# THE EFFECTS OF FETAL AND POST-NATAL GROWTH RATES ON THE DEVELOPMENT OF TYPE 2 DIABETES IN YUCATAN MINIATURE PIGS

LESLIE McKNIGHT

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# The effects of fetal and post-natal growth rates on the development of type 2 diabetes in Yucatan miniature pigs

by

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**Abstract**

Epidemiological studies have linked small birth weight and rapid compensatory growth to a number of chronic diseases, such as type 2 diabetes, hypertension and cardiovascular disease (Chapter 1). Despite their many uses in biomedical research, few studies have used swine as a model for fetal programming. The overall goal of this research was to develop a Yucatan miniature pig model of fetal programming. Specifically we wanted to determine the effects of birth weight, postnatal growth rate and early postnatal nutrition on the development of type 2 diabetes in Yucatan miniature swine (Chapter 2). In order to do so, we needed to demonstrate compensatory growth (Chapter 3) and validate the miniature pig as a model for type 2 diabetes (Chapter 4). Although runt pigs displayed compensatory growth, growth characteristics were not related to any markers of type 2 diabetes development (Chapter 5).

**Key Words:** Yucatan, miniature pig, compensatory growth, type 2 diabetes, fetal programming

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# Chapter 1 Literature Review

## 1.1 Fetal Programming

### The Barker Hypothesis

Epidemiological studies have linked low birth weight and high postnatal growth rate to a number of chronic diseases, including type 2 diabetes, hypertension, obesity, and cardiovascular disease (McMillen and Robinson, 2005). Dr. David Barker of Southampton University, UK first described this relationship between low birth weight and later adult disease as fetal programming in the early 1990s. Fetal programming is defined as the long-term consequences of a nutritional insult experienced in early life. The nutritional insult can be anything from insufficient total nutrients to a specific nutrient deprivation. The nutritional insult permanently re-programs the animal's metabolism in such a way that the animal becomes more susceptible to the development of chronic diseases. The actual mechanism of this re-programming is unknown.

### The Thrifty Phenotype Hypothesis

Hales and Barker described the thrifty phenotype hypothesis in 1992. They proposed that the nutritional environment sensed by the fetus in utero causes changes in fetal metabolism. For example, in response to nutrient shortages in utero, the fetus switches to a conservation metabolism that biologically prepares it for nutrient shortages after birth. The fetus redistributes nutrients to promote the growth of the brain at the expense of other organ systems such as the liver, pancreas and kidneys. This redistribution, termed organ sparing, has profound irreversible effects on cell number and organ size (Kind et al 2003, Ritacco et al 1997). The consequences of this fetal

adaptation are dependent upon postnatal nutrition. In Westernized countries where infants are provided with excessive nutrients, the prenatal switch to conservation metabolism and organ sparing creates a situation where the large metabolic demand of excessive nutrients cannot be handled by the limited cell number. As a result, the infant experiences appetite dysregulation, obesity, and insulin resistance. However, when low birth weight animals receive poor postnatal nutrition from undernourished mothers, the risk of later obesity decreases. It is the mismatch of prenatal and postnatal environments, which leads to later problems.

**Defining Birth Weight**

Another interesting observation is that the risk of chronic disease development decreases with increasing birth weights (McMillen and Robinson, 2005). The observation leads to the question, how does one define low birth weight? Low birth weight in humans is usually defined as an infant born at term weighing less than 2500 g (Dewey, 1998, Rasmussen, 2001). These infants are considered small for their gestational age compared to normal or large birth weight infants in a reference population. There are many factors that influence birth weight including maternal nutrition, smoking, placental disease, birth order, multiple births and genetics. Regardless of what factors contributed to birth size, the absolute birth weight of an infant does not necessarily reflect its intrauterine environment. For example, a lower birth weight infant may have reached its genetic potential for size. This infant would not have experienced any perturbations in growth during gestation to account for its small size at birth. The opposite is true in that normal birth weight babies could have been born larger, but instead were growth-restricted during gestation and unable to reach their genetic potential

for size. It is difficult to determine whether a human infant has reached its genetic potential for size at birth.

It is important to note that the majority of epidemiological studies investigating fetal programming do not include clinical low birth weight infants of <2500 g. Instead, these studies examine infants across a range of normal weights (2500 - 4000 g) (Dewey, 1998, Rasmussen, 2001). Although adult disease risk decreases over an increasing range of normal birth weights, it is not possible to determine which infants had undergone any disruptions in intrauterine growth. Therefore, researchers have looked to early postnatal growth as an indicator of the conditions of the intrauterine environment.

**Animal Models of Fetal Programming**

Although extensive epidemiological evidence has demonstrated an association between low birth weight and later adult disease, what causes this association is unknown. In order to better understand fetal programming several animal models of low birth weight have been developed. The rat, mouse, guinea pig, sheep and domestic pig have all been used as models for examining the fetal origins of adult disease. There are several different approaches to controlling the nutrient supply to the fetus to induce low birth weight animals including surgical methods, global under nutrition and protein deprivation.

**Surgical Methods**

In rodents, intrauterine artery ligation has been used to limit blood flow to the fetus. The result is impaired fetal development and severely reduced birth weight. Jansson and Lambert (1999) ligated the uterine artery of one horn on day 12 of gestation and rat dams delivered very low birth weight pups spontaneously on day 22.

Radiotelemeters were installed in the offspring at 3 months of age and these animals also underwent an intravenous glucose tolerance test. Although there were no differences in blood pressure between animals of very low birth weight and controls, females had significantly higher fasting blood glucose and lower fasting plasma insulin. A similar surgical method has been performed with sheep. The endometrial caruncles are removed (carunclectomy) restricting blood flow to the fetus producing lambs born half the normal size (Bertram and Hanson, 2001). Although low birth weight is achieved in this surgical model, it is excessive and there is increased prenatal mortality. Placental disease in humans can decrease blood flow to the fetus resulting in very low birth weight.

**Global Under Nutrition**

Restriction of total nutrients, often termed global under nutrition, involves restricting the maternal diet to 30-90% of caloric requirement during gestation. Severe nutrient restriction is considered 30-50% of energy requirement, moderate restriction 50-70% and mild ranging from 80-90% of energy requirement (Bertram and Hanson 2001, Kind et al 2003). Maternal feed restriction limits the supply of nutrients to the fetus. The result is slowed fetal growth and development causing low birth weight animals. When rat dams were fed 50% of ad libitum during the second half of gestation, they gave birth to very low birth weight pups (Bertin et al 1999). At 80 days of age, female rats born to feed-restricted mothers had significantly higher glucose, lower insulin and impaired vascular function compared to control rats. Kind and colleagues (2003) found similar results in guinea pigs. The offspring of mild (85% ad libitum) and moderate (70% ad libitum) maternal feed restriction had significantly lowered birth weight compared to offspring of mothers fed ad libitum during gestation. Male offspring of mothers who

suffered moderate feed restriction had significantly higher fasting insulin and ratio of fasting insulin to glucose compared to mild restriction and control animals at 90 days of age. Offspring of mothers fed 50-70% of energy requirement did not experience any symptoms of chronic disease (Kind et al., 2001), suggesting that feeding mothers 30-50% of their requirement may be necessary to observe risk factors of disease in the offspring. Global under nutrition models are reflective of humans that have experienced a period of famine or people living in underdeveloped countries. However, the majority of epidemiological studies examining fetal programming are performed in developed countries where famine situations are less frequent.

**Low Protein**

The most extensively used model for studying fetal programming is the low protein rat model. Since fetal growth is largely dependent upon protein supply, researchers have restricted maternal dietary protein by up to 50%. There are two main low protein diets used by researchers to induce low birth weight, the Southampton and Hope Farm diets. Langley-Evans performed a study that directly compared the two diets (2000). They found that rats whose mothers were fed the low protein Southampton diet had significantly higher systolic blood pressure at 4 weeks than rats whose mothers were fed low protein Hope Farm diet and than offspring of mothers fed adequate protein during gestation. These findings suggest that protein content per se may not lead to fetal programming. Because the fatty acid composition, total fat and methionine content, of the maternal diet were different any of these other nutrients can contribute to fetal programming. Although maternal protein restriction induces low birth weight, the model does not focus on the effects of low birth weight on the development of later disease,

rather the model focuses on how alterations of maternal nutrition during gestation leads to chronic disease development. The majority of epidemiological studies of fetal programming specifically link low birth weight to later chronic disease, and do not focus on maternal nutrition during gestation.

