Insulin dynamics during equine pregnancy

Possible relationship to osteochondrosis in foals
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Foreword

Skeletal disease in growing foals is a significant industry problem. It is commonly referred to as developmental orthopaedic disease (DOD). The debilitating effects of DOD affect all horse breeds. However the underlying cause of the condition is poorly understood.

Skeletal disease in growing horses has been the subject of much research interest for many years. A number of factors including genetics, rate of growth, exercise and nutrition have been identified as possible causal factors. Nutrition is the most cited area and there is evidence that protein and energy levels in the diet and mineral content of the diet significantly effect the development of skeletal diseases. Another interesting aspect of this problem is that it is often seen in very young foals. This suggests that the problems in bone formation occurred prior to birth. Evidence is emerging for this from both human and animal studies, especially with rats and sheep but it has not been examined in the horse. This project tested the hypothesis that insulin resistance in the mare perturbs the uterine environment and in so doing has long term effects on normal bone development and bone turnover in the foal, which was supported.

The results of this project will be of interest to all those who breed and train horses for both racing and pleasure as skeletal disease represents a significant industry cost. It has been estimated that DOD precludes as many as 10% of Thoroughbred foals from being sold as yearlings each year. Aside from the veterinary costs incurred to a breeder and owner after a foal is recognised to have DOD; the presence of skeletal defects reduces the value or sale price of yearlings. There may be additional costs including time lost from racing as well as medical and rehabilitation expenses.

The project was funded in part from industry revenue and funds provided by the Australian Government. Additional financial support was provided by the Hunter Valley Equine Research Foundation and The University of Queensland.

This report is an addition to RIRDC’s diverse range of over 2000 research publications and it forms part of our Horse R&D program, which aims for the Australian horse industry to be nationally and internationally recognised for its excellence as a reputable user and supplier of quality horses, products and services; and for the industry to expand in the global market by having the requisite skills and knowledge for efficient, profitable and sustainable production.

Most of RIRDC’s publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
Managing Director
Rural Industries Research and Development Corporation
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Executive Summary

What the report is about

Skeletal disease in growing horses collectively costs the Thoroughbred breeding industry alone many millions of dollars annually with as many as 10% of foals unable to be sold as yearlings due to the disorder. In addition, it has been suggested that skeletal failure in horses during training may be due to weaknesses linked to developmental lesions. Delineating the cause developmental bone disease has been the subject of much research interest for many years and a number of factors including genetics, rate of growth, exercise and nutrition have been identified as possible causal factors. Nutrition is the most cited area and there is evidence that protein and energy levels in the diet and mineral content of the diet significantly effect the development of skeletal diseases. The other interesting aspect of this problem is that it is often seen in very young foals which suggests that the problems in bone formation occurred prior to birth. Evidence is emerging for this from both human and animal studies, especially with rats and sheep. It is our contention that insulin resistance in the mare perturbs the uterine environment and in so doing has long term effects on normal bone development.

Who is the report targeted at?

The primary target group of this report is the Thoroughbred breeding industry, especially equine stud veterinarians and stud managers. The results of this project will also be relevant to all of those who breed horses. The information will allow the development of on-farm strategies that will reduce the incidence of DOD

Background

Skeletal disease in growing horses collectively costs the Thoroughbred breeding industry alone many millions of dollars annually with as many as 10% of foals unable to be sold as yearlings due to the disorder. In addition, it has been suggested that skeletal failure in horses during training may be due to weaknesses linked to developmental lesions. Delineating the cause developmental bone disease has been the subject of much research interest for many years and a number of factors including genetics, rate of growth, exercise and nutrition have been identified as possible causal factors. Nutrition is the most cited area and there is evidence that protein and energy levels in the diet and mineral content of the diet significantly effect the development of skeletal diseases. The other interesting aspect of this problem is that it is often seen in very young foals which suggests that the problems in bone formation occurred prior to birth. Evidence is emerging for this from both human and animal studies, especially with rats and sheep. It is our contention that insulin resistance in the mare perturbs the uterine environment and in so doing has long term effects on normal bone development.

Aims/objectives

Equine developmental bone disease is often seen in very young foals which suggests that the problems in bone formation occurred prior to birth. It is our contention that insulin resistance in the mare perturbs the uterine environment and in so doing has long term effects on normal bone development of the foal. This study explores this hypothesis by investigating a) the prevalence of insulin resistance in a large population of broodmares and their subsequent foals and its relationship with the incidence of Osteochondritis dissecans (OCD) and b) the influence of differing dietary regimens on insulin sensitivity and OCD.
Methods used

The insulin status of Standardbred mares was monitored throughout pregnancy. During the last trimester of pregnancy mares were offered either a high or low energy diet. Insulin-modified frequently sampled intravenous glucose tolerance tests (FSIGTT) were performed on the mares in early gestation, prior to nutritional manipulation, a few days prior to parturition, and during lactation (Day 120 post-foaling). After birth, FSIGTT were conducted in foals at 2 weeks, 4 months and 10 months of age. Minimal model analysis was used to determine insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response to glucose (AIRg), and disposition index (DI).

In on farm studies, mare and foals were recruited from major Thoroughbred horse studs. Insulin resistance in the mares and foals was predicted by specific proxies, a technique that is widely used in population based human studies.

Standard methods were used for insulin and glucose analysis and for the determination of other metabolic hormones.

Results/key findings

It is clear from the present studies that pregnancy is characterised by a progressive decline in insulin sensitivity of the mare. The results highlight the importance of body condition score in metabolic responses during pregnancy Overall, the results from this project indicate that the maternal diet during late gestation influences the metabolism of the mare and can modulate insulin sensitivity and glucose dynamics in the foal during early post-natal life. The enhanced nutritional plane of HE fed mares resulted in lower basal insulin concentrations in foals during the first year of life. The observed hypoinsulinaemia during early postnatal growth of foals appears be linked to the development of osteochondrosis (OC). This may be a consequence of maternal nutrition on metabolism during pregnancy which directly effects the feto-placental unit and subsequent postnatal metabolism and skeletal development. Further work is warranted to confirm and extend these findings.

Implications

The data presented in this project have assisted in elucidating possible mechanisms by which maternal metabolism can perturb glucose and insulin homeostasis of the foal. The results indicate that maternal diet during late gestation and lactation can influence insulin sensitivity and glucose dynamics in the foal during early post-natal life. The enhanced nutritional plane of high energy fed mares resulted in lower basal insulin concentrations in foals during the first year of life. Furthermore, the results suggest that hypoinsulinaemia during early postnatal growth of foals may be linked to the development of osteochondrosis. These associations highlight the importance of nutrition and the Body Condition Scoring (BCS) of the mare and the complexities of investigations of OC in foals and demonstrate the range of data required to delineate the pathogenesis of this condition. This has obvious implications for the nutritional management of the brood mare.
Introduction

Skeletal disease in growing horses has been the subject of much research interest for many years and a number of factors including genetics, rate of growth, exercise and nutrition have been identified as possible causal factors. Nutrition is the most cited area and there is evidence that protein and energy levels in the diet and mineral content of the diet significantly effect the development of skeletal diseases. The evidence for this is reviewed below. However, there is much data in the literature that is in conflict and we have spent a considerable amount of time trying to delineate the different aetiological factors and come up with a unified hypothesis that would explain skeletal disease. The other interesting aspect of this problem is that it is often seen in very young horses which suggests that the problems in bone formation occurred prior to birth. Evidence is emerging for this from both human and animal studies, especially with rats and sheep. It is our contention that insulin resistance in the mare perturbs the uterine environment and in so doing has long term effects on normal bone development and bone turnover. These aspects are discussed below.

