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ONTOGENETIC PATTERNS OF GROWTH, GLUCOSE, INSULIN, AND NON-ESTERIFIED FATTY ACIDS IN YOUNG GROWING FOALS

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ABSTRACT

The primary objective of this study is to characterize patterns of growth parameters in young growing Thoroughbred foals (0 to 15 months) and to examine associations between those growth patterns and basal plasma glucose, insulin and non-esterified fatty acids concentration. Monthly blood samples and measurements of weight, wither height, girth, and forearm length were obtained for two groups of foals, over two respective years (May 2003 – June 2004 & May 2005 – July 2006). The foals had access to fresh forage, and were provided with concentrates of different energy sources twice daily. Plasma concentrations of glucose, insulin and NEFA concentrations were quantified. Glucose and insulin concentrations were positively correlated with rates of daily gain in weight, height, girth, and forearm length ($P < 0.0001$). NEFA was negatively correlated with weight ($P < 0.0001$) and girth daily gain ($P < 0.05$). This study documents patterns of essential metabolites and their primary regulatory hormone, which could serve as a reference for caring for young growing foals. These patterns have not been previously reported, and such data is valuable to understanding and optimizing equine health. By understanding growth and metabolic changes in foals, a diet may be precisely tailored to control growth, and lower incidence of skeletal disease. This data may also be useful in clinical practice for assessment of healthy foals throughout their development.

Keywords: growth, foal, insulin, non-esterified fatty acids, glucose

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Chapter 1

Review of the Literature

Introduction and Rationale

Developmental Orthopedic Disease is a major problem in the horse industry (Michaël Donabédian, 2006). The causes of this disease are attributed to genetics, growth, feeding practices and exercise conditions (Lepeule et al., 2009). The incidence of this disease in growing animals may be decreased through management. A foal's diet influences concentrations of regulatory hormones. Among the many responsibilities of hormones, is their stimulation of growth. Depending on an animal's diet, the concentration of certain hormones may result in suppression or induction of growth. Altering metabolite concentration through nutrition to ultimately impact hormone levels could be a way to precisely control growth. However, it is necessary to study the normal progression of concentrations of metabolites in the young growing foals. By understanding such patterns, it may be possible to tailor a specific diet to optimize equine growth and health.

Osteochondrosis (OC), osteochondritis dessicans (OCD), subchondral cystic lesions, angular deformities, and cuboidal bone malformation are all considered developmental problems, collectively known as Developmental Orthopedic Disease (DOD) (McIlwraith, 2001). Osteochondrosis is a clinically important and common joint disorder, defined as a focal disturbance of enchochondral ossification. It is a term that pertains to a wide range of lesions among many species (Ytrehus, 2007). The most likely initial step in pathogenesis is thought to be failure of blood supply to growth cartilage (Ytrehus, 2007). The etiologies primarily supported by scientific literature include genetics and anatomic conformation. It has, however, also been

documented that rapid growth, trauma, and dietary imbalances influence the onset of this disorder (Ytrehus, 2007).

Osteochondral fragments in joints compromise horses' athleticism, and jumping performance (Verwilghen, 2012). But this condition does not only debilitate horses - it is a common, and clinically important joint disorder that occurs in humans, and many species of animals such as pigs and dogs. In a study investigating various breeds and feedings, it was shown that pigs with the highest growth rates had the most severe osteochondral lesions compared to those with a more restricted growth rate (Goedegebuure et al., 1980). In dogs, therapeutic effects after receiving surgery were shown to be most effective at ages 6 to 10 months, thereby demonstrating the importance of early diagnosis (Biezyński, 2012). OC is also evident in poultry. Lesions of growth plates or articular cartilage and bone have been reported in chickens and turkeys (Julian, 1985).

Genetics has been confirmed as a substantial contributing risk factor to the prevalence of OC (Lykkjen 2013). However, findings have also elucidated that OC in foals is associated with breeding conditions, specifically maternal nutrition during gestation, and the type of housing of foals during their first year. Mares fed concentrates during gestation and foals housed in box stalls were both identified as factors that increased the incidence of OC (Vander Heyden, 2013). This provides evidence that the risk of OC may be reduced through management.

Osteochondrosis and Growth

Uncontrolled fast growth increases the incidence of OC and DOD. Lepeule et al. (2009) suggested that monitoring growth and reducing nutritional supply in fast growing subjects, limiting pasture areas offered, and providing regular exercise to foals may reduce the prevalence of DOD or OC in foals. Yet no clear indication of when to limit pasture, and what precisely corresponds to "fast growth" is provided. Being mindful of limited nutritional supply is also very

important. Nutrient deficiencies during the post-weaning period may lead to inadequate growth, which has been reposted to compromise skeletal development as well (Thompson, 1988). It is necessary to depict exactly under what circumstances nutritional management should be altered, and how to most effectively intervene.

Van Tilburg and Ellis (2002) correlated the growth rates of 144 Warmblood foals with radiological OC scores. There was no difference in weights at birth. However, the OC positive foals had gained weight significantly in the first three-month after weaning, when compared to the OC negative foals. No difference in average daily gain was evident outside those months in this study. However, other studies documented higher growth rates outside this short time frame. Pagan and Jackson (1996) radiographically diagnosed osteochondrotic lesions in 27 Thoroughbred foals that exhibited a more rapid growth rate between the ages of 3 to 8 months, when compared to 224 normal foals. Van Weeran et al. (1999) also confirmed accelerated growth and incidence of OC. Fifteen Warmblood foals with osteochondritic lesions had higher weight gains during particular periods of growth when compared to a group of 28 foals without lesions.

Osteochondrosis and Nutrition

Studies have identified relationships between intake of types of nutrient and prevalence of DOD (Knight et al. 1985). The introduction of a high-energy diet has been implicated as a risk factor of skeletal issues in growing horses (Savage et al., 1993). This was effectively demonstrated by Glade and Belling (1984). Weanling Thoroughbred horses were fed diets providing 70%, 100%, or 130% of their daily energy requirement for 8 months. It was found that the overfed horses had thicker growth plates when compared to the other groups. Cymbaluk et al. (1990) investigated the effects of limited and ad libitum feeding on 18 colts. This study demonstrated that uncontrolled feeding stimulates rapid, uncontrolled growth.

Not only does the energy of a diet affect growth and metabolic signaling, but constituents of a diet may impact growth. Ringmark et al. (2012) document the growth of Standardbred colts, but only starting at 464 days of age, on a forage-only diet. Yet DOD can occur in the foal from as early as 3 to 5 months (Knight et al., 1987), which implies that further investigation of development and growth patterns in the young growing foal is needed. Kronfeld and Harris (2003) stated that feeding grain-based concentrates to the horse has been connected with an increased risk of numerous metabolic disorders, including OC. Staniar et al. (2001) showed that young horses fed sugar and starch feeds have higher circulating concentrations of insulin like growth factor 1 (IGF-1), a regulatory molecule in cartilage development, when compared with those fed fat and fiber feeds. Gray et al. (2013) documented incidence of altered hormonal and metabolic responses in young foals fed high or low glycemic diets. Wagner and Urschel (2012) demonstrated increased responsiveness to food consumption in yearlings. When compared to mares, yearlings were more receptive to activation of translation initiation factors by diet. Such findings elucidate the necessity to design feeding and management strategies specific for adolescent and mature horses.

Characteristics of Glucose

Glucose is a six-carbon sugar that is the most abundant monosaccharide in nature. It has been conserved across species, as the primary source of energy – mammals, and even microorganisms such as yeast, prefer glucose as their energy source. Consequently, sophisticated regulatory mechanisms have evolved to cope with fluctuating levels of its availability (Johnston, 1999). Glucose is effectively used by a variety of cell types under normal conditions, and its concentration in the blood must be controlled precisely. It plays a central role in metabolism and cellular homeostasis. Most cells in the body are dependent the energy in the molecular bonds of glucose which is liberated by glycolysis. It is primarily controlled by pancreatic hormones

insulin and glucagon. Glucose entry into cells of the skeletal muscle and adipose tissue is an insulin dependent process. Tight regulation of this metabolite is essential. Its homeostatic set point is at approximately 90 mg/dL, although it is not completely understood why that is the case. However, it has been reported that failure to regulate glucose around this set point is indicative of metabolic disorders, such as pre-diabetic and pre-coronary metabolic syndromes (Ford and Giles, 2003). Moreover, literature suggests that higher elevation of blood glucose for longer durations increases the risk of development of chronic diseases and obesity.

Associations between glucose and growth have been shown in various species. When studying blood variables and birth weight gain on the first day of life in piglets, Rootwelt et al. (2012) found that those who did not survive to weaning exhibited decreased glucose and body weight gain when compared to those who survived. Glucose concentrations were decreased to 4.8 ± 0.41 mmol/L, compared to 5.8 ± 0.14 mmol/L. Birth weight gain in those who died before weaning was decreased to 0.0 ± 0.03 kg as oppose to those who survived with a birth weight gain of 0.1 ± 0.01 kg. Changes in blood glucose in response to weaning and feed intake were documented in young calves (Quigley et al., 1991). Concentration of plasma glucose tended to be lower in calves weaned early, averaging at 85.0 mg/dL. Those that were weaned late had a glucose concentration of 90.3 mg/dL. Although there was no statistical significance ($P>0.05$), calves that were weaned early had a lower daily gain (0.51 kg/d) compared to those who were weaned late (0.52 kg/d). Such relationships are yet to be established in a non-ruminant. It is necessary to understand the relationship between glucose and growth in foals because of the effects a high glycemic diet may have on a foal. It has been shown that feeds producing high glycemic and insulinemic peaks may have an increased risk of developing DOD (Pagan et al. 2001; Ralston, 1996, 1995). Various diets can influence metabolism of glucose in horses. Weanlings on high low starch diet have been shown to be less efficient at metabolizing blood glucose than those consuming a high starch diet (Ott, 2005). Glucose and insulin levels have also

been shown to influence the pattern of growth hormone (GH) secretion (Roth et al., 1963). Patterns of glucose and insulin can be manipulated through feeding, to ultimately influence growth. For example, the amplitude of fluctuation in plasma glucose and insulin is reduced when starch and sugar are replaced by fat and fiber meals (Williams et al. 2001).

Characteristics of Insulin

Insulin is a regulatory hormone, released from pancreatic beta cells to uptake glucose for utilization. When insulin interacts with receptors located on tissues, glucose transporters, referred to as GLUTs, translocate to the cell membrane to internalize glucose. Insulin also regulates mobilization of fatty acids by inhibition of hormone sensitive lipase, and increasing lipogenesis. This explains why rising insulin levels are associated with decreasing NEFA concentrations.