**Metabolic Mechanisms**

Through the use of animal models, several hypotheses to explain metabolic mechanisms of fetal programming have been proposed. One theory is that excessive exposure to maternal glucocorticoids in utero causes permanent changes in the hypothalamo-pituitary-adrenal (HPA) axis that lowers birth weight and programs adult hypertension and glucose intolerance (Nyirenda et al., 1998). Another recent hypothesis is that maternal nutritional status can cause epigenetic changes in the fetal genome leading to altered DNA methylation (Wu et al., 2004). Abnormal DNA methylation during fetal development can permanently alter fetal metabolism in such a way that the individual becomes more likely to develop chronic disease later in life. Although the metabolic mechanisms are not yet elucidated, animal models of fetal programming have proved to be invaluable to advance the understanding of fetal programming and its mechanisms.

## 1.2 Compensatory Growth

The majority of low birth weight infants will undergo a period of accelerated postnatal growth, termed compensatory or catch-up growth. During this period, nutrient efficiency is increased allowing for rapid growth. However, this increased efficiency does not last and once over, growth continues according to age and size (Metcalfe and

Monaghan, 2001). The period of compensatory growth usually occurs during the first 6-9 months of life, while milk is the dominant food source (Rasmussen, 2001). It is important to note that low birth weight does not cause compensatory growth. Catch-up growth occurs as a result of periods of nutritional insult or illness; therefore, infants within the normal range of birth weights can also experience compensatory growth. In fact, roughly 30% of infants experience catch-up growth (McMillen and Robinson, 2005). Moreover, infants born large for their gestational age undergo catch-down growth during the first 6-9 months of life (Rasmussen, 2001). There is epidemiological evidence suggesting that small birth weight infants that experience catch-up growth are at even higher risk for the development of chronic diseases (McMillen and Robinson, 2005).

**Mechanisms of Compensatory Growth**

The mechanism of catch-up growth is not well understood. One proposal is that the central nervous centre compares the actual body size to an age-appropriate size and adjusts growth accordingly (Gafni and Baron, 2000). Another theory is that compensatory growth involves the proliferation of growth plate stem cells (Gafni and Baron, 2000). Despite the fact that little is understood about the underlying mechanisms, most animals exhibit compensatory growth including fish, reptiles, amphibians, birds, rats, pigs and humans (Metcalfe and Monaghan, 2001). Compensatory growth increases short-term survival of most animals, allowing them to reach sexual maturity more quickly. The long-term consequences are poorly understood and have received little attention in most species. However, in some studies, life expectancy is compromised as a cost of this catch-up growth in some species such as salmon, zebra fish and rats (Metcalfe and Monaghan, 2001). Rats subjected to protein restriction in utero have reduced cell

numbers in key organs that can not keep up with the increased metabolic demand during compensatory growth (Hales and Barker, 2001). As a result, the rat is more likely to develop chronic diseases that can decrease life expectancy. The fetal programming hypothesis has brought back a resurgence of interest in understanding the metabolic consequences of compensatory growth. Large epidemiological studies in the United Kingdom and Finland have extensively studied the growth patterns of these populations.

**Epidemiological Evidence Linking Catch-Up Growth to Later Disease**

The short-term effects of catch-up growth have been studied extensively in developed nations. Body weight and length records at birth, 2 years and 5 years of age were collected from infants enrolled in the United Kingdom's Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC) in order to examine postnatal growth (Ong et al., 2000). Infants, who experienced catch-up growth during this period, were smaller, shorter and thinner at birth compared to all other infants. When examined at 5 years of age, those infants who experienced compensatory growth had higher body mass indices (BMI), percentage body fat, total fat mass and waist circumference compared to all other infants. Infants who caught up in length from birth to 2 years had higher BMI and fat mass at 5 years of age than all other infants. These findings demonstrate that compensatory growth in body weight and length of low birth weight infants has profound effects on weight gain and fat deposition at five years of age. Further studies have investigated whether this trend persists into adulthood and its consequences on adult health.

A series of retrospective studies was performed using data from the Helsinki Finland cohort, which related birth weight, and growth rates between the ages of 7–15 to

coronary heart disease and type 2 diabetes in adulthood (Forsen et al., 1999 and 2000, Eriksson et al., 1999). In relation to birth weight, the odds ratio for type 2 diabetes was 1.38 for each 1 kg decrease in birth weight. These results show that the odds for type 2 diabetes development increase along a range of decreasing birth weights, suggesting a graded response. Perhaps most interesting, this study also found a strong relationship between growth rate between 7 – 15 years and later type 2 diabetes development (Forsen et al., 2000). The findings of this study suggest a relationship between birth weight and postnatal growth rate and later development of type 2 diabetes.

**Challenges/Problems with Human Studies**

Although postnatal growth rate can be linked to the development of chronic diseases, there are many factors that affect postnatal growth rate. When examining epidemiological studies, it is important to consider the mode of feeding during the first year of life. According to World Health Organization growth records, breast fed babies grow faster during the first 2 months and slower from 3-12 months than formula fed infants (Dewey, 1998). Dewey (1998) performed a meta-analysis of 19 studies from 1980-92 comparing growth of breast-fed and formula fed infants in the same population. Overall, formula fed infants gained more weight in the first year of life compared to breast fed infants. However, there are several limitations and confounding factors that impact studies of this nature. For example, each milk formula composition varies slightly. Also, it is impractical to quantify breast milk intake during this period. Maternal smoking, disease and nutrient intake during pregnancy will all affect the infant's postnatal growth and must be considered when drawing epidemiological conclusions. Using

animals to study compensatory growth is advantageous, as it is easier to control for the confounding factors plaguing human studies

**Animal Models of Compensatory Growth**

As mentioned previously, the low protein rat model is commonly used for inducing low birth weight pups. Restricting maternal protein during lactation provides a unique model for examining the impact of early infant nutrition on postnatal growth. In a recent study by Desai and colleagues (2005), pups of protein-restricted dams that were left on a dam restricted during lactation, remained small throughout life. However, when cross-fostered onto a control dam, pups grew significantly faster and were larger than control pups by 3 weeks of age and remained larger at 9 months of age (Desai et al., 2005). At 9 months of age, these rats had a significantly lower percent lean body mass and higher percent fat mass compared to control animals indicating obesity.

Despite their many uses in medical research, few studies have examined compensatory growth in pigs in relation to fetal programming of later diseases. However, research on compensatory growth in pigs has been widely studied by agricultural scientists. Ritacco and colleagues (1997) compared postnatal growth characteristics of runt piglets to larger same sex littermates from birth until 14 days of age. Runt piglets experienced a faster relative rate of growth than their littermates. Also, the feed efficiency (g feed: g gain) was significantly better (i.e. lower) in runts than larger littermates. Poore and Fowden (2004[a]) also found that the relative rate of growth was significantly higher in runt piglets from 3 –12 months of age compared to larger littermates. In terms of absolute body weight, runts were significantly smaller at 3 months of age than littermates, but not at 12 months of age. These runt pigs were found

to have impaired glucose tolerance at 12 months of age. Although, there is limited research on pigs and fetal programming, they do demonstrate compensatory growth that contributed to later disease.

### 1.3. Diabetes and Fetal Programming

The prevalence of hypertension, cardiovascular disease, obesity and type 2 diabetes in Westernized countries is increasing rapidly (Rader, 2007). Type 2 diabetes has become an epidemic in North America. The explosive rise in the incidence of type 2 diabetes is paralleled with an equally explosive increase of obesity. This observation is not surprising as obesity, especially visceral obesity, is strongly related to type 2 diabetes (Rader, 2007). What is alarming is the extremely high incidence of childhood and adolescent obesity and type 2 diabetes. Studies in Spain, India and France have shown insulin resistance in children as young as 8 years of age (Bavdekar et al., 1999, Ibanez et al., 2003, Leger et al., 1997). Insulin resistance refers to the impaired uptake of glucose by muscle and typically precedes both obesity and diabetes. Insulin resistance is characterized by high fasting plasma insulin needed to keep the individual in euglycemia. However, the pancreas cannot keep up with the insulin demand. Hyperglycemia results and the individual becomes type 2 diabetic. It is now thought that low birth weight and obesity in childhood is a stronger predictor of insulin resistance than of being obese as an adult (Hales and Barker, 2001).

In the ALSPAC prospective cohort study of children born in the United Kingdom, detailed growth records were obtained from birth and compensatory growth was defined as children that had upwardly crossed a centile from 0-3 years of age. In a recent study

by Ong and colleagues (2004) using this cohort, children that experienced catch-up growth had higher BMI, waist circumferences and lower insulin sensitivity at 8 years of age. In children with the highest BMI, low birth weight was strongly associated with insulin resistance. Cohorts of children in India and North America have demonstrated similar results (Dunger and Ong, 2005).