Skeletal disease in growing foals – importance in the industry

Osteochondritis dissecans (OCD) is a relatively common developmental disease caused by a defect in the normal process of bone formation resulting in the thickening, cracking and tearing of the joint cartilage of growing horses. It has been estimated that Developmental Orthopaedic Disease (DOD), encompassing physitis, osteochondrosis (OC), OCD, subchondral cystic lesions, angular limb deformity (ADL), flexural deformities and cuboidal bone malformation (McIlwraith, 2001) collectively costs the Hunter Valley Thoroughbred breeding industry alone in the order of $9.8 million each year (Aldred, 1998) with as many as 10% of foals unable to be sold as yearlings due to the disorder. In addition, it has been suggested that skeletal failure in horses during training may be due to weaknesses linked to these developmental lesions (Krook and Maylin, 1988).

Causes of developmental orthopaedic disease

Current knowledge – mineral levels in growing foal rations

DOD is a multifactorial condition, however nutrition is generally regarded as one of the major causes. Many studies are conflicting and most have been carried out in environments unrelated to Australian conditions. It is generally believed that DOD is more common in large, rapidly growing horses, possibly as a result of excessive weight bearing and other biomechanical stresses on bones and joints whereas other studies have found no difference in the size or average daily gain of horses with and without DOD (Pagan and Jackson, 1996). Excess energy in the growing foal ration has been documented as a cause of the disorder (Savage et al., 1993) as has excess protein, potentially due to excessive calcium loss (Barzel and Massey, 1998). However, it has been suggested that developmental orthopaedic diseases frequently attributed to excessive energy or protein intake in the growing foal diet may instead be caused by an amount of phosphorus, calcium, zinc or copper in the diet inadequate to support the rate of growth permitted by the amount of energy and protein consumed. Investigations on trace minerals and their influence on OCD have received a great deal of attention in recent years. Copper supplementation has resulted in reductions in OC lesions (Hurtig et al., 1990, Knight et al., 1990) and subchondral bone cysts (Hurtig et al., 1990) of
growing foals however some authors believe that copper is not likely to be an important factor in the aetiopathogenesis of osteochondrosis, but there may be a significant effect of high copper status on the natural process of repair of early lesions (van Weeren et al., 2003). The role of trace minerals in the development, resolution or progression of OC lesions remains poorly understood (Gee et al., 2007).

**Recent studies—hyperglycaemia and hyperinsulinaemia in growing foals and its relationship with OCD**

Feeding diets high in soluble carbohydrates to growing horses has been implicated in the development of orthopaedic diseases and it has been suggested that glucose intolerance caused by insulin resistance may be associated with OCD in young horses. Both insulin and insulin–like growth factor–1 (IGF–1) play a role in chondrocyte maturation and perturbations of these hormones as a result of chronic hyperinsulinaemia caused by high carbohydrate diets may be manifested by OCD (Ralston, 1996; Henson et al., 1997).

Pagan et al. (2001), studied the possible implication of hyperglycaemia and hyperinsulinemia with OCD in a field study where young horses were fed concentrate feeds with different glycaemic indexes. The results show a strong positive correlation across all farms between the incidence of OCD and serum glucose and insulin levels 120 minutes after feeding. However, within a farm, there were no significant differences in the glycaemic response between horses that had lesions and those that did not. The authors suggested that diet–induced hyperglycaemia or hyperinsulinemia predisposes weanlings to OCD, but other factors such as biomechanical stress or trauma are needed to produce a clinically relevant lesion and that it would be prudent to feed foals concentrates that produce low glycaemic responses. The authors also suggested that further research is required to determine if a glycaemic response test using a more standardized oral glucose challenge (i.e. dextrose) can be used to identify younger individuals predisposed to OCD.

In a further study, Ott et al. (2005) investigated the influence of starch intake on growth and skeletal development of weanling horses. Body weight and length gains were highest in those weanlings fed high–starch rations but bone osteochondrotic lesions were not found to be related to diet. The authors concluded that at least under the experimental conditions, weanlings need some readily available glucose to support normal growth and that skeletal development of weanling horses was not affected by consumption of high–starch concentrates. In an investigation of dietary energy source on serum concentration of growth hormone, IGF–1, insulin, glucose, and fat metabolites in weanling horses, Ropp et al. (2003) found that IGF–1 did not differ between fat supplemented and conventional carbohydrate supplemented foals. The authors suggested that substitution of fat for soluble carbohydrate may not necessarily alleviate orthopaedic diseases associated with rapid growth in susceptible animals.

Growth and bone development in foals was examined by Hoffman (1999) and shown to be affected by seasonal changes in pasture and dietary supplementation with concentrates rich in sugar and starch or in fat and fibre. In weanlings and yearlings fed the fat and fibre supplement, bone mineral content was lower at certain points, possibly due to a reduction in calcium availability through cation exchange and water–holding capacity of fibre or the formation of calcium soaps with fat in the small intestine, and also a reduction in exercise (as these foals tended to be less active on a daily basis). Some feed companies are now marketing "Low Glycaemic Index" feeds to the stud farm market. The inconsistencies in research data to date suggest that the effects of these "novel" feeding strategies on OCD and growth require further investigation.
Broodmare nutrition and its relationship to OCD in growing foals

The majority of studies conducted to investigate nutritional causes of OCD in growing foals have focused on nutritional strategies from weaning age (approximately six months) onwards. However, several studies have identified the highest incidence of OCD and other skeletal disorders to be at a younger age than this. In one study, Rieck et al., (2000), found that of 98 Thoroughbred foals that underwent physical and radiologic evaluation after birth, 48% had ALD on carpal joints, 6.1% on forelimb fetlocks, 5.1% on tarsal joints and 1% on hindlimb fetlocks showing that ALD are a common finding in neonatal foals, however improvements were noted in some foals by 30 clays of age. The average incidence of DOD in Thoroughbred foals evaluated between 4 through 18 months of age was 16.1% (range 12.9 – 28.8%) and the highest incidence was recorded at 4 months of age (Jelan et al., 1996). Authors noted that affected foals were generally heavier than non affected foals. These studies suggest that while nutritional strategies during the growing period may reduce the incidence of OCD, the causative factor is likely to exist prior to this time, either during early lactation, or during gestation.

Few studies have evaluated the contribution of the mare's ration to the incidence of skeletal disease in growing foals. Some studies have suggested that a reduced copper intake/absorption during gestation could possibly either initiate the development of DOD under certain conditions or may reduce the ability to repair lesions (Knight et al., 1990 Pearce et al., 1998c, Tauson et al., 2006). However in recent study, copper supplementation had no statistically significant effect on the frequency of lesions in 160 day old foals (Gee et al., 2007). In a recent review, Tauson et al. (2006) stated that further research is needed in this area on a much larger scale.

Does "foetal programing" play a role in the development of skeletal disease in growing foals?