In humans, alterations in glucose and insulin concentrations in subjects expressing obesity, over-eating behaviors, and anorexia nervosa have strongly been associated with modifications in GH secretions and bone density (Gray et al., 2013). This evidence is suggestive that characterizations of glucose and insulin in a young growing foal are necessary to conclusively understand their development.

Diabetes mellitus, a disease associated with insulin resistance and high glucose concentrations, is associated with increased risk of osteoporotic fractures and osteoarthiritis. Compromised bone quality may be caused by hyperglycemia, decreased serum concentration of osteocalcin among other reasons. In such cases, insulin was shown to have anabolic effects on bone, and hyperinsulaenimia may explain why Type 2 diabetics exhibit higher bone density (Yan and Li, 2013).

George et al. (2009) have shown that changes in insulin sensitivity and glucose dynamics throughout the pre-weaning period indicate the plasticity of the metabolic system. Throughout this study, the reported baseline insulin concentrations declined steadily, ranging from

3 to 11 mIU/L. A value of 20 mIU/mL is the upper limit of serum insulin concentration in normal horses and ponies, however more research is required to determine values for hyperinsulinaemia, and insulin resistance (Frank et al., 2010).

Insulin resistance is compensated by an increase in insulin secretion (Ahrén and Pacini, 2004). To avoid insulin resistance, it is typically advised to avoid high glycemic feeds such as high starch intakes in grains, and high fructans intakes in grasses to decreases the risk of chronic metabolic disorders associated with insulin resistance (Kronfeld et al., 2005). Reaven (1988) found that a high carbohydrate diet aggravates predisposition to insulin resistance in humans.

Insulin resistance has been associated with obesity in horses and ponies. Jeffcott et al. (1998) suggested that high circulating insulin concentrations from high energy feeding might affect chondrocyte maturation, leading to faulty matric metabolism and mineralization. This may result in a deficient blood supply, and therefore, cartilage cores where a cartilage matrix that is not replaced by bone. As previously stated, insulin patterns can manipulate GH secretions. Glucose and insulin served as triggering factors for the somatotropic axis, with GH and IGF-1 regulating chondrocyte metabolism and maturation (Treiber et al., 2005b). Yet connections between growth patterns and basal insulin concentrations are still yet to be established. Identifying such an association can aid to better understand the physiological changes occurring in a growing horse.

Characteristics of Non-esterified Fatty Acids

Non-esterified fatty acids (NEFA) are the primary energy fuel for most tissues under fasting conditions. Lipoprotein lipase breaks down triglycerides to facilitate the uptake and storage of NEFA. The release of NEFA into the blood stream is dependent on hormone sensitive lipase from adipose in response to glucagon. NEFA removal is dependent on tissue uptake oxidation or re-esterification (Ferrannini, 1997).

Circulating NEFA concentrations are regulated by insulin through a stimulatory effect on lipoprotein lipase, and an inhibitory effect on hormone-sensitive lipase (Ferrannini, 1997). In diabetic patients, insulin action is impaired, leading to elevated plasma NEFA concentration. It is therefore necessary to characterize normal NEFA values in healthy, young growing animals to be able to identify abnormally high levels, which may indicate an underlying disease.

NEFA concentration may vary across breeds. Robie et al. (1975) monitored 19 horses and ponies over a period of 9 months. Morgan horses had higher concentrations of lipids than did Thoroughbreds. In the colder months, plasma lipids appeared lower than those in the warmer months. Shetland ponies had higher NEFA and lower glucose concentrations than did either breed. Insulin induced hypoglycemia can result in various strengths in free fatty acid response depending on the age of the foal. 7-14-day-old foals significantly increased plasma free fatty acids after hypoglycemia, but the rise was less prominent in neonatal foals. This study demonstrates that neonates may exhibit a certain level of insulin resistance (Silver et al., 1987).

Fasting usually results in an increase in plasma NEFA concentration, marking mobilization of fat for energy. Steers that were fed ad libitum had a faster rate of weight gain (1.4 kg/d) compared to diet restricted (50% ad libitum) steers (0.37 kg/d). During refeeding, NEFA concentration decreased markedly, and continued to remain low throughout the compensatory growth phase. The dietary restriction imposed was not severe enough to significantly enhance lipolysis (Ellenberger, 1989). However, further research is necessary to elucidate the relationship between NEFA and its signal for growth to occur.

Summary

Skeletal development is critical in the young growing foal. Rapid growth in the young animal can compromise cartilage development and integrity. Nutrition can be utilized as an intervention tool to optimize growth, and decrease the risk of developmental orthopedic disease. Various diets can induce significant alterations in metabolic and hormonal regulation that may ultimately modify growth. But in order to tailor a specific diet, growth patterns and their association with metabolic changes must first be understood. Through analysis of glucose, insulin and NEFA patterns in the young growing foal, and the specific periods for nutrition intervention, and the metabolic signals required can be identified.

Hypotheses and Objectives

We hypothesize that there is an association between changes in growth patterns and glucose, insulin and NEFA. The objective of this observational study is to characterize ontogenetic growth patterns in young foals from ages 0 to 534 days, and to investigate correlations of these patterns with basal serum glucose, insulin and NEFA concentrations. The measurements and metabolites are quantified on a monthly basis.

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Chapter 2

Characterizing ontogenetic patterns with glucose, insulin, and non-esterified fatty acids in young growing foals

Abstract

The primary objective of this study is to characterize patterns of growth parameters in young growing Thoroughbred foals (0 to 15 months) and to examine associations between those growth patterns and basal plasma glucose, insulin and non-esterified fatty acids concentration. Monthly blood samples and measurements of weight, wither height, girth, and forearm length were obtained for two groups of foals, over two respective years (May 2003 – June 2004 & May 2005 – July 2006). The foals had access to fresh forage, and were provided with concentrates of different energy sources twice daily. Plasma concentrations of glucose, insulin and NEFA concentrations were quantified. Glucose and insulin concentrations were positively correlated with rates of daily gain in weight, height, girth, and forearm length ($P < 0.0001$). NEFA was negatively correlated with weight ($P < 0.0001$) and girth daily gain ($P < 0.05$). This study documents patterns of essential metabolites and their primary regulatory hormone, which could serve as a reference for caring for young growing foals. These patterns have not been previously reported, and such data is valuable to understanding and optimizing equine health. By understanding growth and metabolic changes in foals, a diet may be precisely tailored to control growth, and lower incidence of skeletal disease. This data may also be useful in clinical practice for assessment of healthy foals throughout their development.

Keywords: growth, foal, insulin, non-esterified fatty acids, glucose

Introduction

Growth patterns in young animals can have long-term implications on health. Rapid growth rates may alter skeletal development, and result in osteochondral lesions (Goedegebuure et al., 1980). Controlling and optimizing skeletal development is particularly important in athletic species such as the horse, as it may help to advance performance, and prevent disease or lameness. Animals can recognize and respond to the types of nutrients that they are fed. Feeds that are higher in energy induce a faster growth rate in animals compared to animals on restricted diets (Ellenberger, 1989). Growth is induced in response to hormonal signals in the body, such as GH. Metabolic signaling can alter secretions of such regulatory hormones (Gray et al., 2013).

Glucose and NEFAs are metabolites that are regulated by the hormone insulin. Insulin is secreted from pancreatic beta cells, in response to increased blood glucose concentrations. It signals for the uptake and utilization of glucose, indicating an abundance of energy. This therefore inhibits the need for mobilization of fat stores. Insulin is therefore associated with decreased serum NEFA concentrations.

Meals can result in different metabolic responses, and therefore, altered hormonal signals. It has been suggested that meals that trigger high glycemic and insulinemic peaks may increase the risk of developing DOD (Pagan et al. 2001; Ralston, 1996, 1995). Diets that are that consist of sugar and starch result in higher glycemic and insulinemic responses compared to those of fat and fiber diets (Williams et al. 2001). The degree of diet restriction that would significantly enhance serum concentration of NEFA is not conclusively understood, and requires further investigation (Ellenberger, 1989).

Evidence of plasticity in the young growing animal (Wagner and Urschel, 2012) is suggestive that foals would respond to early dietary intervention. Yet growth and metabolic interactions in young, healthy foals have not been elucidated. Bauer et. al (1984) have established

normal laboratory values from 36 h to 1 year of age. This data is clinically useful to serve as a reference when tending to young foals. In this study, it was found that glucose ranged between 9.2 ± 1.6 mmol/L and 7.5 ± 0.8 mmol/L. Such values have yet to be associated with growth, to aid veterinarians with monitoring foal development. Osteochondrosis is a disease of the growing animal, and OC lesions occur in foals as early as 3 to 5 months of age (Knight et al., 1987). This provides further impetus to investigating patterns of growth in young animals. While many have suggested that high glycemic and insulinemic diets are a risk factor for OC, the precise etiology of this connection has not been determined. If there is a connection, it is likely through the regulation of cartilage growth and development by energy signals that ultimately come from the animal's diet.

We hypothesize that there is an association between changes in growth patterns and glucose, insulin and NEFA. The objective of this observational study is to characterize ontogenetic growth patterns in young foals from ages 0 to 534 days, and to investigate correlations of these patterns with basal serum glucose, insulin and NEFA concentrations. The measurements and metabolites are quantified on a monthly basis.

Materials and Methods

Horses

Twenty-eight foals (10 colts and 18 fillies) from May 2003 were weighed and measured on a monthly basis until June 2004. The same sampling and weights and measures were attained from 18 foals (10 colts and 8 fillies) from May 2005 to July 2006. The measurements included weight, withers height, girth and forearm length. The horses were fed a variety of concentrates twice a day, in addition to forage. A blood samples was obtained between 7:00 to 10:00 h in the morning prior to concentrates feeding. The foals were owned by the Virginia Polytechnic Institute and State University.

Treatments

Foals were born between mid-March and the end of April. All foals in this study were weaned at approximately 180 days of age. Prior to weaning the mares and foals were fed with one another. They were fed concentrates twice a day, between 7:00 to 10:00 h and 14:00 to 16:00 h. In 2003, the concentrates feed offered to the foals was either low or high in fat and fiber. In 2005 foals were fed concentrate feeds that were either sugar and starch or fat and fiber.

Blood Sampling

Twelve-milliliter blood samples were obtained on a monthly basis from the growing horses. The samples were equally distributed into potassium ethylenediaminetetraacetic acid (EDTA) and sodium heparin vacutainer tubes and stored on ice. Within 45 min, blood samples were centrifuged at 1400 x g as instructed by manufacturer at 4°C for 12 minutes. Plasma was separated from erythrocytes and stored at -20°C until analyzed.