Although the data regarding childhood insulin resistance are relatively recent, there is extensive epidemiological evidence demonstrating a relationship between low birth weight and postnatal growth on the development of type 2 diabetes in adults. Poulsen and colleagues (1997) studied the effects of birth weight on the development of type 2 diabetes in monozygotic and dizygotic twins. Twins aged 55-74 were selected from the Danish Twin Register. Twins that participated in the study underwent an oral glucose tolerance test and fasting glucose, insulin, triglycerides and cholesterol were also determined. Individuals of the lowest birth weight had significantly higher 120 minute plasma glucose and glucose area under the curve during the oral glucose tolerance test. When twins were paired, twins that were diagnosed with type 2 diabetes were born at significantly lower birth weight than their non-diabetic co-twins. This finding suggests that birth weight is a strong predictor of later type 2 diabetes development, as factors such as genetics, gestational age, maternal height/weight and birth order were similar in twins.

**Timing of Nutrient Restriction**

Despite the substantial epidemiological evidence linking birth weight and postnatal growth to childhood insulin resistance and adult type 2 diabetes, rat studies have

failed to duplicate these findings. Rats whose mothers were protein-restricted during gestation have better glucose tolerance at weaning than rats that were not restricted in utero (Shepherd et al., 1997). However, later in life, the low protein rats have much worse glucose tolerance than control animals. These results only occurred if the rat protein-deprived in utero is suckled from a well-nourished dam. In order to better understand these findings, researchers looked into the effects of protein deprivation on specific organ systems involved in glucose homeostasis, specifically the pancreas. Pancreatic beta cell number is greatly decreased in protein-deprived pups at birth (Bertram and Hanson, 2001). A reduced beta cell number should decrease the insulin production and secretion capacity compared to non-restricted animals. However, in rats cross-fostered onto a well-nourished mother during lactation, the beta cell mass is restored, whereas, in those left with a dam protein-deprived during lactation, the restoration of beta cell number does not occur. One explanation for this oddity is that rats are altricial species, meaning they experience extensive maturation postnatally. Pancreatic islet cell neogenesis occurs in two periods in rats (Hill and Duvillie, 2000). Pancreatic islets are present during late gestation but are not responsive to glucose, only to amino acids (Hill and Duvillie, 2000). Shortly after birth in rats, the islet cells already present are replaced with new islet cells that are responsive to glucose and able to secrete insulin. It has been suggested that this new population of islet cells will have metabolic control in later life. Therefore, any perturbations to islet cell development in utero have the potential to be corrected postnatally if the second population of islet cells is unaltered (Hill and Duvillie, 2000). Humans, however, are precocial meaning they are born with highly developed cardiovascular and endocrine systems at birth. Unlike the rat, human

pancreatic islet cells are mature and develop glucose responsiveness during the third trimester of pregnancy (Hill and Duvillie, 2000). Therefore, any disruption to pancreatic development during late gestation can lead to altered metabolism in later life.

Since pancreatic organogenesis occurs during the third trimester of human pregnancy, it is not surprising that maternal food restriction during this time would lead to type 2 diabetes later in life. Unique human data were generated on the effects of nutrient restriction in utero during the Dutch winter famine. During the Second World War, food was restricted to the western Netherlands from November 1944 until May 1945. Caloric intakes ranged from 400–1000 kcal/d. Many researchers have studied adults that were in utero during the famine for signs of fetal programming. Adults that were nutrient-restricted in utero during the first trimester of pregnancy had high rates of cardiovascular disease. Those restricted in late gestation experienced insulin resistance, type 2 diabetes and hypertension in adulthood (Barker et al. 1993). Ravelli and colleagues (1998) found that individuals subjected to the famine in utero during mid to late gestation had significantly higher 2-hour glucose levels during an oral glucose tolerance test than those affected during the first trimester. Individuals subjected to famine during late gestation displayed a greater degree of glucose intolerance than those restricted during mid gestation. These results confirm that the timing of nutrient restriction in utero influences which diseases are programmed. More animal models are needed to fully understand the impact of maternal nutrient restriction during specific periods of gestation on organogenesis of the pancreas and later disease development. Specifically, precocial animals such as the pig would make an excellent model of study.

The pig has been used extensively as a model for studying many aspects of both insulin dependent and non-insulin dependent diabetes including pancreatic beta cell function and mass. The pig shares very close nutritional, physiological and metabolic similarities with humans making it an ideal model of study. However, few researchers have used the pig as a model for studying the fetal programming of type 2 diabetes. Recently, Poore and Fowden (2002, 2003, 2004a, 2004b) published several articles introducing the domestic pig as a model for fetal programming. They studied low and high birth weight pigs at 3 and 12 months of age for the development of hypertension, cardiovascular disease, obesity and type 2 diabetes. Growth characteristics including body weight, body length and crown rump length were taken at birth, 1, 3 and 12 months. Fractional growth rates (kg gained/d/kg BW) from 0-1 months, 0-3 months, 3-12 months and 0-12 months were determined. Low birth weight pigs had a faster fractional growth rate than high birth weight animals from 0-1 months of age, which was considered compensatory growth. At 3 and 12 months of age pigs were surgically fitted with venous catheters and intravenous glucose tolerance tests and insulin sensitivity tests were performed. There was no evidence of disease development in the pigs at 3 months of age, but at 12 months of age, the glucose and insulin areas under the curve were significantly higher in low birth weight pigs than high birth weight pigs. Low birth weight pigs also had significantly lower fasting plasma insulin at 12 months. Furthermore, the glucose area under the curve at 12 months was negatively associated with birth weight. These findings support the use of domestic pigs as a model for studying fetal programming, however, more pig studies are needed to validate the model.

**Animal Models of Fetal Programming and Insulin Resistance**

The main animals used to study fetal programming are the sheep and rat. Although both species have proven to be effective models of study, each has its drawbacks. The sheep is a well-established model for fetal human physiology. It is easy to catheterize fetal sheep and study the intrauterine environment. Therefore, the metabolic mechanisms of fetal programming can be determined. However, long-term studies of the consequences of a poor intrauterine environment are rarely performed because the sheep is a ruminant animal. Ruminants have completely different nutrient requirements, feeding behaviours and digestive physiology than humans. The rat is the most extensively used animal for studying fetal programming. Rats are small, easy to handle, maintain and house, and are a widely accepted model for studying many facets of human metabolism and physiology. Rodents are also short-lived and can be forced to develop chronic diseases in aging similar in etiology to humans. However, the rat has very different nutrient requirements and feeding behaviours than humans. Also, rats are altricial, experiencing extensive maturation postnatally during suckling, whereas, humans are precocial, meaning they have a highly developed central nervous system, cardiovascular and endocrine system at birth. Very recently the domestic pig was used as a model for fetal programming. Like rats, pigs are litter-bearing animals, which enables direct comparisons of runts to genetically similar larger littermates. Pigs demonstrate compensatory growth (Ritacco et al. 1997, Wolter et al. 2002, Poore and Fowden 2004a) and are physiologically and nutritionally very similar to humans. Moreover, the pig has been used successfully as a model for many chronic diseases such as diabetes, cardiovascular disease, hypertension, and obesity (Kjems et al. 2001, Larsen et al. 2002, Poore and Fowden 2002, Otis et al. 2003, Xi et al. 2004, Sébert et al. 2005).

## 1.4 Swine Models

Although domestic swine are physiologically very similar to humans, they do have some disadvantages. As a livestock animal, the pig has been genetically selected for rapid growth rates and protein deposition. Therefore, they are less prone to develop obesity. Other breeds of pigs, such as the miniature pig, would make an even better model for fetal programming than the domestic pig. Yucatan miniature pigs are smaller in size than domestic pigs, reaching an adult size of roughly 70 kg. Bred solely for research purposes, the miniature pig grows slowly and has been shown to develop obesity and type 2 diabetes (Larsen et al. 2002, Otis et al. 2003, Sébert et al. 2005). However, no researchers have used the miniature pig as a model for studying the early origins of adult disease.

## Chapter 2: Objectives

The primary goal of this research was to develop a miniature pig model for studying fetal programming. Our specific objectives were as follows:

1. Establish the Yucatan miniature pig as a model for compensatory growth.
2. Validate the use of the Yucatan miniature pig as a model for type 2 diabetes.
3. Determine the effects of birth weight and postnatal growth rate on the development of type 2 diabetes. (This model was also used to investigate other chronic diseases including hypertension, obesity, and cardiovascular disease but those results are not part of this thesis).
4. Determine the impact of early postnatal nutrition (i.e. sow fed vs. formula fed) on the susceptibility to type 2 diabetes.

We hypothesize that low birth weight runt pigs will undergo compensatory growth and develop signs of type 2 diabetes more readily than their larger birth weight littermates. We also hypothesize that suckled piglets will have fewer indicators of disease than either formula fed runts or large littermates.

## Chapter 3 Compensatory Growth

### 3.1 Introduction

Low birth weight infants typically undergo a period of accelerated postnatal growth, termed compensatory or catch-up growth. Epidemiological evidence has shown that low birth weight infants that experience compensatory growth are at an even higher risk for the development of chronic diseases than infants born of low birth weight alone. This recent evidence has stimulated a resurgence of interest in understanding the mechanisms of compensatory growth, which still remain unclear. The purpose of the experiments discussed in this chapter was to validate the Yucatan miniature pig as a model for compensatory growth.