Data from other species suggests that nutrition of the dam and stress on the dam during gestation and lactation could precipitate endocrine responses that alter neonatal physiology. The thrifty phenotype hypothesis is based on the idea of foetal/neonatal programming and proposes that nutrition during gestation and/or lactation turns on different genes in the foetus/neonate, essentially preparing the offspring's endocrine and metabolic systems for an appropriate response to expected energy intake (Hales and Barker, 1992). The "high carbohydrate (HC) rat model" is based on the exposure of rat pups to a high carbohydrate diet during the critical window for postnatal pancreatic development (Tauson et al., 2006). Rat pups were removed from their dams at 4 days of age and artificially reared via gastric feeding of a HC milk formula. The HC formula caused an immediate onset of hyperinsulinaemia (within 24h) and despite the HC formula feeding finishing on day 24, the hyperinsulinaemia persisted into adulthood. In addition, HC pups showed an increased growth rate from day 55 of life, and had developed obesity by an age of approximately 100 days. The model also demonstrated that characteristics of chronic hyperinsulinaemia and adult−onset obesity were spontaneously transmitted from HC dams to their progeny, without involvement of the paternal side. The pregnant and lactating HC dams were normoglycaemic and hyperinsulinaemic but produced milk with identical composition to non HC dams.

Insulin resistance has been generally defined as a state in which normal concentrations of insulin fail to elicit a normal physiological response (Kahn, 1978). In addition to OC, insulin
resistance in horses has been associated with obesity and laminitis (Jeffcott et al., 1986, Pass et al., 1998) and may play a role in colic (Hudson et al., 2001) and exertional rhabdomyolysis (Valentine et al., 2001). Hoffmann et al. (2003) explored the hypothesis that insulin sensitivity would be lower (i.e. more resistant to insulin) in obese vs nonobese horses, and insulin sensitivity would be lower with adaptation to twice daily meals rich in starch and sugar compared with fibre and fat. The authors found that insulin sensitivity was approximately 80% lower in obese horses than in non obese horses, which is similar to that reported in humans (Lee et al., 1992). A higher insulin sensitivity was reported in horses fed a diet high in fat and fibre compared to those fed a ration high in starch and sugar. The authors concluded that horses fed grain meals rich in starch with a high glycaemic index may have a higher risk of developing insulin resistance. This result is in line with previous work carried out by Williams et al. (2001) who measured plasma glucose and insulin responses of Thoroughbred mares. Peak plasma glucose and insulin concentrations were higher in the mares fed a high starch and sugar diet compared to those fed a high fibre and fat diet during early and late lactation. The authors stated that metabolic functions are moderated by the replacement of sugar and starch with fat and fibre and that the metabolic and health impacts are likely to be moderated for meals of fat and fibre, which may be more reflective of the nutritional heritage of the horse. Interestingly, there is now considerable human and animal model data that dietary proteins and amino acids play a significant role in the pathogenesis of insulin resistance (Tramblay et al., 2007). Given that horses usually are fed diets that are high in protein which may have an imbalanced amino acid profile, it is highly likely that protein nutrition is implicated in the development of equine insulin resistance. Amino acid requirements and metabolism is an area that requires further study in the horse.

There have been numerous human studies that have examined nutritional neonatal programming. Krishnaveni et al. (2005) tested the hypothesis that the environment experienced by foetuses of mothers with gestational diabetes mellitus (GDM) and mothers with higher glucose concentrations that are in the normal range causes increased adiposity and altered glucose/insulin metabolism in childhood. The results indicated that newborns of diabetic mothers were larger in all body measurements than control newborns (babies with non diabetic parents). At one year, these differences had diminished and were not statistically significant. However, at five years, female offspring of diabetic mothers had significantly larger subscapular and triceps skinfold thicknesses and higher 30 and 120 min insulin concentrations than control children. Offspring of diabetic fathers were lighter at birth than control children and they showed no differences in anthropometry at 5 years. As maternal GDM is associated with adiposity and higher glucose and insulin concentrations in female offspring at 5 years and the absence of similar associations in offspring of diabetic fathers suggests a programming effect in the diabetic intrauterine environment. Pettitt et al. (1988) reported that non insulin dependent diabetes mellitus (NIDDM) during pregnancy in Pima Indian women results in offspring who have a higher prevalence of NIDDM (45%) at age 20 to 24 yr than in offspring of non diabetic women (1.4%) or offspring of prediabetic women (8.6%), women who developed diabetes only after pregnancy. These differences persist after taking into account paternal diabetes, age at onset of diabetes in the parents, and the offspring's weight relative to height. In another study, the relationship between obesity in children and diabetes during pregnancy in their mothers was examined (Pettitt et al, 1983). Obesity in the offspring was directly related to maternal diabetes and the findings strongly suggested that the prenatal environment of the offspring of diabetic women results in the development of obesity in childhood and early adulthood. In another study, Hillier et al. (2007) found that increasing hyperglycemia in pregnancy is associated with an increased risk of childhood obesity. Studies using rat models indicate that 13 cell function is impaired in offspring from diabetic rat mothers (Han et al., 2006).

The influence of maternal insulin status in mares and its effect on the development of insulin resistance in offspring is unknown. Holdstock et al. (2004) showed that equine 13 cells are
responsive to glucose and arginine and release both insulin and proinsulin during the immediate postnatal period. Further to this, Forehead et al. (2004) examined the effects of intrauterine growth on insulin secretion and resistance in newborn foals. Embryo transfer between small pony and large Thoroughbred mares was used to produce four groups of foals (pony in pony, pony in Thoroughbred, Thoroughbred in Thoroughbred and Thoroughbred in pony). Overgrown pony foals delivered by Thoroughbred mares had high basal insulin levels and greater 13 cell responses to glucose than other groups of foals. The increased supply of nutrients provided by the larger placenta of the pony in Thoroughbred foals may have led to 13 cell proliferation and/or changes in the mechanisms of insulin synthesis and secretion. The results demonstrate that variations in the intrauterine growth rate affect insulin secretion by the foal in the period immediately after birth. The authors concluded that in the horse, enhancing foetal growth above the norm has a greater range of effects on postnatal cardiovascular and endocrine functions than intrauterine growth retardation, at least in the immediate neonatal period. Insulin is known to be an important growth factor in the foetus, and elevated insulin levels may be responsible for larger sized foals (Fowden, 1995). Whether the abnormalities resolve or persist or magnify with increasing postnatal age is unknown but they are likely to affect neonatal metabolism and growth which may in turn programme glucose handling later in life.

The findings of Forhead et al. (2004) suggest that the intrauterine environment is an important determinant of the development of insulin resistance and that its effect is in addition to effects of genetic factors. These results are consistent with previous findings of neonatal hyperinsulinaemia and hypersecretion of insulin in humans and warrant further investigation.

