr).

Joosen, A. M., A. H. Bakker, M. J. Gering, and K. R. Westerterp. 2006. The effect of the PPARgamma ligand rosiglitazone on energy balance regulation. Diabetes Metab Res. Rev. 22:204-210.

Kadowaki, T., T. Yamauchi, N. Kubota, K. Hara, K. Ueki, and K. Tobe. 2006. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J. Clin. Invest 116:1784-1792.

Kageyama, H., T. Hirano, K. Okada, T. Ebara, A. Kageyama, T. Murakami, S. Shioda, and M. Adachi. 2003. Lipoprotein lipase mRNA in white adipose tissue but not in skeletal muscle is increased by pioglitazone through PPAR-gamma. Biochem. Biophys. Res. Commun. 305:22-27.

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

Kerestes, M., V. Faigl, M. Kulcsar, O. Balogh, J. Foldi, H. Febel, Y. Chilliard, and G. Huszenicza. 2009. Periparturient insulin secretion and whole-body insulin responsiveness in dairy cows showing various forms of ketone pattern with or without puerperal metritis. Domest. Anim Endocrinol. 37:250-261.

Kim, J, K. L. Smith, T. R. Overton, and Y. R. Boisclair. 2008. Regulation of adiponectin during the transition from pregnancy to lactation in dairy cattle. Endocrinology (Suppl. 1)(Abstr).

Komatsu, T., F. Itoh, S. Kushibiki, and K. Hodate. 2005. Changes in gene expression of glucose transporters in lactating and nonlactating cows. J. Anim Sci. 83:557-564.

Komatsu, T., F. Itoh, S. Mikawa, and K. Hodate. 2003. Gene expression of resistin in adipose tissue and mammary gland of lactating and non-lactating cows. J. Endocrinol. 178:R1-R5.

Kushibiki, S., K. Hodate, H. Shingu, Y. Ueda, M. Shinoda, Y. Mori, T. Itoh, and Y. Yokomizo. 2001. Insulin resistance induced in dairy steers by tumor necrosis factor alpha is partially reversed by 2,4-thiazolidinedione. Domest. Anim Endocrinol. 21:25-37.

Larsen, P. J., P. B. Jensen, R. V. Sorensen, L. K. Larsen, N. Vrang, E. M. Wulff, and K. Wassermann. 2003. Differential influences of peroxisome proliferator-activated receptors gamma and -alpha on food intake and energy homeostasis. Diabetes 52:2249-2259.

Lemor, A., A. Hosseini, H. Sauerwein, and M. Mielenz. 2009. Transition period-related changes in the abundance of the mRNAs of adiponectin and its receptors, of visfatin, and of fatty acid binding receptors in adipose tissue of high-yielding dairy cows. Domest. Anim Endocrinol. 37:37-44.

Leury, B. J., L. H. Baumgard, S. S. Block, N. Segoale, R. A. Ehrhardt, R. P. Rhoads, D. E. Bauman, A. W. Bell, and Y. R. Boisclair. 2003. Effect of insulin and growth hormone on plasma leptin in periparturient dairy cows. Am. J. Physiol Regul. Integr. Comp Physiol 285:R1107-R1115.

Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology 57:347-358.

Liefers, S. C., R. F. Veerkamp, M. F. Te Pas, C. Delavaud, Y. Chilliard, and L. T. Van der. 2003. Leptin concentrations in relation to energy balance, milk yield, intake, live weight, and estrus in dairy cows. J. Dairy Sci. 86:799-807.

Litherland, N. B., M. Bionaz, R. L. Wallace, J. J. Loor, and J. K. Drackley. 2010. Effects of the peroxisome proliferator-activated receptor-alpha agonists clofibrate and fish oil on hepatic fatty acid metabolism in weaned dairy calves. J Dairy Sci 93:2404-2418.

Maeda, N., M. Takahashi, T. Funahashi, S. Kihara, H. Nishizawa, K. Kishida, H. Nagaretani, M. Matsuda, R. Komuro, N. Ouchi, H. Kuriyama, K. Hotta, T. Nakamura, I. Shimomura, and Y. Matsuzawa. 2001. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 50:2094-2099.