### 3.2 Methods

#### Animals and housing

A total of 18 Yucatan miniature pigs were obtained from the Memorial University of Newfoundland Vivarium, where they were housed until 8 months of age. One day after a sow gave birth, the entire litter was weighed. A runt piglet was defined as weighing less than 900 grams. A same sex larger littermate weighing at least 300 grams more than the runt was chosen as a littermate control. A third littermate (sow-fed control) was selected at this time and left with the sow until weaning. We attempted for the sow-fed control to be the next largest same sex littermate, however, it was not possible in all litters. Therefore, in two of the triplets the sow-fed control is a different sex from the runt and littermate. Overall there was an equal number of males and females used in this study. The runt and larger littermate were taken from the sow at 3 days of age, allowing

for adequate colostrum intake. Piglets were housed together and provided with straw bedding and an infrared heat lamp. Milk replacer (Piglet-Gro, Grober Nutrition, Cambridge Ontario) was rehydrated and was bowl-fed ad libitum eight to ten times daily. Individual milk intake was measured and recorded after each feeding. At one month of age, piglets were adapted to standard pig chow (Eastern Co-op Pig Grower, 16% crude protein) over a 3-day period. At this time, the third (sow-fed) control piglet was introduced and also adapted to chow. Pigs were then housed in triplets, but fed separately for 5 hours daily from 12 – 5pm ad libitum. Feed intake was measured daily and animals had free access to water. The Institutional Animal Care Committee in accordance with the Canadian Council of Animal Care guidelines approved this study.

**Procedures**

Serial growth measurements were taken from birth to 8 months of age. During the milk feeding phase, body weight, snout to tail length, and abdominal circumference were measured 1-2 times weekly. After adaptation to chow, these measurements were made 1-2 times a month. The largest part of the abdomen was measured as the abdominal circumference (cm). Ten millilitre blood samples in EDTA tubes were taken via jugular venipuncture from one month until 3 months of age 1-2 times monthly; pigs were restrained in a V-trough. From 3 months until eight months of age, 20 ml blood samples in EDTA tubes were collected 1–2 times per month. Blood samples were centrifuged for 10 minutes at 4000 x g at 4 °C. The plasma was obtained and stored at –20 °C for later analysis. At roughly 8 months of age pigs were transported to the Health Sciences Center (HSC), Memorial University of Newfoundland where they remained for the remainder of the study. Shortly after arrival at the HSC, two catheters were implanted into the femoral

vein and a radiotelemeter into the femoral artery. Details of the surgical procedure will be discussed in the following chapter. Four weeks after surgery pigs were killed.

**Statistical Analysis**

All statistical analyses were performed using Graph Pad Prism 4 software. The analysis was divided into milk feeding phase (ages 3-31 d) and chow feeding phase (1-8 months). Linear regression was used to determine growth rates during both phases and slopes were compared. During the milk feeding phase, comparisons between runt, littermate and sow-fed were made by repeated measures 1-way ANOVA, with repeated measures analysis used to assess blocking by litter. Because two of the sow-fed piglets were gender mismatched to their littermates, we could not compare gender effect and litter blocking effect simultaneously (2-way ANOVA repeated measures). So to accommodate gender matching in formula-fed piglets, paired students t tests were also used to make runt and littermate comparisons. During the chow feeding phase, we expected gender blocking to have a larger effect than litter matching on growth parameters; so during this phase, differences between groups and gender were determined using non-repeated measures 2 way ANOVAs. In analyses where gender had no effect we presented the data as group means. When gender had an effect, the data was represented as figures. In all analyses, statistical significance was declared if $p < 0.05$. All data are expressed as mean, plus or minus standard deviation.

### 3.3 Results

**Growth characteristics at birth**

The average litter size was 7.0 ± 1.0 with the mean weight being 0.896 ± 0.084 kg. The body measurements of piglets one day after birth are found in Table 3.1. Runt piglets were significantly smaller in weight, length and abdominal circumference than their larger littermate and sow-fed control one day after birth. There were no significant differences in size between littermate and sow-fed controls.

Table 3.1: Body weight (kg) and linear growth and abdominal circumference (cm) (body length and abdominal circumference, cm) one day after birth in runt, littermate and sow-fed control piglets.

| | Runt | Littermate | Sow-fed Control |
|---|---|---|---|
| Body weight (kg) | 0.730 ± 0.106 [a] | 1.1097 ± 0.1337 [b] | 1.0149 ± 0.1609 [b] |
| Body length (cm) | 31.7 ± 2.7 [a] | 35.5 ± 1.5 [b] | 35.6 ± 2.1 [b] |
| Abdominal circumference (cm) | 23.1 ± 1.6 [a] | 27.6 ± 1.1 [b] | 27.4 ± 0.5 [b] |

[a] $p < 0.001$ (ANOVA)

**Growth characteristics during the milk feeding phase (3 days-1 month of age)**

**Feed intake**

Milk intake was measured in runts and littermates only, as it was not possible to quantify sow-fed control sow milk intake while with their mothers. Large littermates consumed more milk replacer than runts on a daily basis (Figure 3.1).

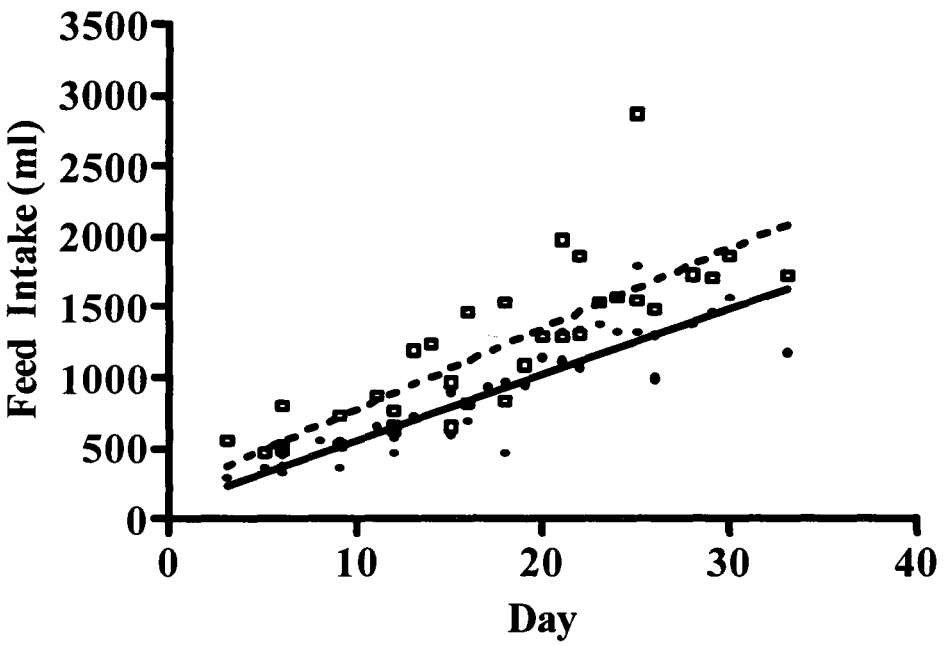


Figure 3.1: Average daily milk replacer intake (ml/d) of runt and littermate pigs during milk feeding.

- Runt
- Littermate
- Line of regression of Runt ($r^2$=0.80)
- Line of regression of Littermate ($r^2$=0.66)

Feed efficiency was calculated as the kilograms of feed required to produce one kilogram of gain. The overall average feed efficiency for the month of milk feeding was not different between runts and littermates. Therefore, weekly averages were determined to look for changes in feed efficiency over time (Table 3.2). The most efficient growth was experienced during the first week of milk feeding, and declined over time. During the second week of formula feeding only, runt piglets had significantly better feed efficiency than their littermates (p=0.03). This short period of increased nutrient efficiency is characteristic of compensatory growth.

Table 3.2: Average feed efficiency (kg feed: kg gain) from day 3-31, 3-10, 11-17, 18-24 and 25-31 in runt and littermate piglets.

| | Runt | Littermate |
|---|---|---|
| Average feed efficiency (Day 3-31) | 1.3 ± 0.2 | 1.3 ± 0.1 |
| Feed efficiency (Day 3-10) | 0.7 ± 0.2 | 0.8± 0.2 |
| Feed efficiency (Day 11-17) | 1.0 ± 0.1 [a] | 1.3 ± 0.2 [b] |
| Feed efficiency (Day 18-24) | 1.2 ± 0.1 | 1.2 ± 0.2 |
| Feed efficiency (Day 25-31) | 1.7 ± 0.3 | 1.8 ± 0.2 |

[a] p=0.03

**Growth rates during milk feeding**

During the milk-feeding phase, the growth rates of all piglets were linear (Table 3.3). Runt piglets grew at a significantly slower rate than other pigs (p=0.008) (Figure

3.2, table 3.3). Large littermates grew at the same rate as sow-fed controls. Body length and abdominal circumference also increased linearly during milk feeding (Table 3.3). The rate of length gain and rate of abdominal gain was not significantly different between pigs.