Summary

Taken together, these studies suggest a relationship may exist between the metabolic status of mares and their likelihood of producing hyperinsulinaemic foals. As insulin plays a role in chondrocyte maturation, exacerbations of this hormone as well as IGF−1 as a result of chronic hyperinsulinaemia may be manifested by OCD, or by other metabolic conditions later in life such as exertional myopathy, laminitis and Cushings Disease. This study explores this hypothesis by investigating a) the prevalence of insulin resistance in a large population of broodmares and their subsequent foals and its relationship with the incidence of OCD and b) the influence of differing dietary regimens on insulin sensitivity and OCD.
Objectives

Equine developmental bone disease is often seen in very young foals which suggests that the problems in bone formation occurred prior to birth. It is our contention that insulin resistance in the mare perturbs the uterine environment and in so doing has long term effects on normal bone development of the foal. This study explores this hypothesis by investigating a) the prevalence of insulin resistance in a large population of broodmares and their subsequent foals and its relationship with the incidence of OCD and b) the influence of differing dietary regimens on insulin sensitivity and OCD. Specific objectives were to;

1. determine insulin sensitivity in the mare during gestation and lactation,
2. investigate the effect of ration and body condition score on insulin sensitivity in the mare during gestation,
3. determine the influence of maternal insulin sensitivity on the foal from birth to one year of age, and
4. explore the relationship between insulin status of Thoroughbred yearlings and the occurrence of OC.
Methodology

The experiments described in this report were conducted at the University of Queensland Gatton Campus in the Equine Unit. Samples were analysed from a number of Thoroughbred studs in the Hunter Valley region and southern region of New South Wales (Figure 1). The University of Queensland Animal Ethics Committee approved experimental procedures.

Figure 1:  Thoroughbred mares and foals grazing in paddocks in the Hunter Valley

Horses

The horses (mares, foals and stallions) used and sampled in these studies are described in the relevant Experiment along their husbandry and management. The horses were predominantly Thoroughbred or Standardbred.

Collection of Blood

Basal samples

Blood samples were collected via jugular venipuncture using an 18-gauge needle and one sterile vacutainer tube (10ml) containing lithium heparin to prevent clotting. A small drop of blood was placed on a glucometer™ Glucocare/Biosensore technology) glucose strip to provide an immediate glucose concentration reading. Blood samples were immediately stored on ice and centrifuged within 30-minutes of collection at 2,300rpm for 10-minutes, at 4°C. Plasmas were collected and stored frozen at -20°C pending assay analysis for hormone concentrations.

Catheterisation

For the collection of multiple blood samples, the jugular vein was catheterised as follows. Areas on the neck around the left and right jugular vein were shaved and cleaned thoroughly with Chlorohexidine solution and an alcohol swab to remove dirt and potential infectious material. Prilocaine™ (2mls), a local anaesthetic, was administered to each site to allow easy insertion of catheters into the left and right jugular veins. Sterile gloves were worn and a angiocath (mare:
16gauge, 5 ¼ inch; foals 14gauge 3 ¾ inch) catheter was inserted into each vein and were maintained in place with super-glue (Super glue 5000). Extension sets (4-6 inch) were flushed with heparinised saline (5,000IU/L) and attached to the catheter. Upon instalment, the catheters and extension sets were secured with elastoplast and flushed out with heparinised saline to help maintain blood flow and prevent clotting.

**Biochemical Analysis**

For the series of studies reported within this thesis, biochemical analysis of plasma insulin and glucose was used to examine the glucose and insulin dynamics of mares and their foals.

**Insulin**

Plasma insulin concentrations were determined by RIA kits (Coat-A-Count Insulin, Diagnostics Products Corp., Los Angeles, CA), previously validated for use with equine plasma (Sessions et al., 2004). Intra-assay coefficients of variation were <1% for glucose and <15% for insulin between duplicate samples. Total percentage binding was an average of 30% and three internal laboratory quality control (QC) samples of pooled equine plasma were assayed in duplicate at the start of each assay to determine intra-assay variation. Within assay variation was 9.8%, 10.2% and 13.5% for QC1, QC2 and QC3 respectively. Between assay variations were 9.4%, 15.5% and 15.9% respectively. The limit of detection for insulin assays were 0.79μIU/ml and high insulin plasma insulin concentrations that were observed in FSIGT minimal model analysis were diluted with buffer (bovine calf serum) in either a 1:2, 1:4 or 1:8 solutions and re-assayed for parallelism.

**Glucose**

Plasma glucose concentrations were analysed enzymatically (Hexokinase method, Olympus kit on an Olympus AU 400 Analyser, Diagnostics Systems Division, Melville NY). This analysis was conducted by the University of Queensland Veterinary Laboratory.

**Frequent Sampling Intravenous Glucose Tolerance Test (FSIGTT)**

On the morning of the FSIGTT, each horse was weighed and catheterised (Section 3.2.2) one hour prior to commencement of the test. Baseline samples were collected 10 minutes prior to commencement of the FSIGTT to determine basal concentrations of insulin and glucose. The blood collection procedure involved discarding the first 10ml of blood (heperinised saline and blood mix in catheter and extension set) taken, followed by a 10ml blood sample directly into lithium heperanised vacutainer tubes (Figure 3.1). The catheter and extension set were kept patent by flushing with heperinised saline following blood collection intervals exceeding 5minutes to prevent clotting (Figure 3.2). Concentrations of blood glucose were assessed immediately using a handheld glucometer™ (Glucocare/Biosensor Technology) for each sample. The blood samples were stored in ice and centrifuged within 30 minutes of collection at 2,300rpm for 10-minutes at 4°C. Plasma was removed and frozen at -20°C pending analysis of insulin and glucose concentrations.

Following collection of baseline samples, each horse was administered a bolus of glucose (0.3g/kg/bodyweight; dextrose solution 50% w/v) rapidly (within 2 ½ minutes) through the catheter which was then flushed with heparinised saline solution to ensure complete administration of the dose into the systemic circulation. Blood samples were then collected at 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16 and 19 minutes following glucose administration. At 20 minute after the glucose bolus, an insulin (Elili Lilly Aust/Humalog) bolus (0.02 International Unit per kilogram of body weight (IU/kg/BW)) was administered (within 10 seconds) through the catheter and flushed with heparinised saline. Further blood samples were drawn as 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180 and 240 minutes after the glucose bolus.
Thirty-one venous samples were collected from each mare and foal over the 4-hour FSIGT period. The blood samples were immediately stored in ice and centrifuged within 30 minutes of collection for the separation of plasma and the determination of insulin and glucose concentrations.

The FSIGT schedule was developed to provide comprehensive curves for mathematical analysis, with more frequent sampling when rapid changes in glucose and insulin are expected, following bolus injections. Data obtained from the FSIGT were used for Minimal Model analysis as described below.

**Minimal Model Analysis**

Minimal Model analysis takes raw glucose and insulin data from a FSIGT and calculates the minimal model compartments including insulin sensitivity (SI), glucose effectiveness (Sg), endogenous insulin secretion (AIRg) and overall insulin response and effectiveness (DI) (Bergman et al., 1987). The minimal model compartments used to describe the glucose and insulin dynamics from a FSIGT are shown by Figure 2. The equations used in the model have been previously validated in the horse (Geor et al., 2005). The program used in the current studies was the MinMod Millenium as described by Boston et al., (2003).