McCutcheon, S. N. and D. E. Bauman. 1986. Effect of chronic growth hormone treatment on responses to epinephrine and thyrotropin-releasing hormone in lactating cows. J. Dairy Sci. 69:44-51.

McNamara, J. P. and J. K. Hillers. 1986a. Regulation of Bovine Adipose Tissue Metabolism During Lactation. 1. Lipid Synthesis in Response to Increased Milk Production and Decreased Energy Intake1. J. Dairy Sci. 69:3032-3041.

McNamara, J. P. and J. K. Hillers. 1986b. Regulation of Bovine Adipose Tissue Metabolism During Lactation. 2. Lipolysis Response to Milk Production and Energy Intake1. J. Dairy Sci. 69:3042-3050.

McNamara, J. P., D. C. McFarland, and S. Bai. 1987. Regulation of bovine adipose tissue metabolism during lactation. 3. Adaptations of hormone-sensitive and lipoprotein lipases. J. Dairy Sci. 70:1377-1384.

McNamara, J. P. and F. Valdez. 2005. Adipose tissue metabolism and production responses to calcium propionate and chromium propionate. J. Dairy Sci. 88:2498-2507.

McTernan, Philip G., A. L. Harte, L. A. Anderson, A. Green, S. A. Smith, J. C. Holder, A. H. Barnett, M. C. Eggo, and S. Kumar. 2002. Insulin and rosiglitazone regulation of lipolysis and lipogenesis in human adipose tissue in vitro. Diabetes 51:1493-1498.

Moate, Peter J., J. R. Roche, L. M. Chagas, and R. C. Boston. 2007. Evaluation of a compartmental model to describe non-esterified fatty acid kinetics in Holstein dairy cows. Journal of Dairy Research. 74:430-437.

Mokdad, Ali H., E. S. Ford, B. A. Bowman, W. H. Dietz, F. Vinicor, V. S. Bales, and J. S. Marks. 2003. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. JAMA 289:76-79.

Morino, K., K. F. Petersen, and G. I. Shulman. 2006. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. Diabetes 55 Suppl 2:S9-S15.

Muniyappa, R., S. Lee, H. Chen, and M. J. Quon. 2008. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am. J. Physiol Endocrinol. Metab 294:E15-E26.

Nikkhah, A., J. J. Loor, R. L. Wallace, D. Graugnard, J. Vasquez, B. Richards, and J. K. Drackley. 2008. Moderate excesses of dietary energy markedly increase visceral adipose tissue mass in non-lactating dairy cows. J. Dairy Sci. 86:(E-Suppl. 1) (Abstr).

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Academy Press. Washington, DC.

Oba, M. and M. S. Allen. 2003. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:174-183.

Ohtsuka, H., M. Koiwa, A. Hatsugaya, K. Kudo, F. Hoshi, N. Itoh, H. Yokota, H. Okada, and S. Kawamura. 2001. Relationship between serum TNF activity and insulin resistance in dairy cows affected with naturally occurring fatty liver. J. Vet. Med. Sci. 63:1021-1025.

Owecki, M., A. Miczke, E. Nikisch, D. Pupek-Musialik, and J. Sowinski. 2010. Serum Resistin Concentrations are Higher in Human Obesity but Independent from Insulin Resistance. Exp. Clin. Endocrinol. Diabetes (EPub).

Park, K. S., B. D. Rhee, K. U. Lee, H. K. Lee, C. S. Koh, and H. K. Min. 1990. Hyperglycemia per se can reduce plasma free fatty acid and glycerol levels in the acutely insulin-deficient dog. Metabolism 39:595-597.

Petersen, K. F. and G. I. Shulman. 2006. Etiology of insulin resistance. Am. J. Med. 119:S10-S16.

Petterson, J. A., F. R. Dunshea, R. A. Ehrhardt, and A. W. Bell. 1993. Pregnancy and undernutrition alter glucose metabolic responses to insulin in sheep. J. Nutr. 123:1286-1295.

Picard, F. and J. Auwerx. 2002. PPAR(gamma) and glucose homeostasis. Annu. Rev. Nutr. 22:167-197.

Piepenbrink, M. S. and T. R. Overton. 2003. Liver metabolism and production of cows fed increasing amounts of rumen-protected choline during the periparturient period. J. Dairy Sci. 86:1722-1733.