Table 3.3: Growth rates (body weight, kg/d, body length and abdominal circumference, cm/d) of runt, littermate and sow-fed control piglets during milk feeding (1st month of life)

| **Growth Rates** | Runt | Littermate | Sow-fed |
|---|---|---|---|
| Body weight (kg/d) | $0.1389 \pm 0.0060$ [a] $r^2=0.93$ | $0.1668 \pm 0.0087$ [b] $r^2=0.89$ | $0.1780 \pm 0.0118$ [b] $r^2=0.85$ |
| Body length (cm/d) | $0.74 \pm 0.05$ $r^2=0.83$ | $0.82 \pm 0.03$ $r^2=0.93$ | $0.76 \pm 0.05$ $r^2=0.88$ |
| Abdominal circumference (cm/d) | $0.71 \pm 0.03$ $r^2=0.92$ | $0.69 \pm 0.03$ $r^2=0.90$ | $0.63 \pm 0.06$ $r^2=0.81$ |

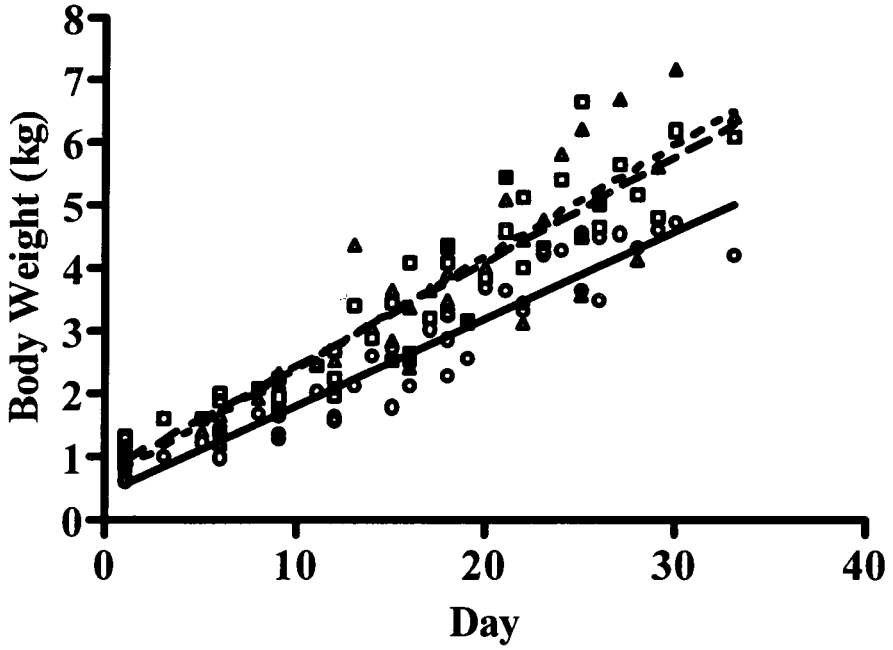


Figure 3.2: Lines of regression of runt, littermate and sow-fed control pigs for growth rate (kg/d) during milk feeding

- Runt — Runt $r^2$=0.93
- Littermate – – Littermate $r^2$=0.89
- Sow-fed ---- Sow-fed $r^2$=0.85

Runt and sow-fed piglets grew at a similar rate as a percentage of their initial body weight. Large littermates grew significantly slower as a percentage of their initial weight compared to runt and sow-fed controls (p=0.001) (Figure 3.3). When examining body length and abdominal circumference as a percentage of initial values, there were no significant differences between runts, littermate and sow-fed controls (Table 3.4).

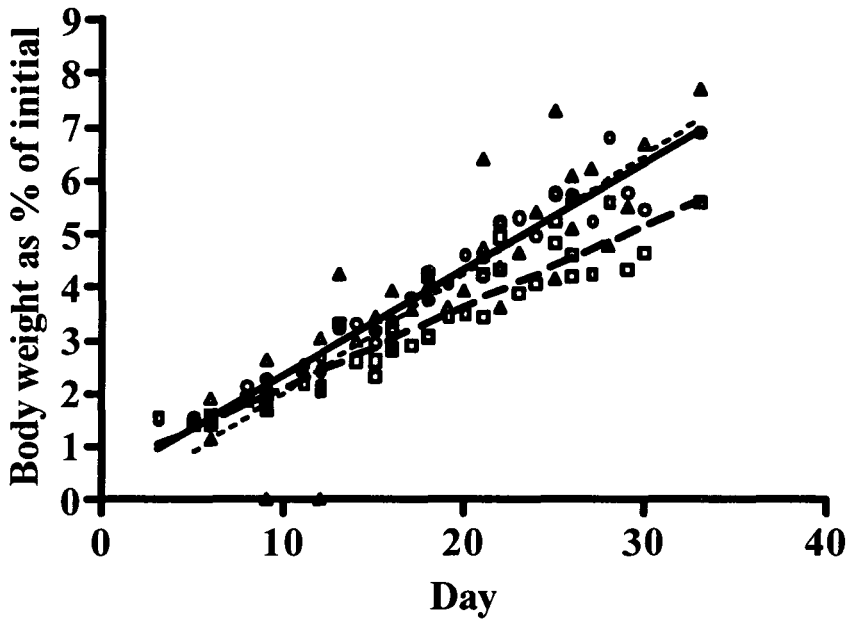


Figure 3.3: Relative rate of body weight gain (rate of body weight gain as a percentage of initial body weight) expressed as line of regression in runt, littermate and sow-fed control pigs.

- ○ Runt —— Runt $r^2$=0.95
- □ Littermate – – Littermate $r^2$=0.89
- ▲ Sow-fed ····· Sow-fed $r^2$=0.75

Table 3.4: Relative rate of body weight, body length and abdominal circumference gain in runts, littermates and sow-fed control piglets during milk feeding. The rate of gain expressed is as a percentage of initial weight, length and circumference measurements.

| | Runt | Littermate | Sow-fed |
|---|---|---|---|
| Relative rate of body weight gain | $0.20 \pm 0.01^{b}$ $r^2=0.95$ | $0.22 \pm 0.02^{a}$ $r^2=0.89$ | $0.15 \pm 0.01^{b}$ $r^2=0.75$ |
| Relative rate of body length gain | $0.02 \pm 0.001$ $r^2=0.80$ | $0.02 \pm 0.001$ $r^2=0.91$ | $0.03 \pm 0.006$ $r^2=0.49$ |
| Relative rate of body abdominal circumference gain | $0.03 \pm 0.002$ $r^2=0.76$ | $0.02 \pm 0.002$ $r^2=0.83$ | $0.02 \pm 0.003$ $r^2=0.80$ |

Data within a row [a] $p>0.05$

**Absolute growth during milk feeding.**

In terms of absolute body weight and body length runts remained significantly smaller than other piglets at week 1, 2, 3, 4 (Table 3.5, 3.6). However, by four weeks the there was no significant difference in abdominal circumference between runts and other pigs ($p=0.06$) (Table 3.7).

Table 3.5: Average weekly absolute body weights, kg (day 3-10, 11-17, 18-24, 25-31) of runt, littermate and sow-fed piglets during milk feeding.

| Average Body Weight (kg) | Runt | Littermate | Sow-fed |
|---|---|---|---|
| Week 1 (Day 3-10) | 1.1863 ± 0.1559 [a] | 1.7418 ± 0.2385 [b] | 1.6068 ± 0.4156 [b] |
| Week 2 (Day 11-17) | 1.9460 ± 0.3901 [a] | 2.7575 ± 0.8369 [b] | 3.0064 ± 0.8492 [b] |
| Week 3 (Day 18-24) | 2.9102 ± 0.6169 [a] | 3.8698 ± 0.9688 [b] | 3.9121 ± 0.9421 [b] |
| Week 4 (Day 25-31) | 4.1005 ± 0.4723 [a] | 5.3765 ± 0.7964 [b] | 5.2919 ± 1.1096 [b] |

Table 3.6: Average weekly absolute body lengths, cm (day 3-10, 11-17, 18-24, 25-31) of runt, littermate and sow-fed piglets during milk feeding.