![Diagram of minimal model compartments used to interpret glucose-insulin dynamics from an FSIGT (Hoffman et al., 2003).](image)

The glucose and insulin curves were interpreted according to the minimal model of glucose and insulin dynamics as described by the following equations:

\[ G'(t) = G(t) \times [Sg + X(t)] + Sg \times Gb \]

Where \( G'(t) \) is the net rate (mg ∙dL⁻¹∙ min⁻¹) of change in plasma glucose. Glucose effectiveness (Sg) describes one component of this plasma disposal rate (min⁻¹), which is the capacity of the cells to take up glucose without insulin mediation (Boston et al., 2003). The plasma glucose concentration (mg/L) at time = t is \( G(t) \); Gb is the basal glucose concentration (mg/dL), maintained primarily by hepatic production. Insulin action, X(t), represents the insulin mediated component (min⁻¹) of the plasma glucose disposal rate via the acceleration of glucose uptake in response to an increment change in the insulin concentration. This component is further described by:

\[ X'(t) = p3 \times [I(t) – Ib] – p2 \times X(t) \]

Where \( X'(t) \) is the rate of change of the insulin action, p3 describes delivery of insulin to the interstitium, and p2 describes the disposal of insulin from the interstitial fluid, possible reflecting hepatic extraction of plasma insulin. Insulin sensitivity (SI, L∙min⁻¹∙ mI⁻¹) is the ratio of these
parameters: SI = p3/p2, and represents the efficiency of insulin to accelerate glucose uptake by the cells (Boston et al., 2003).

The increase in plasma insulin above basal concentration integrated from 0 to 10 minutes after glucose dose results from the β-cells responsiveness to the glucose load that is described by the acute response of insulin to glucose (AIRg, mIU/[L·min]) (Bergman 1997). The suitability of the response of β-cells, in relation to the degree of insulin resistance in the tissue or the disposition index (DI) is determined by the product of the AIRg and SI. Once both glucose and insulin levels were determined, the MINMOD Millennium computer program was applied to the data to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. This software iteratively fits the above equations to the sampling curve for plasma glucose and insulin, initially solving for the unknown parameters Sg, p2, and p3 (Boston et al., 2003).

Later analysis involves determining insulin concentration levels for each of the blood samples. The investigation was sure to take into account each horse’s previous and current diet, age, sex, body condition, bodyweight, physiological condition, breed and whether the horse’s showed any signs of stress, as all these variables can potentially affect the glucose and insulin concentrations in the blood.

Statistical Analysis

Means (±S.E) were calculated for all plasma insulin and glucose concentrations, body weights, BCSs and minimal model parameters derived from FSIGTT analysis. Statistical significance was assessed using a repeat measures ANOVA, with main effects being treatment (high energy versus low energy and NA versus OCD), farm and time. If a significant treatment x time effect or farm x time interaction was observed, then a one/two-way ANOVA and t-test was performed at each time point. For FSIGT results, the areas under the response curve (AUC) for glucose and insulin were calculated as well as peak plasma glucose and insulin concentrations. Correlations between variables including basal insulin and glucose, BCS and body weights were determined for pregnant and non-pregnant mares and their foals as well as yearlings involved in all experimental studies.
Experimental Studies

Experiment 1

Rationale and objectives

In animals pregnancy is characterized by a progressive decline in insulin sensitivity, a natural adaptation that parallels growth of the feto-placental unit ensuring sufficient glucose supply to the fetus. Ample evidence shows an adverse intrauterine environment can have deleterious consequences for health later in life. In particular, perturbations in glucose and insulin metabolism during gestation in mares can result in changes in metabolism in the resulting neonatal foal potentially predisposing the foal to metabolic disorders associated with insulin resistance later in life.

Experiment 1 investigated the relationship between glucose and insulin dynamics in pregnant mares and their subsequent foal.

Protocol

Pregnant (n=12) mares were used in the study and offered either a high (HE: n=6) or low energy (LE: n=6) diet during the last trimester of pregnancy and throughout lactation. Insulin-modified frequently sampled intravenous glucose tolerance (FSIGT) tests were performed the pregnant mares in early gestation prior to nutritional manipulation, a few days prior to parturition, and in lactation (Day 120 post-foaling). After birth, FSIGT tests were conducted in foals at 2 weeks, 4 months and 10 months of age. Minimal model analysis was used to determine insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response to glucose (AIRg), and disposition index (DI).

Results

In mares diet had a significant (P<0.01) effect in late pregnancy, with higher basal glucose (93.0 ± 2.7 vs. 85.0 ± 1.5 mg/dL) and insulin concentrations (5.0 ± 1.3 vs. 3.4 ± 0.9 mIU/L) being observed in HE fed mares compared to LE fed mares respectively. However no significant difference in any minimal model parameter was observed from the FSIGT test immediately prior to parturition, or later in lactation.

In foals, at 2 weeks of age basal insulin concentrations were significantly (P<0.01) lower in foals from HE fed mares than foals from LE fed mares (5.2 ± 0.8 vs. 13.4 ± 2.8 mIU/L, respectively), but no differences in glucose levels were observed. Further at this time, both Si (9.2 ± 1.2 vs. 3.9 ± 0.6 x10^-4 L.mU^-1.min^-1) and Sg (6.6 ± 0.4 vs. 4.1 ± 0.6 x10^2/min) were significantly (P<0.01) higher in HE foals than LE foals. Prior to weaning, basal insulin was significantly (P<0.05) lower in HE foals than LE foals, but there were no significant differences in minimal model parameters at 4 months of age. Similarly, at 10 months of age, basal insulin concentrations (0.7 ± 0.1 vs. 2.0 ± 0.2 mIU/L) remained lower in HE foals, but again no differences in minimal model parameters were observed.
Experiment 2

Rationale and objectives

The results of Experiment 1 indicate that maternal diet during late gestation and lactation can influence insulin sensitivity and glucose dynamics in the foal during early post-natal life. The enhanced nutritional plane of high energy fed mares resulted in lower basal insulin concentrations in foals during the first year of life. These results indicate that the management of mares on stud farms, including feeding practices may influence maternal metabolism and metabolic changes of their offspring.

The objective of Experiment 2 was to further examine the relationship between insulin and glucose dynamics throughout pregnancy and lactation on the insulin metabolism of foals on commercial Thoroughbred stud farms.

Protocol

A total of 71 mares were recruited from Thoroughbred stud farms in the Hunter Valley and southern Highlands regions of NSW. All farms maintained their mares on semi-improved pasture during the early stages of pregnancy and were moved to improved pastures during late pregnancy and lactation. The mares and foals sampled on each farm were nominated by the stud managers.

Jugular blood samples were obtained from pregnant (Farms A, C and D) and lactating (Farms A, B, C and D) mares during 2008 and 2009 and from their foals (born in 2008) at 2, 5 and 14 months of age. However as mares were immediately put back in foal following parturition, mares were at mid-gestation when samples were collected post-weaning. All blood samples were collected after an overnight graze on pasture. At the time of blood sampling, body condition score (BCS, determined on a scale of 1-5) of the mares were estimated. Foals were weighed at birth.
Results

Body condition scores of mares increased ($P<0.05$) from mid gestation to 2 weeks prior to birth across all farms. Two months after parturition, BCSs of mares had decreased ($P<0.01$) across all farms.