Pires, J. A., J. B. Pescara, and R. R. Grummer. 2007a. Reduction of plasma NEFA concentration by nicotinic acid enhances the response to insulin in feed-restricted Holstein cows. J. Dairy Sci. 90:4635-4642.

Pires, J. A., A. H. Souza, and R. R. Grummer. 2007b. Induction of hyperlipidemia by intravenous infusion of tallow emulsion causes insulin resistance in Holstein cows. J. Dairy Sci. 90:2735-2744.

Pullen, D. L., D. L. Palmquist, and R. S. Emery. 1989. Effect on days of lactation and methionine hydroxy analog on incorporation of plasma fatty acids into plasma triglycerides. J. Dairy Sci. 72:49-58.

Ranheim, T., F. Haugen, A. C. Staff, K. Braekke, N. K. Harsem, and C. A. Drevon. 2004. Adiponectin is reduced in gestational diabetes mellitus in normal weight women. Acta Obstet. Gynecol. Scand. 83:341-347.

Regnault, T. R., H. V. Oddy, C. Nancarrow, N. Sriskandarajah, and R. J. Scaramuzzi. 2004. Glucose-stimulated insulin response in pregnant sheep following acute suppression of plasma non-esterified fatty acid concentrations. Reprod. Biol. Endocrinol. 2:64-74.

Reynolds, C. K., P. C. Aikman, B. Lupoli, D. J. Humphries, and D. E. Beever. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. J. Dairy Sci. 86:1201-1217.

Richards, B. F., N. A. Janovick, K. M. B. D. E. Moyes, and J. K. Drackley. 2010. Comparison of a controlled-energy high-fiber diet fed throughout the dry period to a two-stage far-off and close-up dietary strategy. J. Dairy Sci. 87:(E-Suppl) (Abstr).

Rukkwamsuk, T., T. A. Kruip, G. A. Meijer, and T. Wensing. 1999a. Hepatic fatty acid composition in periparturient dairy cows with fatty liver induced by intake of a high energy diet in the dry period. J. Dairy Sci. 82:280-287.

Rukkwamsuk, T., T. Wensing, and M. J. Geelen. 1998. Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. J. Dairy Sci. 81:2904-2911.

Rukkwamsuk, T., T. Wensing, and M. J. Geelen. 1999b. Effect of overfeeding during the dry period on the rate of esterification in adipose tissue of dairy cows during the periparturient period. J. Dairy Sci. 82:1164-1169.

Sakai, Takeo, M. Hamakawa, and S. Kubo. 1996. Glucose and Xylitol Tolerance Tests for Ketotic and Healthy Dairy Cows. J. Dairy Sci. 79:372-377.

Santos, J. E. and F. S. Lima. 2009. Feeding rumen-protected choline to transition dairy cows. Proceedings 20th Annual Florida Ruminant Nutrition Symposium149-159.

Sasaki, S. 2002. Mechanism of insulin action on glucose metabolism in ruminants. Animal Science Journal 73:423-433.

Schinner, S., W. A. Scherbaum, S. R. Bornstein, and A. Barthel. 2005. Molecular mechanisms of insulin resistance. Diabet. Med. 22:674-682.

Schoenberg, K. M., R. M. Ehrhardt, and T. R. Overton. 2009. Effect of plane of nutrition and feed deprivation on insulin responses in dairy cattle during late gestation. J. Dairy Sci. 92(E-Suppl.):701(Abstr.).

Schoenberg, K. M. and T. R. Overton. 2010. The changing roles of insulin during the transition period. Proceedings of the Cornell Nutrition Conference for Feed Manufacturers 72:175-185.

Schoenberg, K. M., K. L. Perfield, S. L. Giesy, Y. R. Boisclair, and T. R. Overton. 2010. Prepartum 2,4-thiazolidinedione alters gene expression of peroxisome proliferator-activated receptor gamma and leptin in the adipose tissue of dairy cows. J. Dairy Sci. 93(E-Suppl.):200(Abstr.).