| Average Body Length (cm) | Runt | Littermate | Sow-fed |
|---|---|---|---|
| Week 1 (Day 3-10) | 34.5 ± 2.3 [a] | 38.5 ± 2.4 [b] | 36.8 ± 2.8 [b] |
| Week 2 (Day 11-17) | 39.5 ± 4.9 [a] | 43.3 ± 4.1 [b] | 43.5 ± 5.6 [b] |
| Week 3 (Day 18-24) | 45.7 ± 2.4 [a] | 51.0 ± 2.8 [b] | 50.4 ± 4.2 [b] |
| Week 4 (Day 25-31) | 51.3 ± 2.8 [a] | 58.1 ± 2.6 [b] | 55.8 ± 1.8 [b] |

Table 3.7: Average weekly absolute abdominal circumference, cm (day 3-10, 11-17, 18-24, 25-31) of runt, littermate and sow-fed piglets during milk feeding

| Average Abdominal Circumference (cm) | Runt | Littermate | Sow-fed |
|---|---|---|---|
| Week 1 (Day 3-10) | 25.6 ± 0.9 [a] | 29.3 ± 1.4 [b] | 27.5 ± 0.7 [b] |
| Week 2 (Day 11-17) | 31.0 ± 3.0 [a] | 35.0 ± 3.9 [b] | 34.8 ± 3.5 [b] |
| Week 3 (Day 18-24) | 38.0 ± 2.2 [a] | 42.1 ± 3.4 [b] | 39.7 ± 2.6 [b] |
| Week 4 (Day 25-31) | 41.7 ± 1.6 [b] | 45.2 ± 2.2 [b] | 43.7 ± 3.2 [b] |

**Growth characteristics during the chow feeding phase (1 – 9 months of age)**

**Feed intake**

Average daily feed intake (kg/d) did not significantly differ between pigs throughout the study. Upon visual inspection, it increased linearly from day 40 until approximately day 110 of study. Feed intake then became more variable as animals reached sexual maturity (Figure 3.4). Feed efficiency (kg feed per kg gain) steadily declined over time and was not different between groups of animals (Figure 3.5).

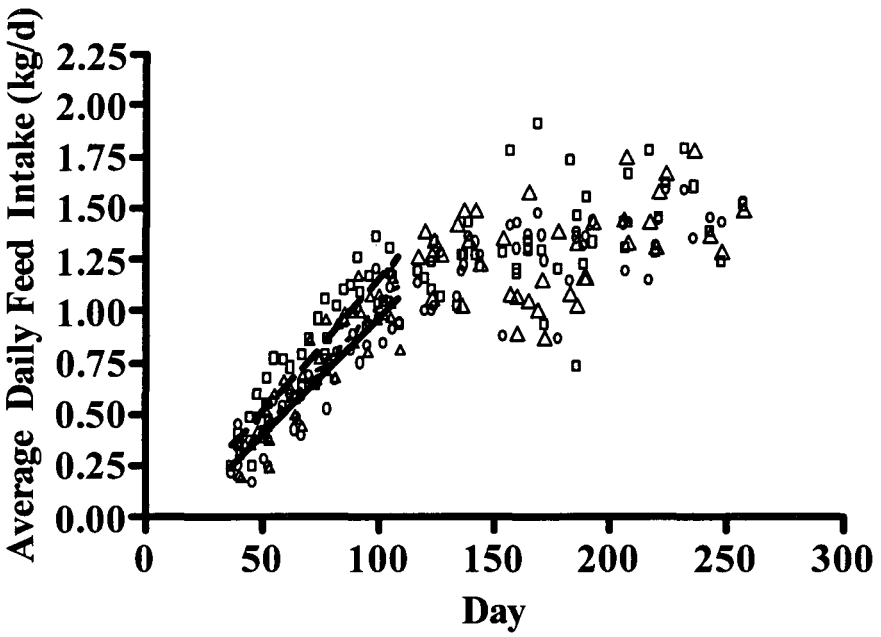


Figure 3.4: Average daily feed intake (kg/d) from adaptation to chow (day 40) to the end of study in runt, littermate and sow-fed controls.

- ○ Runt — Runt $r^2$=0.83
- □ Littermate - - Littermate $r^2$=0.82
- △ Sow-fed ····· Sow-fed $r^2$=0.80

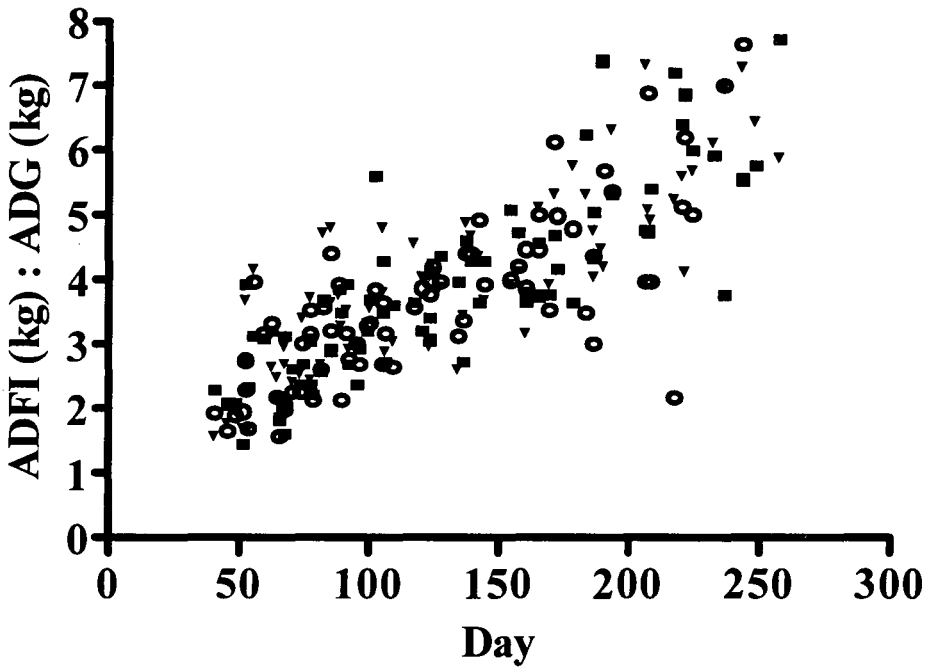


Figure 3.5: Average feed efficiency (kg feed per kg gain) during chow feeding in runt, littermate and sow-fed control pigs.
ADFI = Average daily feed intake
ADG = Average daily gain

- Runt $r^2 = 0.68$
- Littermate $r^2 = 0.68$
- Sow-fed $r^2 = 0.62$

**Growth rates**

Weight gain, body length and abdominal circumference once the piglets were adapted to chow increased linearly throughout the study (Table 3.8). Runt pigs grew at a significantly slower rate than other pigs from day 40 –110 of study (p=0.002) but not from day 110 until the end of study (Figure 3.6). Body length and abdominal

circumference increased significantly more rapidly from day 40 until 110 than from day 110 until the end of the study ($p<0.0001$) (Figure 3.7, 3.8). There were no differences in these indices between runts, littermates or sow-fed controls at either time period.

Table 3.8: Rate of body weight gain (kg/d), body length and abdominal circumference gain (cm/d) from day 40 –110 and 110 until the end of study in runt, littermate and sow-fed pigs

| | Runt | Littermate | Sow-fed |
|---|---|---|---|
| Growth rate (kg/d) | | | |
| Day 40-110 | $0.270 \pm 0.011$ [a] | $0.321 \pm 0.011$ [b] | $0.309 \pm 0.011$ [b] |
| | $r^2=0.94$ | $r^2=0.95$ | $r^2=0.95$ |
| Day 110-220 | $0.301 \pm 0.016$ | $0.301 \pm 0.011$ | $0.277 \pm 0.017$ |
| | $r^2=0.89$ | $r^2=0.95$ | $r^2=0.87$ |
| Body length gain (cm/d) | | | |
| Day 40 –110 | $0.5 \pm 0.022$ | $0.5 \pm 0.02$ | $0.6 \pm 0.02$ |
| | $r^2=0.97$ | $r^2=0.94$ | $r^2=0.95$ |
| Day 110 – 220 | $0.2 \pm 0.01$ | $0.2 \pm 0.02$ | $0.2 \pm 0.02$ |
| | $r^2=0.89$ | $r^2=0.71$ | $r^2=0.82$ |
| Abdominal Circumference gain (cm/d) Day 40-110 | $0.4 \pm 0.02$ | $0.4 \pm 0.02$ | $0.4 \pm 0.02$ |
| | $r^2=0.85$ | $r^2=0.93$ | $r^2=0.91$ |
| Day 110-220 | $0.2 \pm 0.02$ | $0.19 \pm 0.01$ | $0.17 \pm 0.03$ |
| | $r^2=0.65$ | $r^2=0.75$ | $r^2=0.56$ |