Hormone and Metabolite Analysis

Glucose, insulin and NEFA were measured from both sample blocks (2003 to 2004 and 2005 to 2006). Plasma glucose was analyzed using an enzymatic colorimetric assay (Stanbio, Boerne, TX). NEFAs were also quantified by enzymatic colorimetric assays (Wako Diagnostics, Richmond, VA). A previously validated radioimmunoassay for insulin was used (Freestone et al., 1991).

Statistical Analysis:

SAS version 9.2 (SAS Institute, Cary, NC) was used to formulate Pearson correlation coefficients for glucose, insulin and non-esterified fatty acids with each of the growth parameters (weight

daily gain, height daily gain, girth daily gain and forearm daily gain). A stepwise linear regression was then employed to evaluate the relationship between glucose, insulin and non-esterified fatty acids collectively, with each of the daily gains. $P < 0.05$ was declared statistically significant.

Results

The relationship between glucose and daily weight, height, girth and forearm gain was positively correlated ($P < 0.0001$) as depicted by Table 2-1, and Figures 2-18 to 2-21. Insulin also exhibited a positive correlation with all gains, ($P < 0.0001$) displayed in Figures 2-22 – 2-25. NEFA displayed negatively correlated trend with weight daily gain ($P < 0.0001$) shown in Figure 2-26, and girth daily gain ($P < 0.05$), exhibited in Figure 2-27. Significant correlations were not detected for the relationship between NEFA's and height and forearm daily gain.

The average basal concentrations for the hormone and metabolites analyzed are depicted in Table 2-2. The average basal glucose concentration measured in the study was 116 ± 25 mg/dL. The 10^{th} percentile was 93.8 mg/dL, and the 90^{th} percentile was 146.0mg/dL. Insulin's average basal concentration was 4 ± 3.6 mIU/L, with10^{th} and 90^{th} percentiles of 0.7 mIU/L and 8.2 mIU/L respectively. The average basal NEFA concentration was 337 ± 0.5 μEq/L with a 10^{th} percentile of 188.8 μEq/L, and 519.5 μEq/L 90^{th} percentile. The average monthly concentrations of glucose, insulin and NEFA for each year are presented in Figures 2-1, 2-2, and 2-3. "Day of year" on the x-axis describes the day of the year that the foal's blood sample was taken. After 365 days (1 year), the number of days since the previous collection resumed to be added, to maintain continuity on the figure.

The day of year on which samples were taken was different for 2003 and 2005. Therefore, the metabolite concentration for a given sample did not correspond to the same days of age for both years. Because of that, foals were classified into "age groups" to compile averages

from 2003 and 2005. Figures 2-7 to 2-11 show the average changes in growth rates for the years 2003 and 2005 separately. The combined averages of growth rates from both years are displayed in Figures 2-12 to 2-16.

The stepwise linear regression was utilized to evaluate the combination of glucose, insulin and non-esterified fatty acids for weight daily gain. Glucose R-square was 0.29. The next stepwise selection entered insulin, and improved R-square to 0.328. Lastly NEFA was selected, ultimately increasing R-square to 0.338. Ultimately, all variables were entered into the model.

Discussion

Glucose was positively correlated with all growth parameters at a significant level ($P < 0.0001$). It had stronger correlation with weight daily gain and girth daily gain. Insulin was also positively correlated with all growth parameters ($P < 0.0001$). Like glucose, its strongest correlations were with daily weight gain and daily girth gain. The growth parameter with the weakest relationship for both glucose and insulin was forearm daily gain. Forearm length in young foals is a measurement that is difficult to obtain due to small changes in size from month to month. Although this relationship was weak, its statistical significance provides reason for further investigation

Non-esterified fatty acids displayed weak negatively correlated relationships with all growth parameters. However, only two of these were statistically significant; girth daily gain and NEFA ($P < 0.05$), and weight daily gain ($P < 0.0001$). Although the relationships are weak, the significant associations necessitate further investigation. The relationship between NEFA, forearm, and height daily gain was not found to be statistically significant.

Basal glucose, insulin and NEFA plasma concentration exhibited very similar patterns throughout the years 2003 and 2005, as shown in Figure 2-1. The patterns are also evident in Figure 2-2, where the concentrations from both years were averaged. Slight variations from year to year may be due to environmental factors. There were also larger variations in standard deviations when the foals were younger. Throughout the first 120 days, the inhibitory effects of insulin on NEFA were not evident, possibly due to stresses of birth, and rapid adaptation. Younger foals had higher circulating glucose, and therefore, also exhibited increased levels of insulin for regulation. Normal serum concentrations of insulin in a horse are usually < 20 mIU/L (Frank et al., 2010). However, throughout the course of the study, insulin's average concentration was 4 ± 3.6 mIU/L. This could suggest that as horses age, their ability to respond to insulin may decrease. As the foals passed 120 days of age, insulin's inhibitory effects on NEFA became clearer. As the concentration of insulin decreased, an increase in NEFA became prominent. The highest concentration of NEFA corresponds to the month of December, when the foals were 240 to 271 days old. This is may be due to limited pasture, and therefore a limited glucose supply. The foals appear to respond to this environmental change by mobilization of fat for energy.

Foals were born throughout the months of March through April and pasture is primarily affected by the time of year. Such high levels of glucose, and therefore insulin, may be due to high availability of forage, and lactose as they nurse from their dams. Throughout the subsequent Spring, at 330 days of age, the basal concentrations of glucose and insulin are not reported to be as high as they were when the foals were a few days old. Figures 2-3, 2-4, 2-5 and 2-7 depict the changes in growth rates of various parameters as the foals continue to grow. Similar to the increased concentrations of metabolites, foals displayed faster growth rates when they were younger. As the foals reached weaning their growth rates had significantly declined. However, at 271 to 390 days of age, there is a deducible increase in weight daily gain. A corresponding rise in glucose throughout the same period is also evident. Compensatory growth occurs after a period of

restricted growth, usually due to reduced feed intake, in which re-fed animals reach the weight of animals whose growth was not restricted (Hornick, e al. 2000). The foals were weaned at approximately 181 to 210 days of age. Although the foals were not restricted by diet, the pressures of weaning may have been a contributing factor to the declining growth rate from ages 181 to 300 days of age. There is a detectible decrease in glucose and insulin concentrations, and a rise in NEFA concentration. These may also be explained by the foals' stress of adaptation to a new diet.

Because of the relationship between glucose, insulin and NEFA with growth patterns, areas of possible nutrition intervention could be identified to control growth, and thereby attempt to decreases incidence of osteochondrosis. An example of this time may be at age 270 to 390 days, when rapid growth occurs. Diet can be utilized to increase growth rate throughout the winter months.

A diet that controls insulin response could preserve the integrity of cartilage. Alteration of this hormone through feeds during maternal gestation and its effect on growth was demonstrated in the ewe (Long et. al, 2010). The offspring showed increased fasted plasma glucose and insulin, and increased birth weight. Understanding correlations of growth, and how a foal responds to the nutrients it is fed, can serve as valuable information for caring of the young animal. Intervening early in the foal's life may aid in improving horses' health, and athleticism throughout their lifetime. Diet can be utilized to stimulate growth via metabolic and hormonal signaling. The relationship between chondrocytes and metabolic signaling is yet to be conclusively understood. The implications of this research are to design diets that may induce growth throughout the winter, to prevent rapid growth in later months, and therefore decrease the risk of osteochondrosis.

Conclusion

Glucose and insulin are positively correlated with the rate of daily gain in weight, height, girth and forearm length. Non-esterified fatty acids are negatively correlated with rate of weight and girth daily gain. Further research is needed to examine the effect of nutrition on these parameters in young growing foals.

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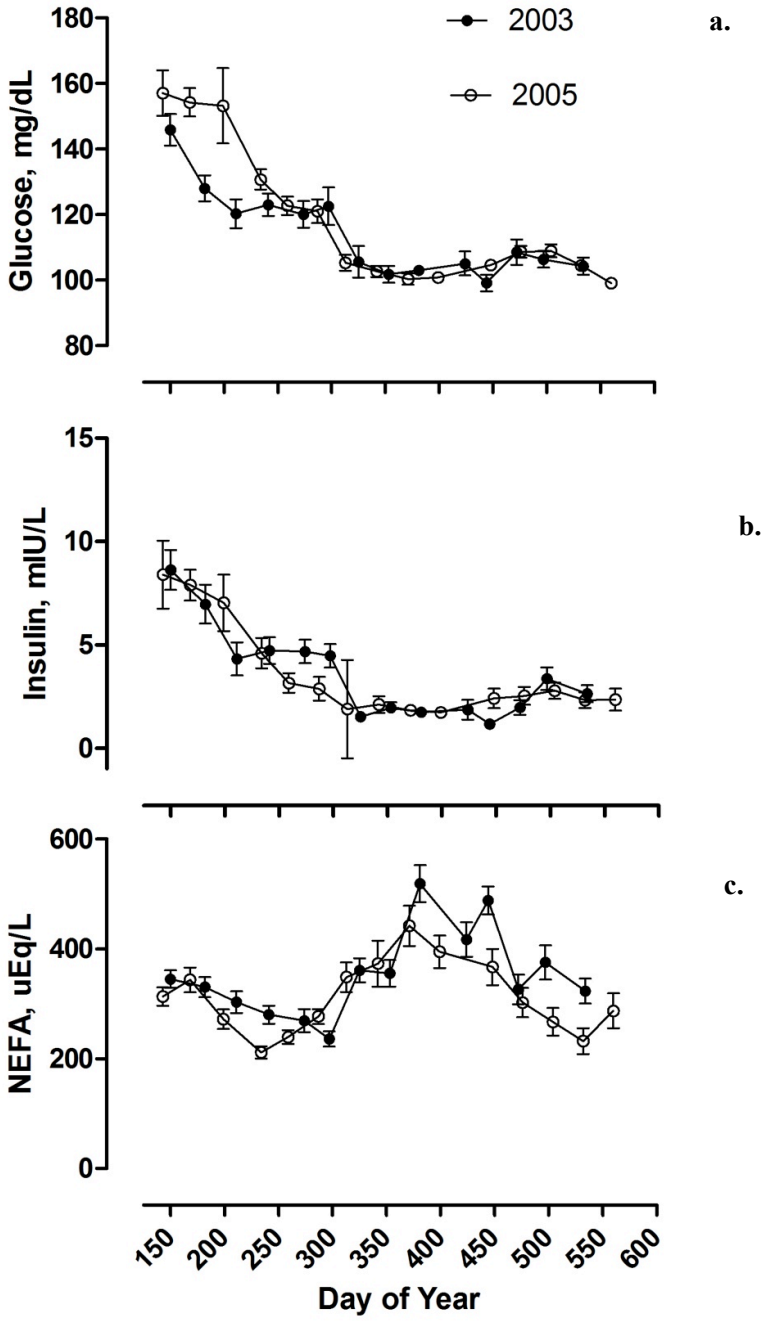

Figure 2-1.a,b,c. Basal glucose, insulin, and NEFA concentrations for 2003 and 2005 over a period of 15 months, presented as means (± standard deviation).