Schoenberg, K. M., K. L. Smith, R. M. Ehrhardt, Y. R. Boisclair, and T. R. Overton. 2008. Effects of prepartum 2,4-thiazolidinedione on plasma leptin and insulin sensitivity in transition dairy cows. J. Dairy Sci. 91(E-Suppl.):183(Abstr).

Sharma, A. M. and B. Staels. 2007. Review: Peroxisome proliferator-activated receptor gamma and adipose tissue--understanding obesity-related changes in regulation of lipid and glucose metabolism. J. Clin. Endocrinol. Metab 92:386-395.

Shen, W. J., S. Patel, H. Miyoshi, A. S. Greenberg, and F. B. Kraemer. 2009. Functional interaction of hormone-sensitive lipase and perilipin in lipolysis. J Lipid Res. 50:2306-2313.

Shimizu, H., T. Tsuchiya, N. Sato, Y. Shimomura, I. Kobayashi, and M. Mori. 1998. Troglitazone reduces plasma leptin concentration but increases hunger in NIDDM patients. Diabetes Care 21:1470-1474.

Sirois, P. K., M. J. Reuter, C. M. Laughlin, and P. J. Lockwood. 1994. A method for determining macro and micro elements in forages and feeds by inductively coupled plasma atomic emission spectrometry. Spectroscopist 3:6-9.

Smith, K. L., W. R. Butler, and T. R. Overton. 2009. Effects of prepartum 2,4-thiazolidinedione on metabolism and performance in transition dairy cows. J. Dairy Sci. 92:3623-3633.

Smith, K. L., S. E. Stebulis, M. R. Waldron, and T. R. Overton. 2007. Prepartum 2,4-thiazolidinedione alters metabolic dynamics and dry matter intake of dairy cows. J. Dairy Sci. 90:3660-3670.

Spears, J. W. 2010. Chromium in dairy cattle nutrition. Proceedings of the Cornell Nutrition Conference for Feed Manufacturers 72:38-47.

Spears, J. W., C. S. Whisnant, G. B. Huntington, K. E. Lloyd, K. Krafka, and A. Lamptey. 2010. Effects of dietary chromium propionate on glucose metabolism and insulin sensitivity in growing cattle. J. Dairy Sci. 93:(Suppl. 1) 780 (Abstr).

Stanley, T. A., R. C. Cochran, E. S. Vanzant, D. L. Harmon, and L. R. Corah. 1993. Periparturient changes in intake, ruminal capacity, and digestive characteristics in beef cows consuming alfalfa hay. Journal of Animal Science 71:788-795.

Steppan, C. M., S. T. Bailey, S. Bhat, E. J. Brown, R. R. Banerjee, C. M. Wright, H. R. Patel, R. S. Ahima, and M. A. Lazar. 2001. The hormone resistin links obesity to diabetes. Nature 409:307-312.

Stienstra, R., C. Duval, M. Ller, and S. Kersten. 2006. PPARs, Obesity, and Inflammation. PPAR. Res. 2007:95974-95984.

Sumner, A. E., R. N. Bergman, G. L. Vega, D. J. Genovese, C. S. Cochran, K. Pacak, R. M. Watanabe, and R. C. Boston. 2004. The multiphasic profile of free fatty acids during the intravenous glucose tolerance test is unresponsive to exogenous insulin. Metabolism 53:1202-1207.

Sumner, J. M. and J. P. McNamara. 2007. Expression of lipolytic genes in the adipose tissue of pregnant and lactating Holstein dairy cattle. J. Dairy Sci. 90:5237-5246.

Sumner, J. M., F. Valdez, and J. P. McNamara. 2007. Effects of chromium propionate on response to an intravenous glucose tolerance test in growing Holstein heifers. J. Dairy Sci. 90:3467-3474.

Sundvold, H., A. Brzozowska, and S. Lien. 1997. Characterisation of bovine peroxisome proliferator-activated receptors gamma 1 and gamma 2: genetic mapping and differential expression of the two isoforms. Biochem. Biophys. Res. Commun. 239:857-861.

Thorn, S. R., S. Purup, W. S. Cohick, M. Vestergaard, K. Sejrsen, and Y. R. Boisclair. 2006. Leptin does not act directly on mammary epithelial cells in prepubertal dairy heifers. J. Dairy Sci. 89:1467-1477.