$p=0.002$

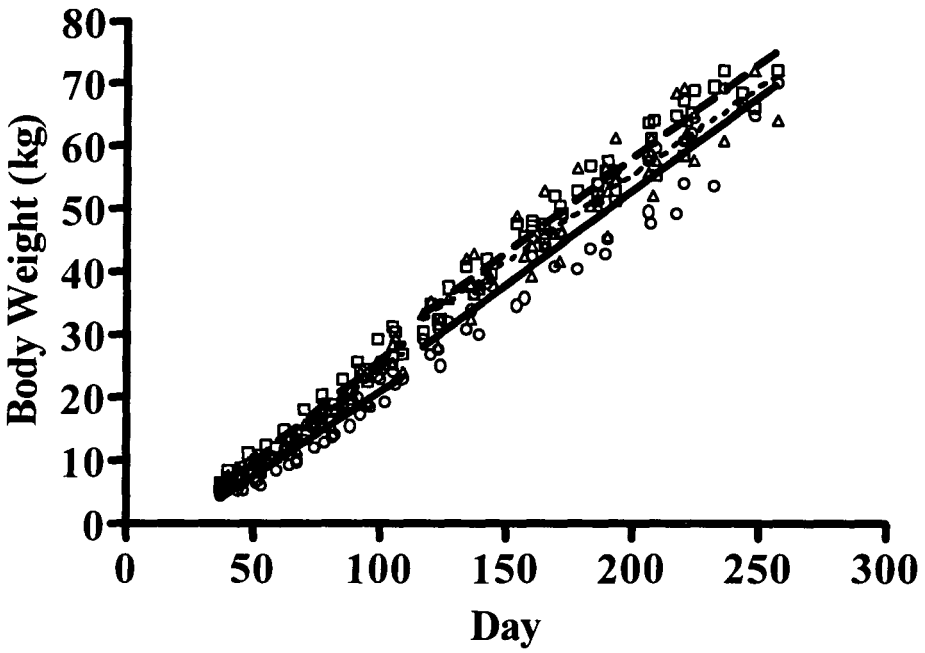


Figure 3.6: Lines of regression for rate of body weight gain (kg/d) during chow feeding in runt, littermate and sow-fed pigs

- ○ Runt — Runt $r^2 = 0.89$
- □ Littermate – – Littermate $r^2 = 0.95$
- △ Sow-fed ····· Sow-fed $r^2 = 0.87$

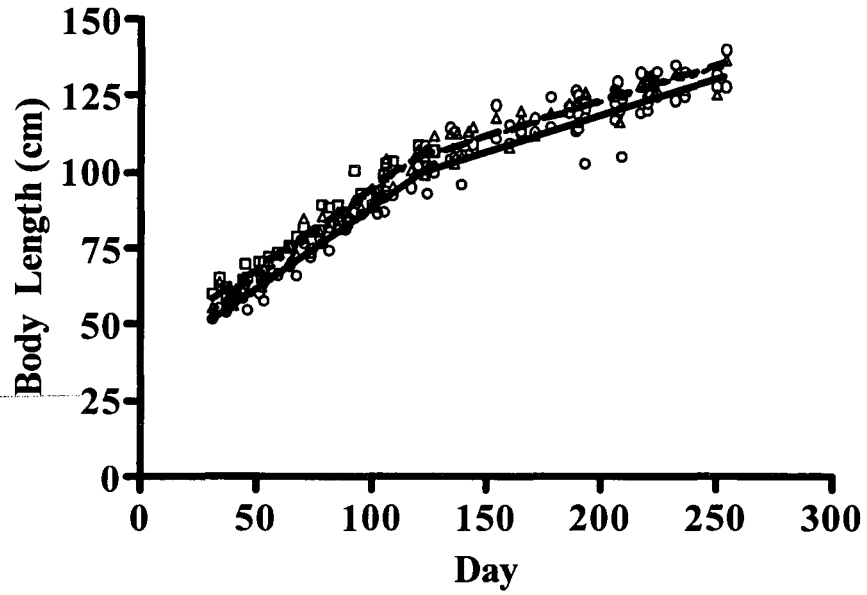


Figure 3.7: Lines of regression for the rate of body length gain (cm/d) from adaptation to chow to day 110 and day 110 until the end of study in runt, littermate and sow-fed control pigs

—— Runt (d40 - d110, $r^2 = 0.97$; d110 to end, $r^2 = 0.89$)

– – Littermate (d40 - d110, $r^2 = 0.94$; d110 to end $r^2 = 0.71$)

----- Sow-fed (d40 - d110, $r^2 = 0.95$; d110 to end $r^2 = 0.82$)

○ Runt

□ Littermate

△ Sow-fed

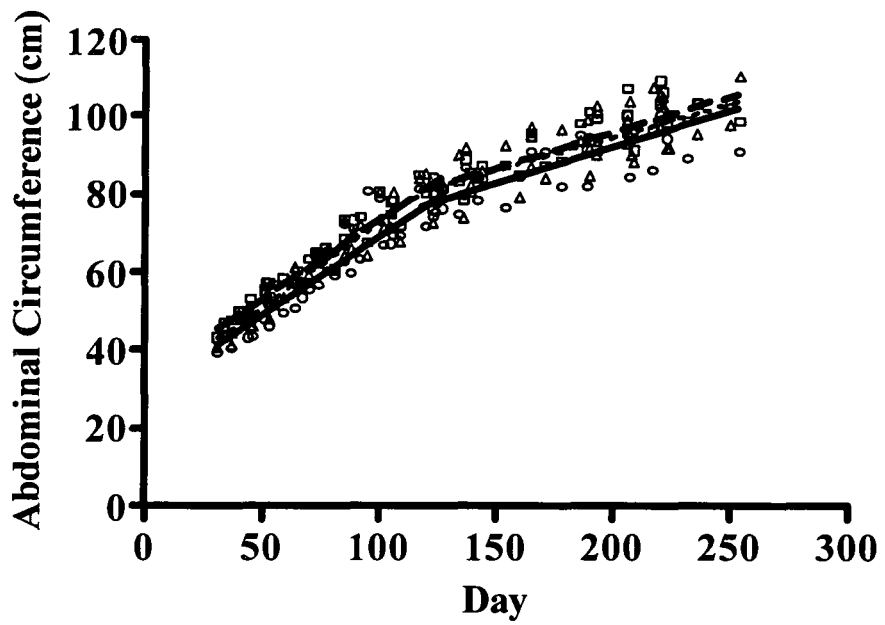


Figure 3.8: Lines of regression for the rate of abdominal circumference gain (cm/d) from adaptation to chow to day 110 and day 110 until the end of study in runt, littermate and sow-fed control pigs

—— Runt (d40 - d110, $r^2 = 0.85$; d110 to end, $r^2 = 0.66$)

– – · Littermate (d40 - d110, $r^2 = 0.93$; d110 to end $r^2 = 0.75$)

····· Sow-fed (d40 - d110, $r^2 = 0.91$; d110 to end $r^2 = 0.56$)

- ◦ Runt
- ▫ Littermate
- ▵ Sow-fed

**Absolute Growth.**

Absolute growth was examined at two time points, day 110 and day 220 (Tables 3.9, 3.10). In analyses where gender had no effect the data was presented as group means. When gender had a significant effect, the data was presented as figures (Figures 3.9, 3.10, 3.11). At day 110, the pigs approximately 3.5 months of age and their rate of

length and abdominal growth was just beginning to slow down. Most animals reach sexually maturity around 3.5 - 4 months of age (i.e. when we observed standing heat in females). Day 220 was toward the end of the study and the pigs were roughly seven months of age. Runts were significantly smaller than other pigs in terms of body weight and body mass index at day 110 of study ($p=0.0048$). Abdominal circumference was not different between groups of pigs, but was greater in females than males ($p=0.0101$) (Figure 3.9). Runts were significantly smaller in terms of body length than other pigs ($p=0.005$). Female large littermates were significantly shorter in body length than male littermates ($p=0.02$) (Figure 3.10). Towards the end of study at day 220, there were no differences in body weight, length, body mass index or abdominal circumference between pigs. Abdominal circumference was greater in females than males ($p<0.0001$) (Figure 3.11).