Overall plasma glucose and insulin concentrations increased ($P<0.01$) in mares from mid to late gestation for all farms (Table 1). For plasma glucose, a significant ($P<0.01$) interaction between farm and time was shown, however this was not seen for insulin. Individual farm analysis from mid to late gestation followed a similar trend for increased glucose concentrations across all farms, although significant differences were only observed in mares from cohort D ($78.5 \pm 4.7$ vs. $100.4 \pm 1.4$ mIU/L, respectively). Plasma insulin concentrations, increased significantly from mid to late gestation in mares across all farms.

Overall, glucose and insulin concentrations were found to decrease ($P<0.01$) from late gestation to early lactation with a significant interaction between farms and time was shown (Table 1). During lactation, overall glucose concentrations were maintained but insulin concentrations tended to increase and a significant interaction between farm and time was observed.

Table 1: Plasma glucose and insulin concentrations (mean ± SEM) in Thoroughbred mares during gestation* and lactation#

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mid pregnancy (n=71)</th>
<th>Late pregnancy (n=71)</th>
<th>Prior to birth (n=71)</th>
<th>Early Lactation (n=71)</th>
<th>Post-weaning (n=71)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>84.7 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.6 ± 1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>94.3 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.1 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.5 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>3.5 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.3 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Time until parturition: Mid-pregnancy (4 months), Late pregnancy (2 months), Prior to birth (2 weeks)
- Time after birth: Early lactation (2 months), Post-weaning (5 months)

Means in rows bearing different superscripts <sup>abc</sup> are significantly different (two-way ANOVA and t-test). ANOVA was done for pregnant and lactating mares separately.

All Thoroughbred mares involved in the study gave birth to normal foals. Foal birth weights were significantly ($P<0.05$) heavier on Farm C, although no other differences were observed between any other Thoroughbred farm (Table 2).

Table 2: Foal birth weights (mean ± SEM) on Thoroughbred farms

<table>
<thead>
<tr>
<th>Farm</th>
<th>Foal Birth weights (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>55.7 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>52.3 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>59.4 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>D</td>
<td>53.9 ± 7.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means in column bearing different superscripts <sup>ab</sup> are significantly different.
Glucose and insulin concentrations of foals from all farms decreased \((P<0.01)\) with advancing age and an overall significant interaction between farms and time was observed (Table 6.5). Further, glucose concentrations decreased more markedly \((P<0.01)\) than insulin concentrations with advancing age for all farms, thus a significant change in glucose-to-insulin and insulin-to-glucose rations were also observed over time (Table 6.5).

**Table 3:** Plasma glucose and insulin concentrations (mean ± SEM) in Thoroughbred foals of different ages

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2 months ((n=71))</th>
<th>5 months ((n=71))</th>
<th>14 months (yearling) ((n=71))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>124.9 ± 2.2(^a)</td>
<td>102.4 ± 1.9(^b)</td>
<td>79.5 ± 4.6(^c)</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>2.8 ± 0.2(^a)</td>
<td>2.3 ± 0.2(^a)</td>
<td>1.9 ± 0.2(^b)</td>
</tr>
</tbody>
</table>

Means in rows bearing different superscripts \(^abc\) are significantly different

Examination of mare and foal data for all farms showed a negative correlation \((P<0.05, r = -0.4)\) between mare glucose concentrations during late gestation and overall insulin concentration in their foals from 2 to 14 months of age. A significant negative correlations \((P<0.05, r = -0.4)\) were observed between mare insulin concentrations and with insulin concentrations in their foals at 2 months of age.

**Experiment 3**

**Rationale and objectives**

In the horse, the effects of diet and exercise on insulin resistance have been examined, but few studies have investigated insulin resistance and bone development. Osteochondrosis (OC) is a developmental disease caused by a defect in the normal process of bone formation resulting in the thickening, cracking and tearing of the joint cartilage of growing horses. In the most advanced stages the condition is termed osteochondrosis dissecans (OCD). This condition is known to be associated with dietary deficiencies and/or nutrient imbalances, biomechanical stress or trauma, rapid growth rates, and genetic influences. In regard to feeding practises and growth rates there is a current hypothesis that elevated post-feeding insulin levels may predispose growing horses to develop the condition.

Experiment 2 investigated the relationship between insulin status and the occurrence of OC in Thoroughbred yearlings.

**Protocol**

Briefly, yearlings \((n=191)\) born in ‘2007’ and ‘2008’ were recruited from four NSW Thoroughbred stud farms (A, B, C, D) and fasting blood samples were obtained following stabling overnight. Plasma insulin and glucose concentrations were determined by radioimmunoassay and Hexokinase methods respectively. Skeletal abnormalities were determined by radiography analysed by experienced veterinarians, and the data were retrospectively classified (see Appendix Table A1) as either osteochondrosis related lesions (OC), other bone abnormalities, or no abnormality (NA).
Results

Across all farms 27% of yearlings examined exhibited OC lesions whilst 48% of yearlings had NA. A significantly \((P<0.01)\) higher proportion of male compared to female yearlings presented with OC lesions. Analysis of insulin revealed significantly lower \((P<0.05)\) fasting insulin concentrations (Figure 4) in yearlings with OC compared to those with NA \((2.3 \pm 0.2 \text{ vs. } 3.4 \pm 0.2 \text{ mIU/L})\) respectively. In contrast plasma glucose concentrations were not different \((100.8 \pm 1.1 \text{ vs. } 101.0 \pm 1.4 \text{ mg/dL})\).

Analysis of the blood samples from the 2008 yearling cohort that had been obtained earlier in postnatal life, as foals (2 month) and weanlings (5 months), and also from their mothers during late gestation. In foals, insulin levels were again significantly \((P<0.01)\) lower in horses that developed OC as yearlings \((n=13)\) compared to those classified with NA as yearlings \((n=52)\). Overall mean plasma insulin concentrations from 2 to 14 months were \(1.5 \pm 0.1 \text{ vs. } 2.8 \pm 0.2 \text{ mIU/L}\) for OC and NA groups respectively, with no differences in glucose concentrations noted at any age. Interestingly, a moderate inverse relationship \((r = -0.4, P<0.05)\) was noted between insulin levels in the foals in postnatal life and mare glucose levels during late gestation. Indeed mares that gave birth to foals that developed OC as yearlings had significantly \((P<0.01)\) higher plasma glucose levels \((93.2 \pm 2.0 \text{ vs. } 86.7 \pm 1.1 \text{ mg/dL})\) and body condition scores \((4.1 \pm 0.1 \text{ vs. } 3.8 \pm 0.1; \text{ out of 5.0})\) during gestation compared to mares that had foals that developed normally.

![Figure 4: The relationship between fasting insulin concentrations and radiographic lesions in ‘2007’ and ‘2008’ yearlings. Significant differences are represented by *P<0.05 and **P<0.01 (one-way ANOVA and t-test).](image-url)
Experiment 4

Rationale and objectives

Osteochondrosis (OC) is an important developmental orthopedic disease in horses. The primary lesion is a defect in endochondral ossification, within the growing cartilage. The pathogenesis of OC is complex, but generally thought to involve abnormal chondrocyte maturation or failure of differentiation. The resultant changes in extracellular matrix composition (dyschondroplasia) can lead to subchondral fractures and cysts, cartilage flaps, presence of loose cartilage fragment (osteochondrosis dissecans), and synovitis. Much research has examined possible causative factors, including biomechanical influences, vascularization, nutritional factors (mineral imbalances and excess energy), and genetic predisposition, but the relative contribution of these appears to vary. However a key role for the endocrine system in metabolic signaling to bone appears important in the pathogenesis of OC. During the past decade a major hypothesis has been proposed; that high energy diets fed to rapidly growing animals, induces post-feeding hyperinsulinaemia and this is associated with OC. However OC lesions are observed in many horses fed low to moderate energy diets.