Tonelli, J., W. Li, P. Kishore, U. B. Pajvani, E. Kwon, C. Weaver, P. E. Scherer, and M. Hawkins. 2004. Mechanisms of early insulin-sensitizing effects of thiazolidinediones in type 2 diabetes. Diabetes 53:1621-1629.

Tordjman, J., G. Chauvet, J. Quette, E. G. Beale, C. Forest, and B. Antoine. 2003a. Thiazolidinediones block fatty acid release by inducing glyceroneogenesis in fat cells. J. Biol. Chem. 278:18785-18790.

Tordjman, J., W. Khazen, B. Antoine, G. Chauvet, J. Quette, F. Fouque, E. G. Beale, C. Benelli, and C. Forest. 2003b. Regulation of glyceroneogenesis and phosphoenolpyruvate carboxykinase by fatty acids, retinoic acids and thiazolidinediones: potential relevance to type 2 diabetes. Biochimie 85:1213-1218.

Trout, K. K., C. Homko, and N. C. Tkacs. 2007. Methods of measuring insulin sensitivity. Biol. Res. Nurs. 8:305-318.

Tylutki, T. P., D. G. Fox, V. M. Durbal, L. O. Tedeschi, J. B. Russell, M. E. Van Amburgh, T. R. Overton, L. E. Chase, and A. N. Pell. 2008. Cornell Net Carbohydrate and Protein System: A model for precision feeding of dairy cattle. Animal Feed Science and Technology 143:174-202.

Van den Top, A. M., T. Wensing, M. J. Geelen, G. H. Wentink, A. T. van't Klooster, and A. C. Beynen. 1995. Time trends of plasma lipids and enzymes synthesizing hepatic triacylglycerol during postpartum development of fatty liver in dairy cows. J. Dairy Sci. 78:2208-2220.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Vernon, R. G. 1989. Endocrine control of metabolic adaptation during lactation. Proc. Nutr. Soc. 48:23-32.

Vernon, R. G. 2005. Lipid metabolism during lactation: a review of adipose tissue-liver interactions and the development of fatty liver. J. Dairy Res. 72:460-469.

Vernon, R. G., R. A. Clegg, and D. J. Flint. 1981. Metabolism of sheep adipose tissue during pregnancy and lactation. Adaptation and regulation. Biochem. J. 200:307-314.

Vernon, R. G., R. G. Denis, and A. Sorensen. 2001. Signals of adiposity. Domest. Anim Endocrinol. 21:197-214.

Vernon, R. G. and C. M. Pond. 1997. Adaptations of maternal adipose tissue to lactation. J. Mammary. Gland. Biol. Neoplasia. 2:231-241.

Vidal-Puig, A. J., R. V. Considine, M. Jimenez-Linan, A. Werman, W. J. Pories, J. F. Caro, and J. S. Flier. 1997. Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. Journal of Clinical Investigation 99:2416-2422.

Way, J. M., C. Z. Gorgun, Q. Tong, K. T. Uysal, K. K. Brown, W. W. Harrington, W. R. Oliver, Jr., T. M. Willson, S. A. Kliewer, and G. S. Hotamisligil. 2001. Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. J. Biol. Chem. 276:25651-25653.

Wildman, E.E., G. M. Jones, P. E. Wagner, R. L. Boman, J. Troutt, and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495-501.

Xiang, Anny H., R. K. Peters, S. L. Kjos, A. Marroquin, J. Goico, C. Ochoa, M. Kawakubo, and T. A. Buchanan. 2006. Effect of pioglitazone on pancreatic β-cell function and diabetes risk in Hispanic women with prior gestational Diabetes. Diabetes 55:517-522.

Yang, W. Z., D. N. Mowat, A. Subiyatno, and R. M. Liptrap. 1996. Effects of chromium supplementation on early lactation performance of Holstein cows. Canadian Journal of Animal Science 76:221-230.

Yang, X. and U. Smith. 2007. Adipose tissue distribution and risk of metabolic disease: does thiazolidinedione-induced adipose tissue redistribution provide a clue to the answer? Diabetologia 50:1127-1139.

Yki-Jarvinen, H. 2004. Thiazolidinediones. N. Engl. J. Med. 351:1106-1118.