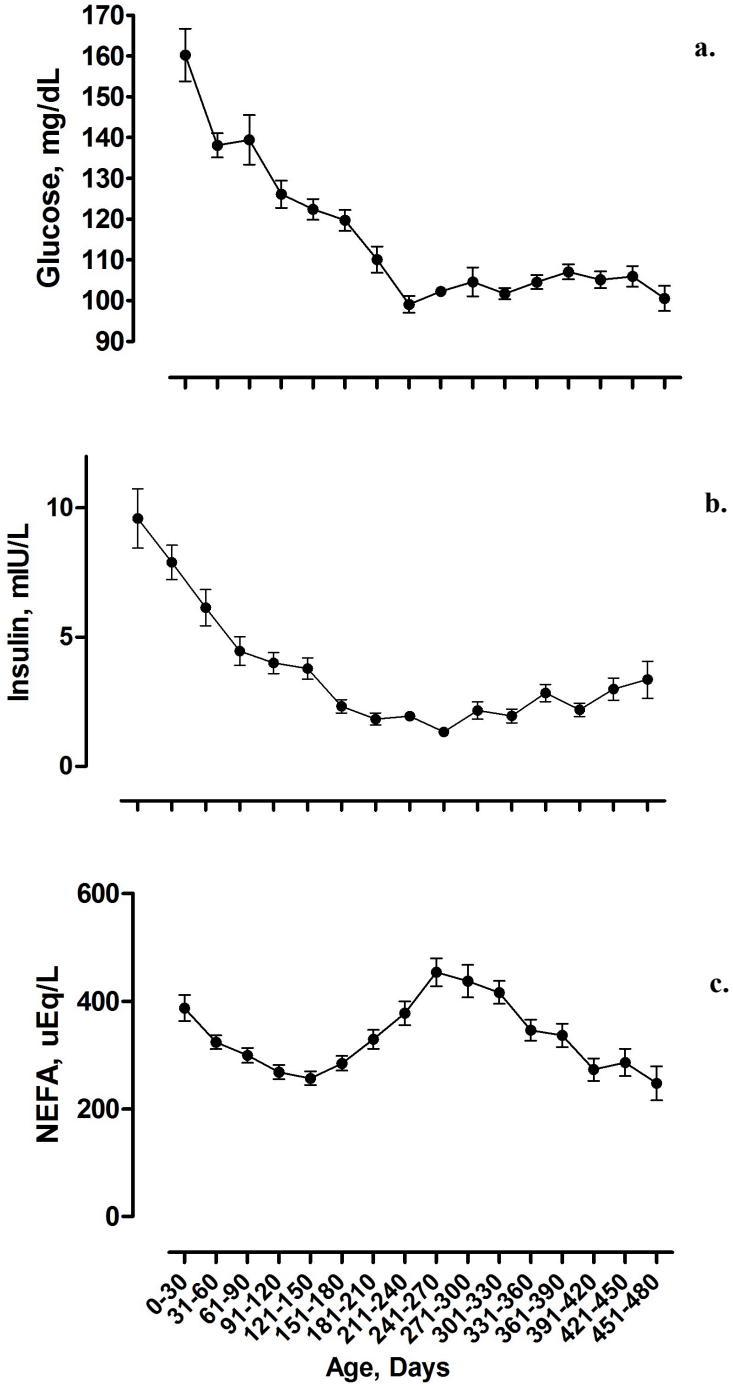

Figure 2-2.a,b,c. Basal glucose, insulin, and NEFA concentrations over a period of 15 months, presented as means (± standard deviation).

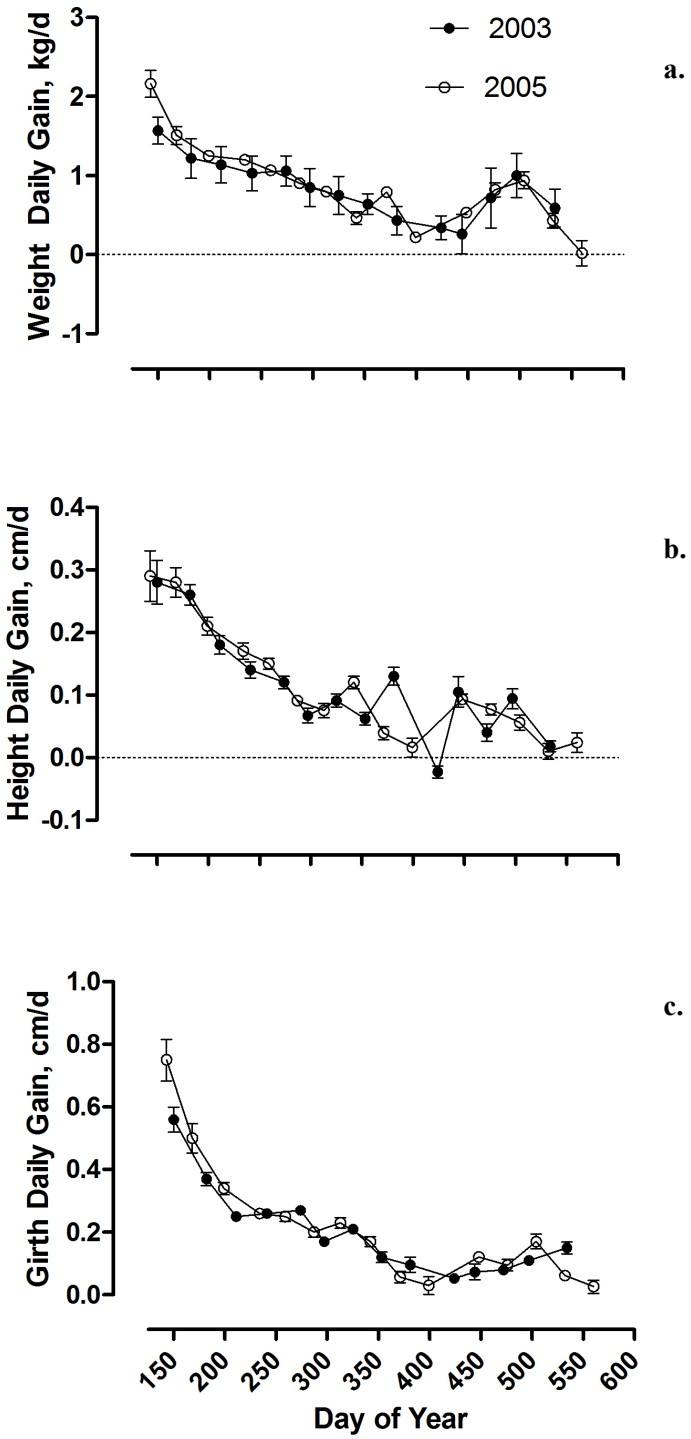

Figure 2-3.a,b,c. Average weight, height, and girth daily gains for 2003 and 2005, over a period of 15 months, presented as means (± standard deviation).

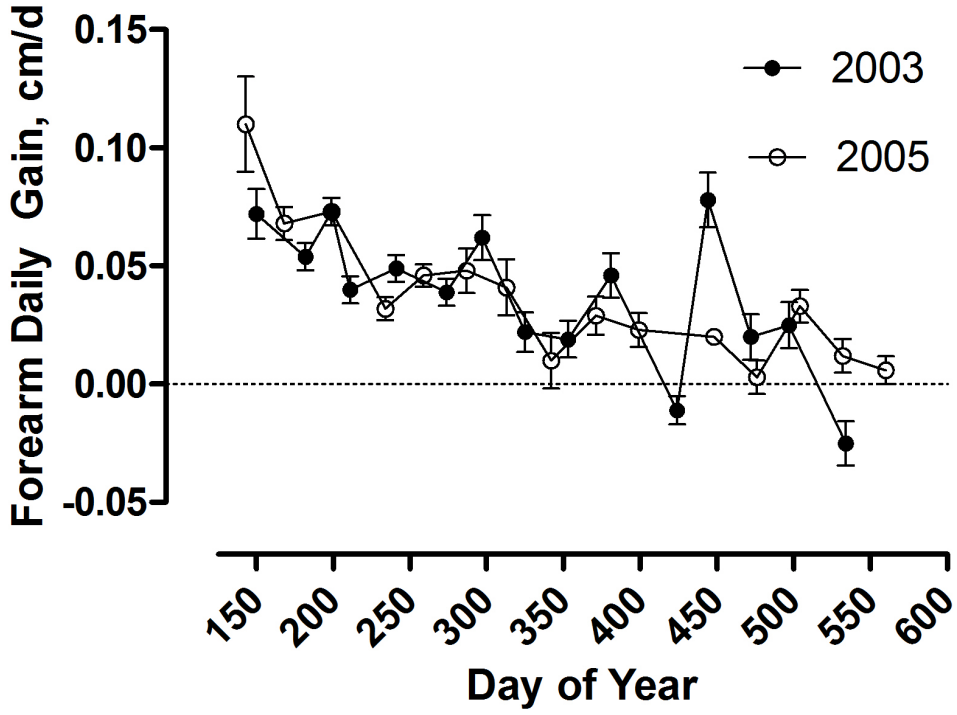

Figure 2-4. Average forearm daily gain for 2003 and 2005, over a period of 15 months, presented as means (± standard deviation).