The aim of Experiment 3 was to extend the observations of Experiment 2 with the objective of defining the association of insulin and other metabolic hormones with OC.

Protocol

The same cohort of Thoroughbred yearlings was examined as in Experiment 2. Briefly, yearlings \( n=191 \) born in ‘2007’ and ‘2008’ were recruited from four NSW stud farms and fasting blood samples were obtained following stabling overnight. Plasma concentrations of metabolic hormones (insulin, IGF1, thyroxine, leptin and adiponectin) were determined by standard immunoradiometric and radioimmunoassay. Skeletal abnormalities were determined by radiography analysed by experienced veterinarians, and the data were retrospectively classified as either osteochondrosis related lesions (OC), other bone abnormalities, or no abnormality (NA).

Results

Hormone analysis revealed significantly \( P<0.05 \) lower fasting insulin concentrations in yearlings with OC compared to those with NA \( 2.3 \pm 0.2 \text{ vs. } 3.4 \pm 0.2 \text{ mIU/L, respectively} \). Also significantly \( P<0.05 \) higher IGF1 levels (Figure 5) were found in yearlings with OC than NA \( 264.1 \pm 40.9 \text{ vs. } 192.1 \pm 13.1 \text{ng/ml, respectively} \).

In contrast there was no significant difference in total thyroxine \( 1.8 \pm 0.2 \text{ vs. } 1.8 \pm 0.1 \mu g/dL \), leptin \( 2.0 \pm 0.2 \text{ vs. } 2.0 \pm 0.1 \text{ng/ml} \) or adiponectin \( 2.5 \pm 0.1 \text{ vs. } 2.4 \pm 0.1 \text{ng/ml} \) concentrations between OC and NA groups respectively. Correlation analysis across all animals revealed significant moderate correlations between insulin and glucose \( r = +0.30 \), adiponectin and glucose \( r = +0.24 \) and IGF1 and total T4 \( r = +0.21 \). However in OC animals, significant relationships between insulin and total T4 \( r = -0.32 \), and IGF1 and total T4 \( r = +0.37 \) were only found.
Experiment 5

Rationale and objectives

In pregnancy dramatic changes in maternal metabolism are necessary to ensure normal development of the foetus. Partitioning of nutrients from maternal tissues to the feto-placental unit is associated with decreased insulin sensitivity, increased basal insulin secretion, enhanced β cell responsiveness, decreased insulin clearance, and higher post-prandial glucose and insulin responses. Such metabolic changes can be influenced by diet. In Experiment 1 it was noted that feeding a high energy diet to mares in the last trimester of pregnancy increased basal insulin and glucose concentrations, but did not affect insulin sensitivity, glucose effectiveness, or β cell responsiveness. However in that study, only small differences in the bodyweight and body condition of mares were obtained.

Experiment 5 was a follow-up study to Experiment 1 with the objective of examining insulin and glucose responses in pregnant mares with disparate body condition scores.

Protocol

Pregnant (n=13) mares were used in the study and given either a high (HE: n=6) or low energy (LE: n=7) diet for the last trimester of pregnancy. Body condition score (BCS) was assessed on a scale of 1-9. Insulin-modified frequently sampled intravenous glucose tolerance (FSIGT) tests were performed on the pregnant mares on Day 290 and Day 320 of gestation. Minimal model analysis was used to determine insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response to glucose (AIRg), and disposition index (DI).

Results

There were no significant differences in bodyweights between HE and LE fed mares in late gestation. However BCSs in HE fed mares were significantly (P<0.001) higher than LE fed mares on Days 290 and 320. Further BCS significantly (P<0.05) increased in HE fed mares between Days 290 (6.3 ± 0.4) and 320 (7.2 ± 0.5), whereas in LE fed mares BCS significantly (P<0.01) decreased over these days (4.1 ± 0.3 vs. 3.3 ± 0.3). Basal insulin and glucose concentrations were not significantly between HE and LE fed mares on either Day 290 or Day 320. In HE mares, minimal model parameters did not significantly change from Day 290 to Day 320. On Day 320 values in HE fed mares were Si (1.1 ± 0.4, x10-4L.mU-1.min-1), Sg (2.3 ± 0.4 x 102. min-1), AIRg (347.5 ± 60.8 min.mIU/L) and DI (282.9 ± 79.7). In comparison, insulin sensitivity and the disposition index in LE fed mares were significantly (P<0.01) lower on both Day 290 (Si 0.2 ± 0.2, x10-4L.mU-1.min-1; DI 33.9 ± 28.8) and Day 320 (Si 0.04 ± 0.01, x10-4L.mU-1.min-1; DI 4.3 ± 2.7) compared to HE fed mares. Further the acute insulin response to glucose was similar between the mare groups on Day 290, but significantly (P<0.01) lower in LE mares compared to HE mares on Day 320. In contrast, glucose effectiveness was not different at any time.
Discussion of Results

Insulin resistance is a complex pathophysiological condition that appears to be associated with a number of chronic conditions grouped together and called ‘equine metabolic syndrome’, including laminitis, obesity, and osteochondrosis (OC). In the horse, the effects of diet and exercise on insulin resistance have been examined in some detail but there have been few studies during pregnancy. Pregnancy is characterized by a progressive decline in insulin sensitivity, a natural adaptation that parallels growth of the fetoplacental unit ensuring sufficient glucose supply to the foetus. Evidence from human and rodent studies show that significant changes to the intrauterine environment can have deleterious consequences for health later in life of the offspring. These studies suggest a relationship may exist between the metabolic status of mares, in particular their insulin sensitivity and subsequent modification of foal metabolism. This may be manifested by OC, or by other metabolic conditions later in life. However, there is little data available on insulin and glucose dynamics in mares during pregnancy and lactation and their foals.

The aim of these studies was therefore to examine the glucose and insulin dynamics in the mare during pregnancy and lactation. Experiment 1 investigated the relationship between glucose and insulin dynamics in pregnant mares offered either a low or high energy diet during the last trimester of pregnancy. Insulin-modified frequently sampled intravenous glucose tolerance (FSIGT) tests were performed on the pregnant mares in early gestation prior to nutritional supplementation and a few days prior to parturition. Minimal model analysis was used to determine insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response to glucose (AIRg), and disposition index (DI). Diet had a significant (P<0.01) effect in late pregnancy, with higher basal glucose (93.0 ± 2.7 vs. 81.0 ± 1.9 mg/dL) and insulin concentrations (4.3 ± 0.9 vs. 1.7 ± 0.2 mIU/L) being observed in HE fed mares compared to LE fed mares respectively. However no significant difference in any minimal model parameter was observed from the FSIGT test immediately prior to parturition.