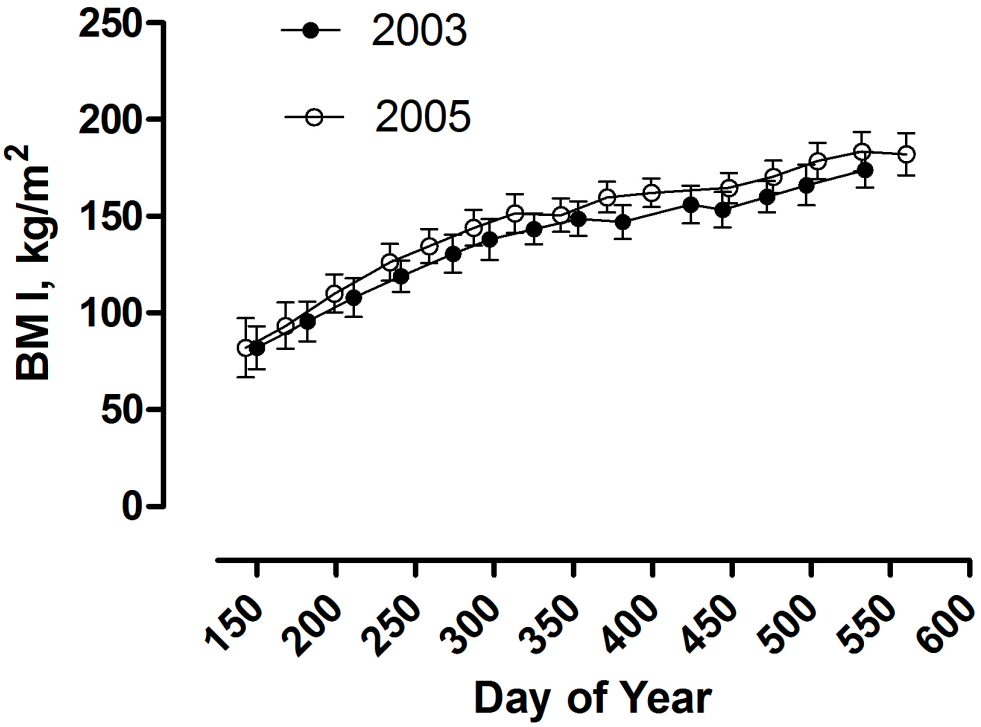

Figure 2-5. Average BMI for 2003 and 2005, over a period of 15 months, presented as means (± standard deviation).

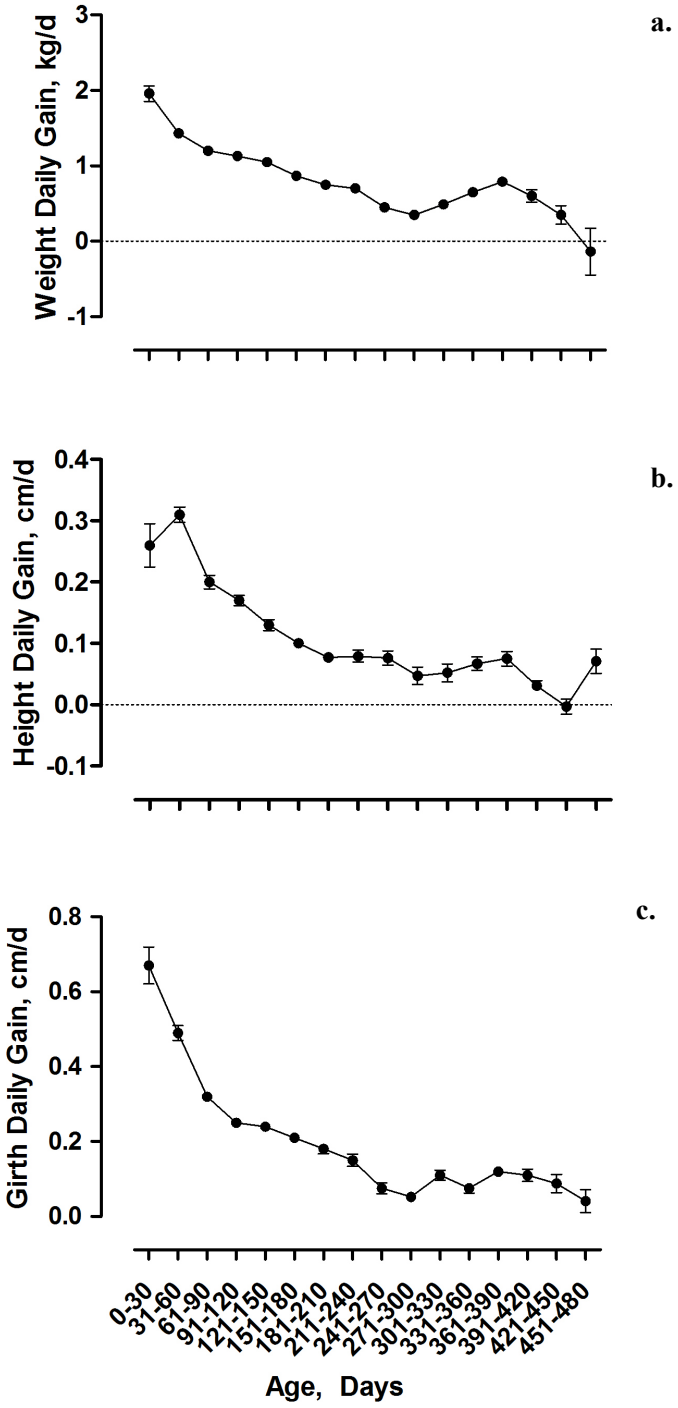

Figure 2-6.a,b,c.. Average weight, height and girth daily gain (± standard deviation) of foals between ages 0-480 days.

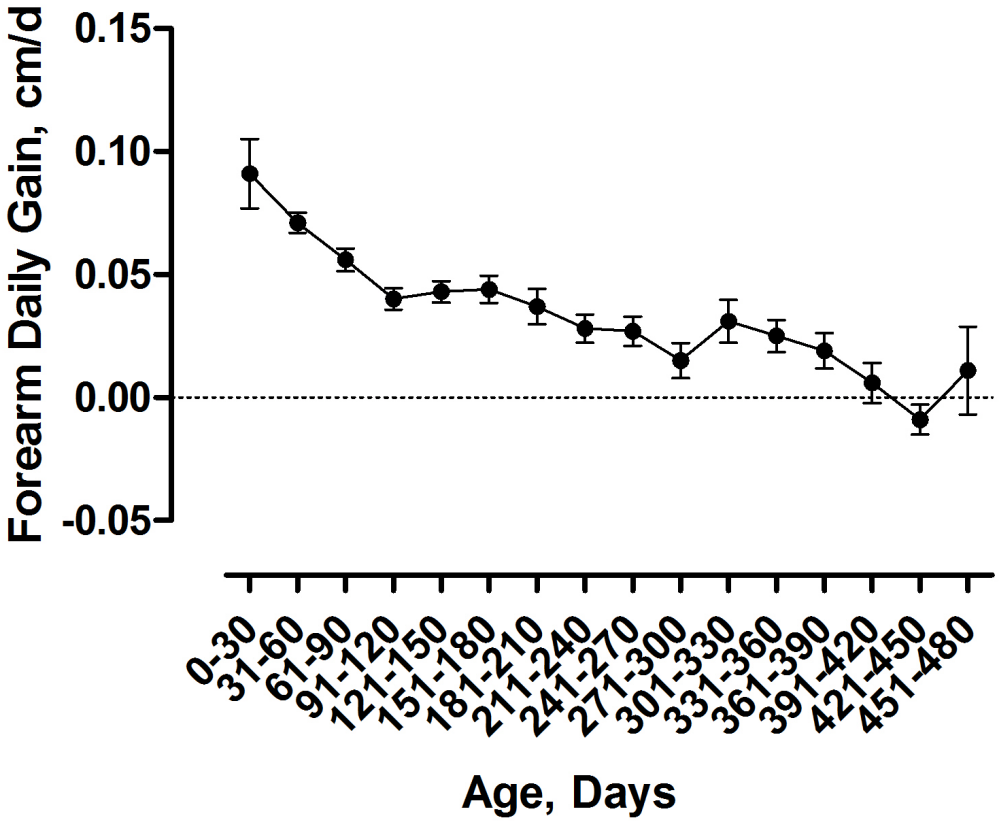

Figure 2-7. Average forearm daily, over a period of 15 months, presented as means (± standard deviation).

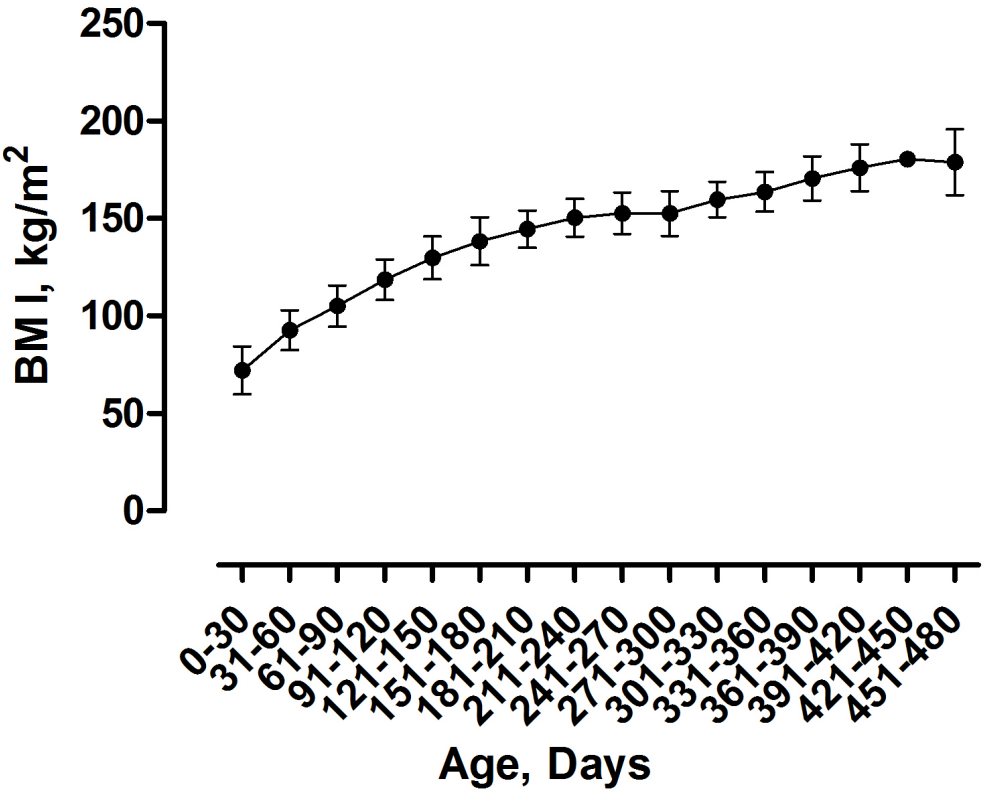

Figure 2-8. Average BMI (± standard deviation) of foals between ages 0-480 days.