The relationship between diet and metabolism during pregnancy was extended in Experiment 5. The results indicate that LE fed mares became insulin resistant in late pregnancy and exhibit diminished β cell responsiveness. With increasing insulin resistance in pregnancy, the expected homeostatic response is enhanced β cell secretion to compensate for decreased insulin-mediated glucose uptake. However we observed inadequate acute β cell secretion in LE fed mares. Such a defect in pancreatic β cell function, accompanying severe insulin resistance, is a prime characteristic of human gestational diabetes mellitus (GDM). Lean women with GDM exhibit pronounced insulin resistance and inadequate insulin secretion, similar to our LE fed mares with low BCS. These novel results highlight the importance of BCS in metabolic responses during pregnancy.

After parturition of the mares in Experiment 1 there were no significant differences in any minimal model parameter was observed from the FSIGT test in mares upto 120 days of lactation or at the time the foals were weaned. In contrast, at 2 weeks of age, basal insulin concentrations were significantly (P<0.01) lower in foals from HE fed mares than foals from LE fed mares and this difference persisted till weaning. The results indicate that maternal diet during late gestation and lactation can influence insulin sensitivity and glucose dynamics in the foal during early postnatal life. The enhanced nutritional plane of high energy fed mares resulted in lower basal insulin concentrations in foals during the first year of life. Whether such dynamic changes predispose foals to metabolic problems later in life remains to be determined.

The relationship between insulin and glucose dynamics of the mare and the insulin metabolism of their foals was further examined on commercial Thoroughbred farms in Experiment 2. Results from this study also showed significantly higher basal insulin and glucose concentrations in Thoroughbred mares during late gestation compared to mid gestation and lactation. Interestingly, a significant inverse relationship (P<0.05, r = -0.4) was noted between insulin concentrations in the foals in postnatal life and mare glucose levels during late gestation. Again indicating that the maternal uterine environment influences the subsequent metabolic development and capacity of the foal.
In Experiment 3, the relationship between insulin status after an overnight fast and the occurrence of OC in a population of 191 Thoroughbred yearlings recruited from NSW Thoroughbred stud farms and fasting blood samples were obtained following stabling overnight. Across all farms 27% of yearlings examined exhibited OC lesions whilst 48% of yearlings had NA. A significantly (P<0.01) higher proportion of male compared to female yearlings presented with OC lesions. Analysis of insulin revealed lower fasting insulin concentrations in yearlings with OC compared to those with NA (P<0.01). It was also shown that mares that gave birth to foals that developed OC as yearlings had significantly (P<0.01) higher plasma glucose concentrations and body condition scores during gestation compared to mares that had foals that developed without bone lesions. Taken together the results suggest that hypoinsulinaemia during early postnatal growth of foals may be linked to the development of osteochondrosis. Furthermore the influence of maternal nutrition on metabolism during pregnancy may have consequential effects upon postnatal metabolism and normal skeletal development.

To further explore the possible endocrine basis of OC in yearlings, Experiment 4 examined other metabolic hormones. The results suggest that low insulin and high IGF1 concentrations in fasted yearlings are associated with OC. Whether these hormonal alterations are involved in the pathogenesis of OC remains to be determined. The observation of higher IGF1 levels in yearlings with OC may be indicative of joint repair occurring in these animals with lesions, rather than being related to the development of OC. In regards to insulin, these yearlings had lower resting insulin concentrations throughout early postnatal life which provides more evidence that insulin is involved in the pathogenesis of OC.

Overall, the results from this project indicate that the maternal diet during late gestation influences the metabolism of the mare and can modulate insulin sensitivity and glucose dynamics in the foal during early post-natal life. The enhanced nutritional plane of HE fed mares resulted in lower basal insulin concentrations in foals during the first year of life. The observed hypoinsulinaemia during early postnatal growth of foals appears be linked to the development of OC. This may be a consequence of maternal nutrition on metabolism during pregnancy which directly effects the feto-placental unit and subsequent postnatal metabolism and skeletal development. Further work is warranted to confirm and extend these findings.
**Implications**

It is clear from the present studies that pregnancy is characterised by a progressive decline in insulin sensitivity. Moreover, nutrition of the mare during late gestation may also influence glucose and insulin metabolism of foals during the first year of life. This appears to result from an in utero effect. The results also suggest that abnormalities in the insulin dynamics of foals may predispose them to the development of OC. The results also indicate that nutritional management during pregnancy may be an important risk factor in abnormal bone development in foals.

The results of the current studies pose as many questions as they answer, and this reflects the lack of information available and the cost and resources required to conduct this type of research. However, the findings do provide new insights into the possible relationship between maternal nutrition and the insulin dynamics and skeletal abnormalities in growing horses. The findings also suggest a role for maternal nutrition in the pathogenesis of the skeletal abnormalities in growing foals. Further research is warranted to refine the relationship between maternal glucose and insulin dynamics and foal metabolism, especially during critical windows of foetal and neonate growth and development. This aspect of foetal and neonatal metabolism should be explored in detail.

The data presented in this project have assisted in elucidating possible mechanisms by which maternal metabolism can perturb glucose and insulin homeostasis of the foal. The results indicate that maternal diet during late gestation and lactation can influence insulin sensitivity and glucose dynamics in the foal during early post-natal life. The enhanced nutritional plane of high energy fed mares resulted in lower basal insulin concentrations in foals during the first year of life. Furthermore, the results suggest that hypoinsulinaemia during early postnatal growth of foals may be linked to the development of osteochondrosis. These associations highlight the importance of nutrition and the BCS of the mare and the complexities of investigations of OC in foals and demonstrate the range of data required to delineate the pathogenesis of this condition. This has obvious implications for the nutritional management of the brood mare.
Publications and Presentations Associated with this Research


## Table A1: Criteria for classification of radiographic findings in Thoroughbred yearlings

<table>
<thead>
<tr>
<th>Criteria for clinical classification</th>
<th>OC Lesions (OC)</th>
<th>Other Bone Lesions (OB)</th>
<th>No Abnormalities (NA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OCD (hock, stifle, fetlock)</td>
<td>Bone modeling and spurring (unspecified)</td>
<td>No abnormalities</td>
</tr>
<tr>
<td></td>
<td>Subchondral bone cyst (Stifle, fetlock, pastern</td>
<td>Sesamoiditis (fetlock)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Physitis</td>
<td>Sesamoid fracture (fetlock)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sagital ridge fracture</td>
<td>LH fetlock palmaromed axial fragment</td>
<td></td>
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<tr>
<td></td>
<td>Subchondral lucency (stifle)</td>
<td>Knee spurring (unspecified)</td>
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<tr>
<td></td>
<td>Lysis medial condoyle</td>
<td>Carpal bone modeling</td>
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<tr>
<td></td>
<td>Cervical Vertebral Malformation (wobbler)</td>
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<tr>
<td></td>
<td>Distal Sagital Ridge (DSR) lucency and lysis</td>
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References


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Insulin dynamics during equine pregnancy

By W.L. Bryden, C.E. Foote, A.J. Cawdell-Smith and S.T. Anderson

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Skeletal disease in growing foals is a significant industry problem. It is commonly referred to as developmental orthopaedic disease (DOD). The debilitating effects of DOD affect all horse breeds and the underlying causes of the condition is poorly understood. It is often seen in very young foals which suggests that the problems in bone formation occurred prior to birth. This project investigated insulin resistance in the mare and the long term effects on normal bone development in the foal.

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