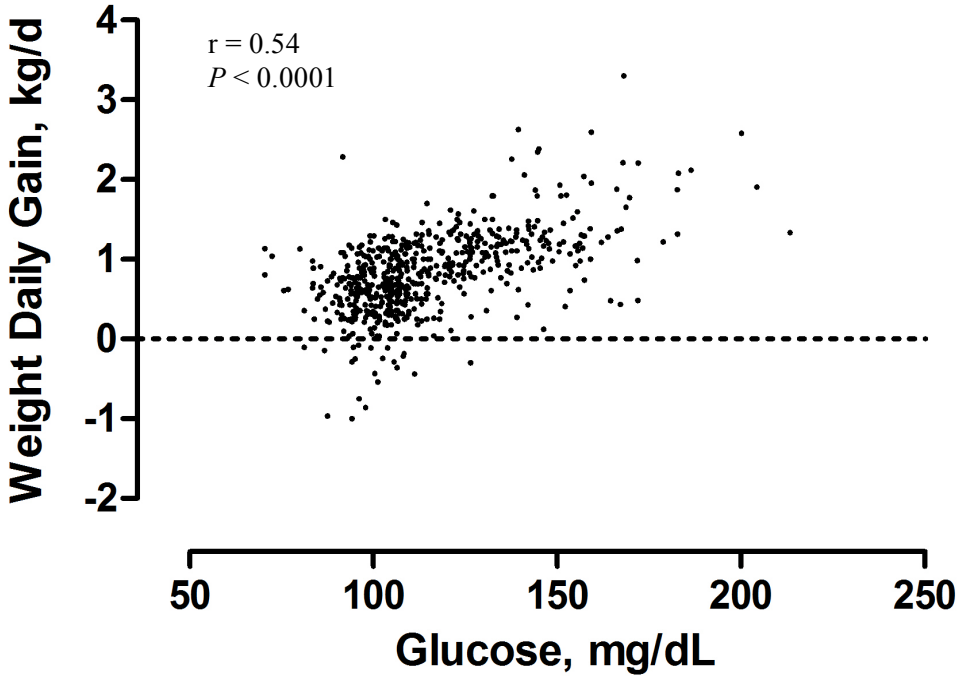

Figure 2-9. Scatterplot of plasma glucose and weight daily gain in Thoroughbred foals from 0 to 534 days of age.

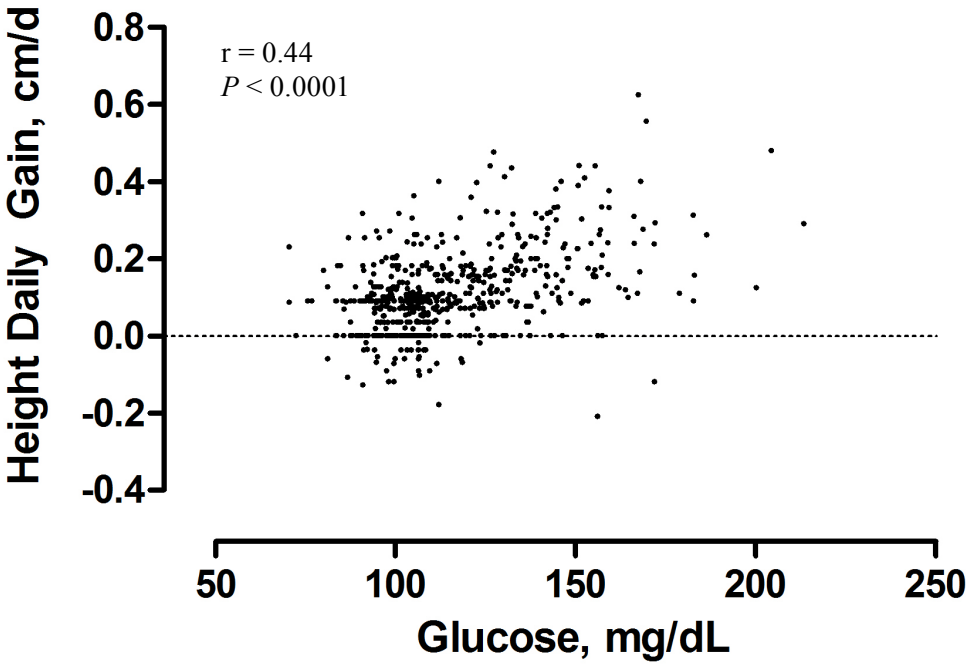

Figure 2-10. Scatterplot of plasma glucose and height daily gain in Thoroughbred foals from 0 to 534 days of age.

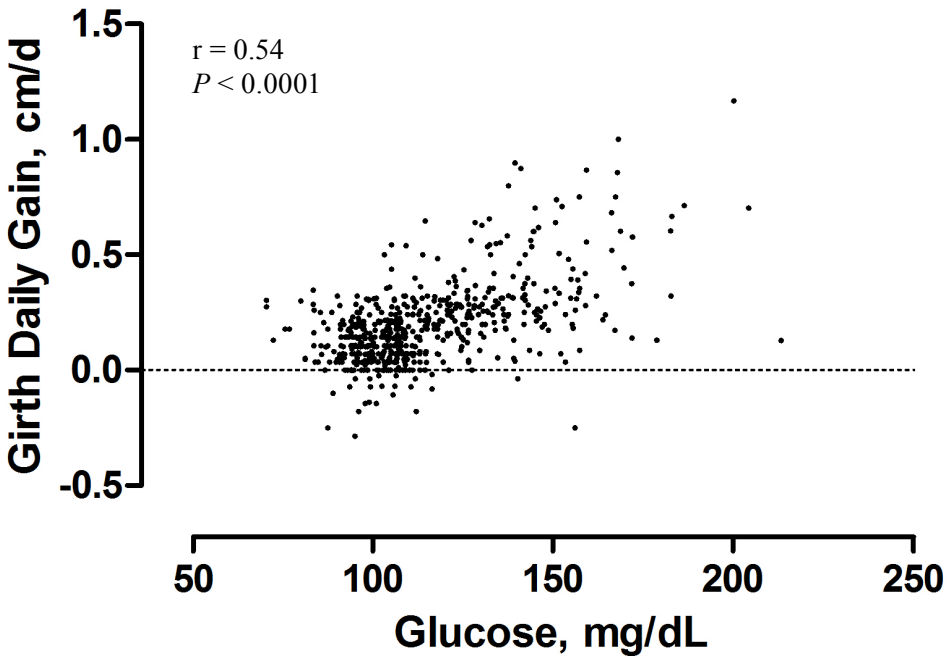

Figure 2-11. Scatterplot of plasma glucose and girth daily gain in Thoroughbred foals from 0 to 534 days of age.

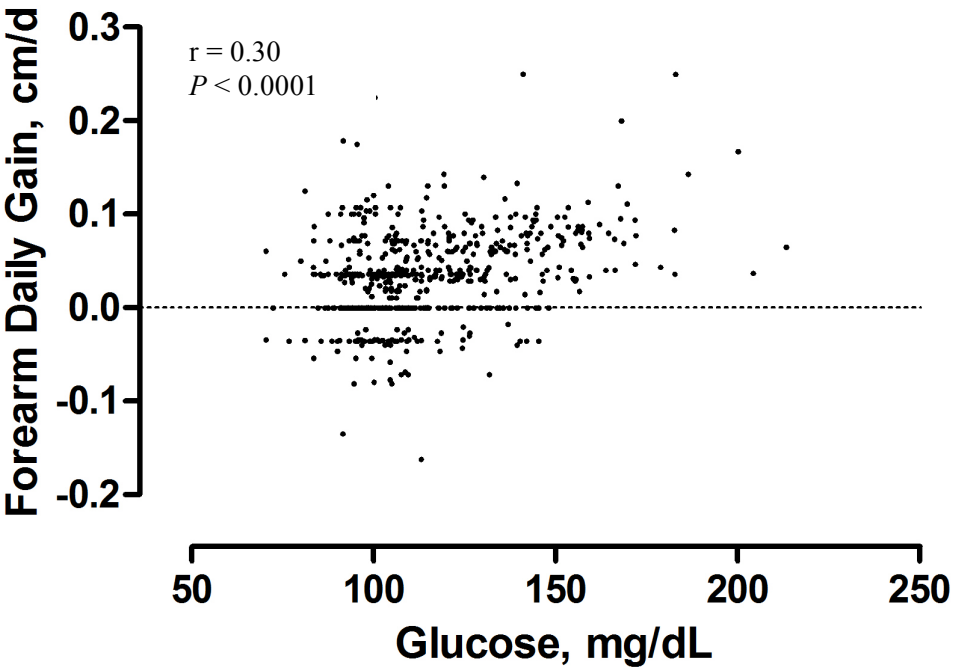

Figure 2-12. Scatterplot of plasma glucose and forearm daily gain in Thoroughbred foals from 0 to 534 days of age.

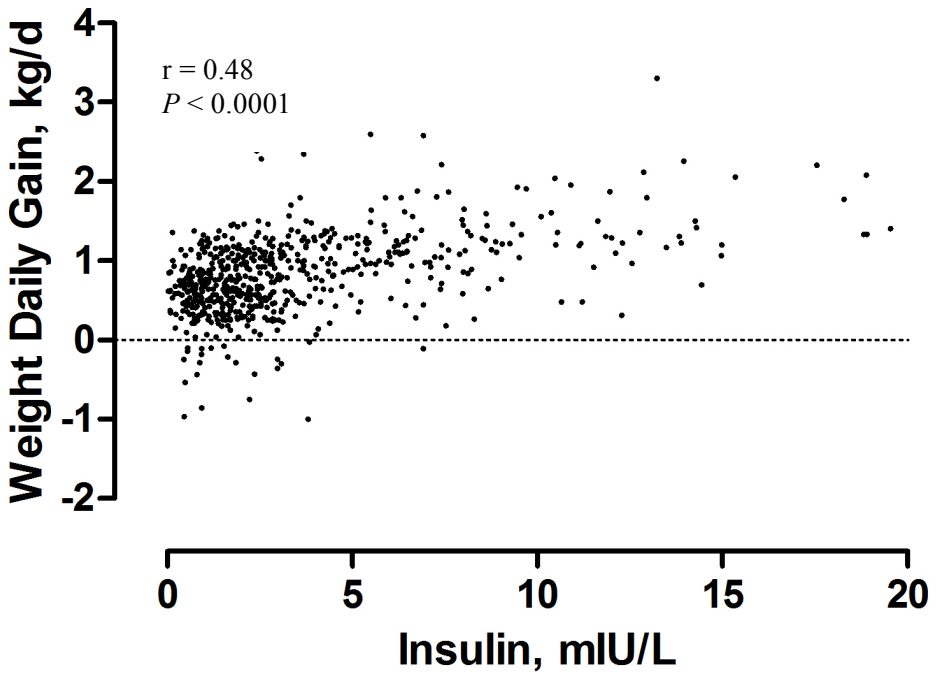

Figure 2-13. Scatterplot of plasma insulin and weight daily gain in Thoroughbred foals from 0 to 534 days of age.

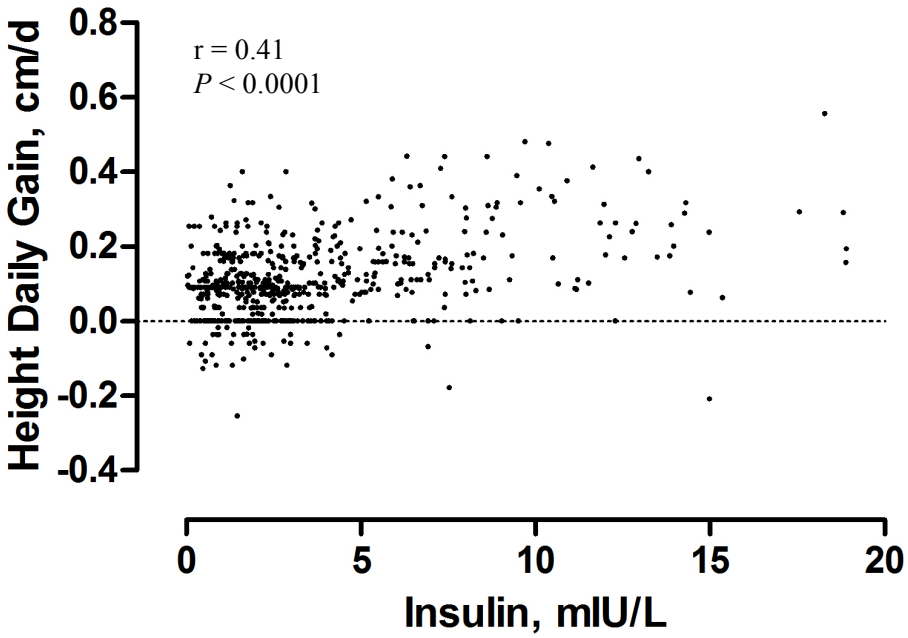

Figure 2-14. Scatterplot of plasma insulin and height daily gain in Thoroughbred foals from 0 to 534 days of age.

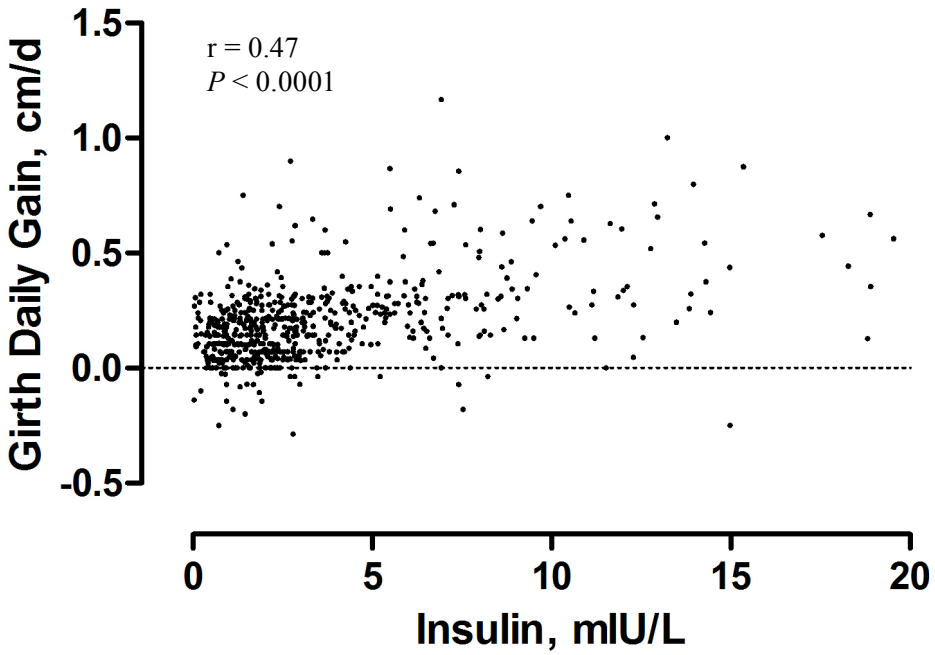

Figure 2-15. Scatterplot of plasma insulin and girth daily gain in Thoroughbred foals from 0 to 534 days of age.

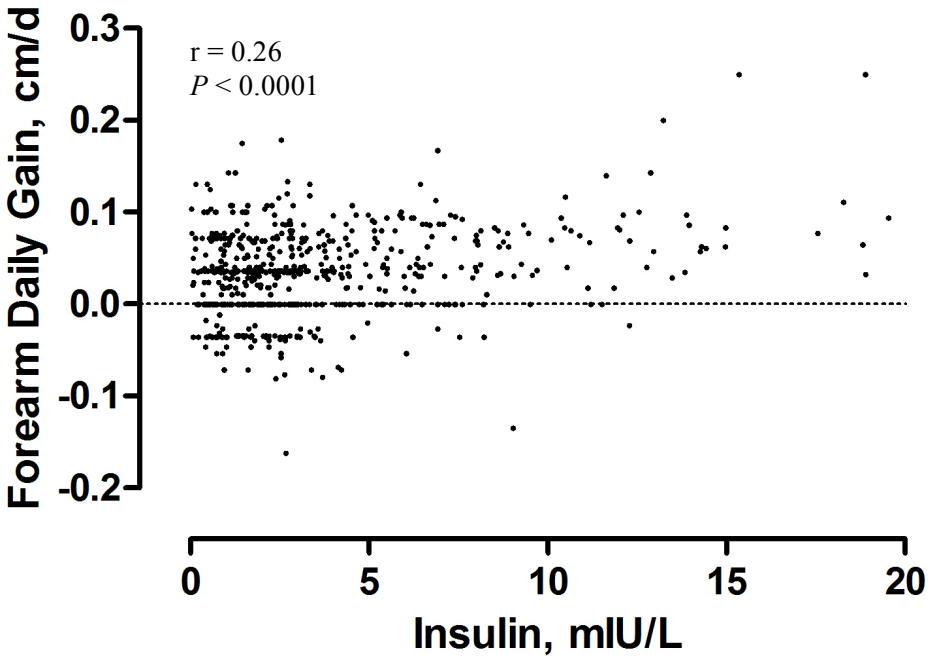

Figure 2-16. Scatterplot of plasma insulin and forearm daily gain in Thoroughbred foals from 0 to 534 days of age.

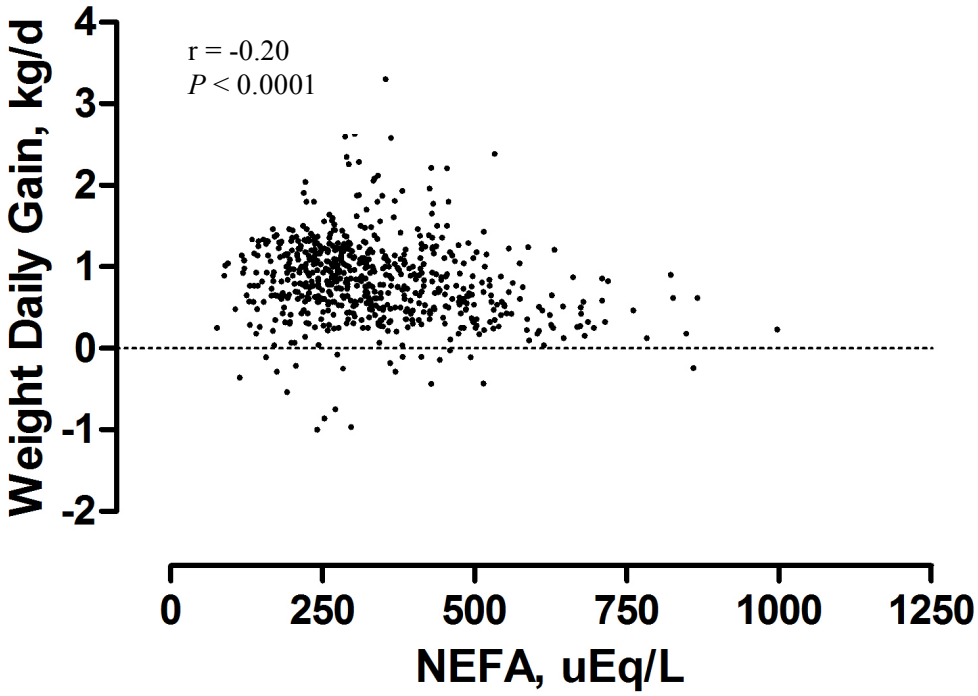

Figure 2-17. Scatterplot of plasma NEFA and weight daily gain in Thoroughbred foals from 0 to 534 days of age.

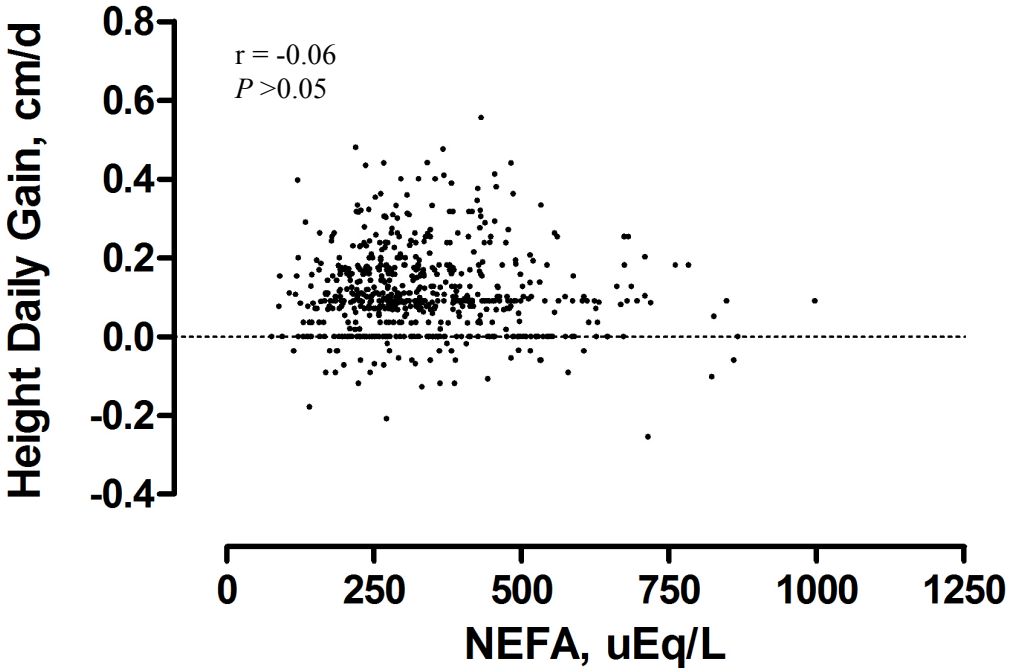

Figure 2-18. Scatterplot of plasma NEFA and height daily gain in Thoroughbred foals from 0 to 534 days of age.

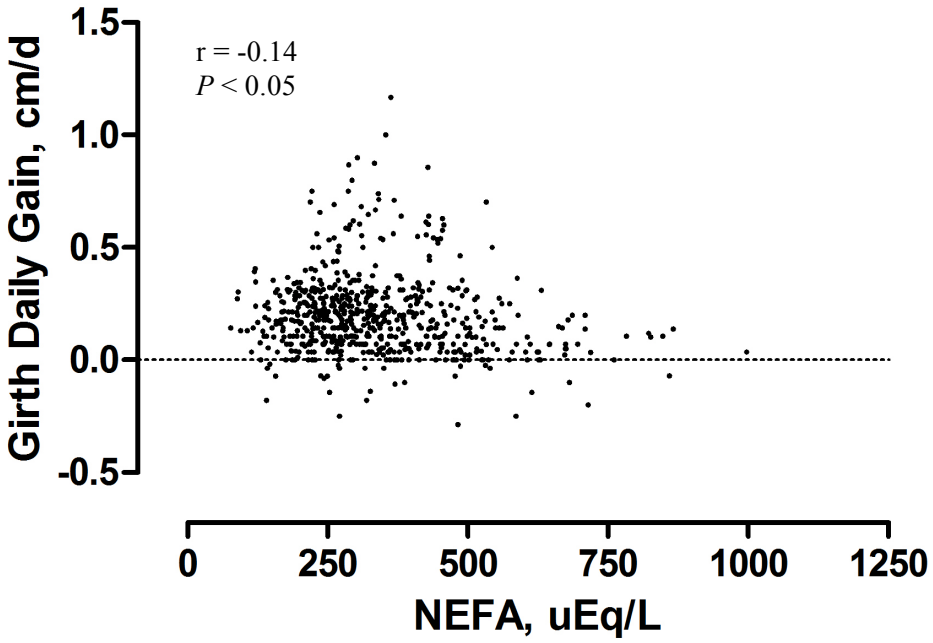

Figure 2-19. Scatterplot of plasma NEFA and girth daily gain in Thoroughbred foals from 0 to 534 days of age.

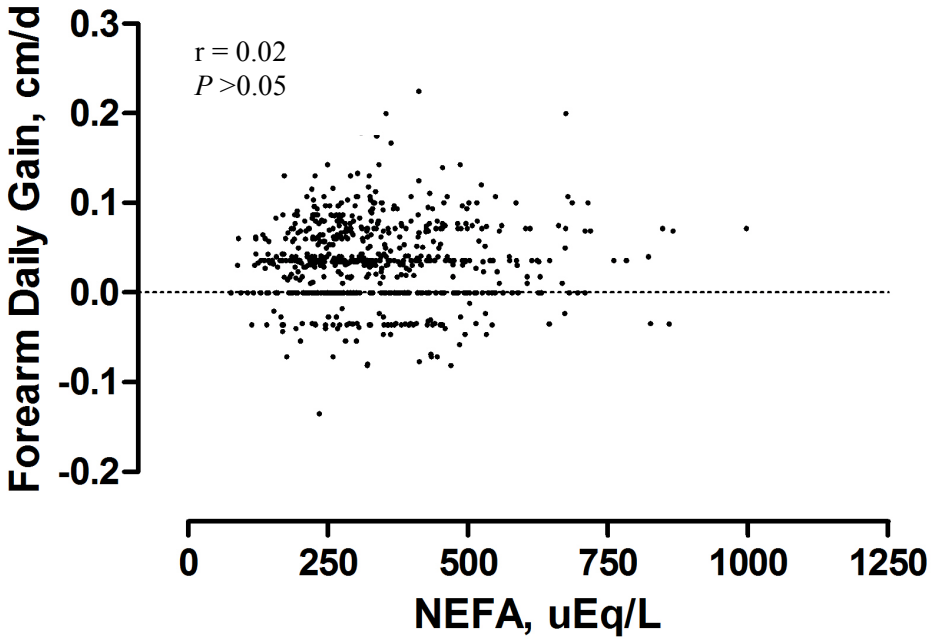

Figure 2-20. Scatterplot of plasma NEFA and forearm daily gain in Thoroughbred foals from 0 to 534 days of age.

Table 2-1. Pearson Correlation Coefficients for Variables Studied

Variable	Glucose	Insulin	NEFA	WDG	HDG	GDG	FDG
Glucose	1						
Insulin	0.644**	1					
NEFA	-0.135*	-0.104*	1				
WDG	0.539**	0.476**	-0.204**	1			
HDG	0.438**	0.405**	-0.063	0.544**	1		
GDG	0.540**	0.465**	-0.137*	0.735**	0.624**	1	
FDG	0.303**	0.261**	0.018	0.333**	0.288**	0.337**	1

*. Correlation significant at <0.05
**. Correlation significant at <0.0001

Table 2-2. Statistical Averages for Variables Studied

Variable	N	Mean
Glucose (mg/dL)	590	116 ± 25
Insulin (mIU/L)	620	4 ± 3.6
NEFA (μEq/L)	649	337 ± 0.5
WDG (kg/day)	645	0.8 ± 0.1
HDG (cm/day)	645	0.1 ± 0.1
GDG (cm/day)	646	0.2 ± 0.2
FDG (cm/day)	644	0.04 ± 0.05

WDG – Weight Daily Gain
HDG – Height Daily Gain
GDG – Girth Daily Gain
FDG – Forearm Daily Gain

Table 2-3. Statistical Averages for Variables Studied by Age Group

Age Group (d)	Glucose (mg/dL)	Insulin (mIU/L)	NEFA (uEq/L)	WDG (kg/d)	HDG (cm/d)	GDG (cm/d)	FDG (cm/d)	BMI (kg/m²)
0-30	160.3 ± 36.3	9.6 ± 6.5	387.0 ± 139.2	2.0 ± 0.5	0.3 ± 0.2	0.7 ± 0.3	0.1 ± 0.1	72.2 ± 12.3
31-60	138.1 ± 17.97	7.9 ± 4.2	323.6 ± 84.1	1.4 ± 0.3	0.3 ± 0.1	0.5 ± 0.1	0.1 ± 0.03	92.7 ± 10.1
61-90	139.5 ± 37.1	6.1 ± 4.4	299.2 ± 90.4	1.2 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.03	105.1 ± 10.5
91-120	126.1 ± 21.9	4.5 ± 3.7	268.2 ± 90.3	1.1 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.04 ± 0.03	118.6 ± 10.5
121-150	122.4 ± 16.5	4.0 ± 2.7	256.7 ± 85.3	1.0 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.04 ± 0.03	129.8 ± 11.0
151-180	119.7 ± 17.7	3.8 ± 3.0	284.4 ± 100.4	0.9 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.04 ± 0.04	138.2 ± 12.3
181-210	110.1 ± 21.3	2.3 ± 1.7	329.0 ± 121.8	0.8 ± 0.3	0.1 ± 0.1	0.2 ± 0.1	0.04 ± 0.05	144.6 ± 9.4
211-240	99.1 ± 13.5	1.8 ± 1.6	377.2 ± 152.3	0.7 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.04 ± 0.04	150.4 ± 9.8
241-270	102.3 ± 7.8	1.9 ± 1.1	453.4 ± 174.3	0.5 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.03 ± 0.04	152.6 ± 10.7
271-300	104.6 ± 18.5	1.3 ± 0.9	436.9 ± 167.4	0.3 ± 0.3	0.05 ± 0.1	0.1 ± 0.1	0.02 ± 0.04	152.6 ± 11.4
301-330	101.7 ± 8.8	2.2 ± 2.2	416.1 ± 146.3	0.5 ± 0.4	0.05 ± 0.1	0.1 ± 0.1	0.03 ± 0.06	159.7 ± 9.1
331-360	104.6 ± 12.0	2.0 ± 1.9	346.0 ± 142.7	0.7 ± 0.4	0.1 ± 0.1	0.1 ± 0.1	0.03 ± 0.05	163.6 ± 10.0
361-390	107.1 ± 12.0	2.8 ± 2.2	336.4 ± 147.7	0.8 ± 0.4	0.1 ± 0.1	0.1 ± 0.1	0.02 ± 0.05	170.5 ± 11.3
391-420	105.2 ± 12.0	2.19 ± 1.5	272.8 ± 122.5	0.6 ± 0.3	0.03 ± 0.1	0.1 ± 0.1	0.01 ± 0.05	176.1 ± 12.1
421-450	106.0 ± 12.0	2.99 ± 2.1	286.0 ± 122.1	0.35 ± 0.6	− 0.003 ± 0.1	0.1 ± 0.1	− 0.01 ± 0.03	180.5 ± 10.9
451-480	100.6 ± 6.9	3.4 ± 1.6	247.6 ± 71.1	− 0.1 ± 0.7	0.071 ± 0.04	0.04 ± 0.1	0.01 ± 0.04	178.9 ± 16.9

WDG – Weight Daily Gain
HDG – Height Daily Gain
GDG – Girth Daily Gain
FDG – Forearm Daily Gain

ACADEMIC VITA

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Education

B.S., Nutritional Sciences, 2013

The Pennsylvania State University, University Park, Pa

Honors in Animal Science

Research Interests

Particularly interested in nutritional aspects of disease, and desires to study

Diabetes mellitus due to its prevalence in her region of the world.

Activities

Kuwaiti Student Association, 2009-2013

Khaleeji Student Association, 2009, 2013

Penn State Equine Research Team (PSERT), 2011-2012

Languages

English (fluent), Arabic (fluent), French (basic)