Table 3.9: The effects (p value) of group (runt, littermate, sow-fed) and gender on absolute body weight (kg), body length and abdominal circumference (cm) and body mass index ($kg/m^2$) on day 110

| | Runt | Littermate | Sow-fed | Group p value | Gender p value | Group X Gender p value |
|---|---|---|---|---|---|---|
| Body weight (kg) | 22.81 ± 2.00 | 27.53 ± 2.74 | 26.42 ± 2.16 | 0.005 | 0.16 | 0.13 |
| Body length | 90.1 ± 2.9 | 96.7 ± 6.0 | 96.2 ± 4.3 | 0.005 | 0.008 | 0.02 |

| | | | | | | |
|---|---|---|---|---|---|---|
| (cm) | | | | | | |
| Abdominal circumference (cm) | 70.8 ± 4.8 | 75.9 ± 3.4 | 74.4 ± 5.1 | 0.08 | 0.01 | 0.39 |
| Body mass index ($kg/m^2$) | 22.8 ± 1.0 | 27.5 ± 2.9 | 26.4 ± 0.8 | 0.005 | 0.16 | 0.13 |

Table 3.10: The effects (p value) of group (runt, littermate, sow-fed) and gender on absolute body weight (kg), body length and abdominal circumference (cm) and body mass index ($kg/m^2$) on day 220

| | Runt | Littermate | Sow-fed | Group p value | Gender p value | Group X Gender p value |
|---|---|---|---|---|---|---|
| Body weight (kg) | 58.60 ± 5.75 | 64.86 ± 3.55 | 63.34 ± 4.58 | 0.09 | 0.60 | 0.21 |
| Body length (cm) | 124.3 ± 4.5 | 129.4 ± 3.7 | 129.1 ± 2.3 | 0.051 | 0.46 | 0.25 |
| Abdominal circumference (cm) | 97.3 ± 6.3 | 102.1 ± 4.9 | 99.8 ± 7.0 | 0.10 | $p<0.0001$ | 0.48 |
| Body mass index ($kg/m^2$) | 58.6 ± 0. 2 | 64.9 ± 2.2 | 63.3 ± 4.7 | 0.09 | 0.60 | 0.21 |

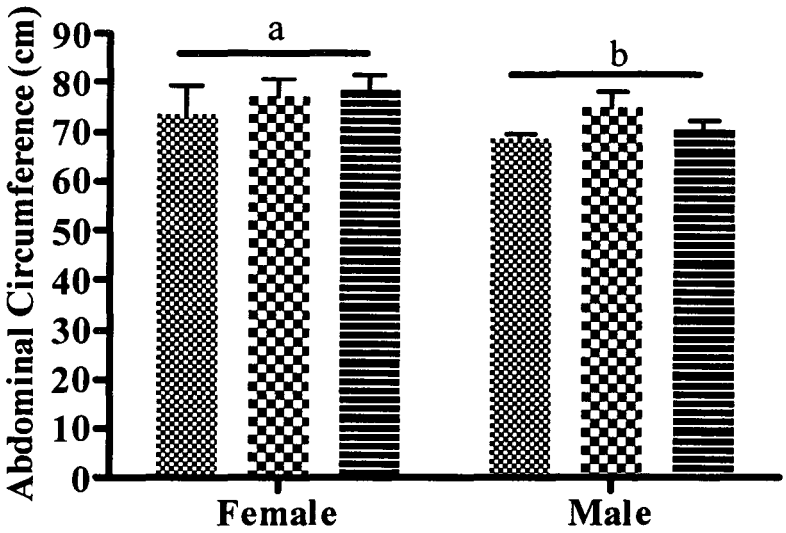


Figure 3.9: Abdominal circumference (day 110) in runt, littermate and sow-fed control pigs. Female pigs had statistically significantly higher abdominal circumference at day 110 than male pigs (p=0.010). There were no statistically significant differences between runt, littermate and sow-fed control pigs (p=0.08)

Runt

Littermate

Sow-fed

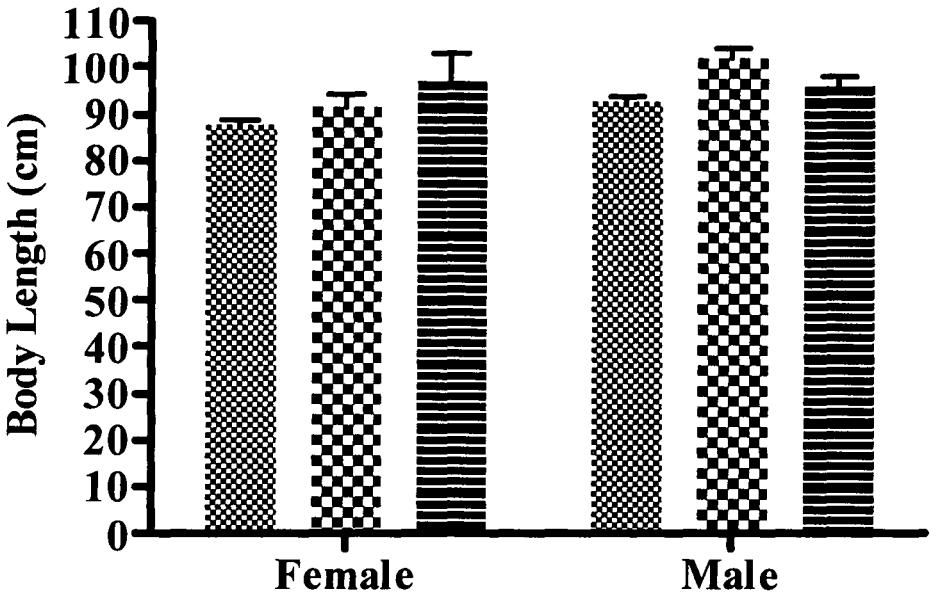


Figure 3.10: Body length (cm) at day 110 in runt, littermate and sow-fed control pigs. Runts were significantly smaller than other pigs (p=0.005). Female large littermates were significantly shorter in body length than male littermates (p=0.02)

Runt

Littermate

Sow-fed

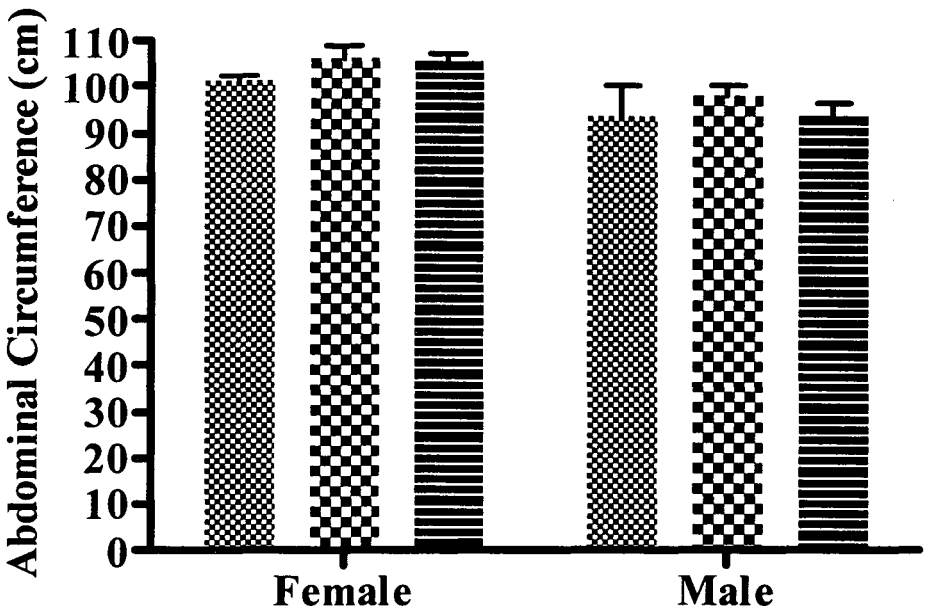


Figure 3.11: Abdominal circumference (cm) on day 220 in runt, littermate and sow-fed control pigs. Female pigs had significantly greater abdominal circumference (cm) than male pigs ($p<0.0001$).

Runt

Littermate

Sow-fed

### 3.4 Discussion

Low birth weight is often the result of an insufficient supply of nutrients to the fetus. In litter-bearing species such as the pig, naturally occurring low birth weight neonates (runts) are born as small as one-third the size of their littermates. Runting is thought to be caused by placental insufficiency resulting in decreased oxygen and nutrient delivery to the fetus (Foxcroft et al., 2006). Often, runts do not gain access to an adequate teat at birth due to litter competition and as a result, have lower feed intakes, slower growth and decreased survival rates. In addition to limited postnatal nutrient supply, runt piglets are born with less muscle fibres leading to slower growth rates making them less capable to achieve the same size as their littermates (Foxcroft et al., 2006). Powell and Aberle found that runt piglets grow less efficiently and produce carcasses with more fat than their littermates (Powell and Aberle, 1980). Because runt piglets in our study were provided ad libitum nutrients via individual formula feeding (eliminating competition), we did not expect to see this typical marked decrease in growth rate. Therefore, in this chapter we wanted to examine and compare the growth patterns of well-nourished runt piglets from birth to early adulthood to their larger littermates.

In response to nutritional insults in utero, neonates will undergo a period of accelerated postnatal growth, termed compensatory or catch-up growth. During this period, nutrient efficiency (utilization) is increased allowing for rapid growth. However, this increased efficiency is transient and once over, growth continues along a trajectory according to age and size. If the animal continues to receive abundant nutrients once the period of compensatory growth is complete, the excess nutrients will be catabolized and deposited as fat. We expected runt piglets to experience a period of increased nutrient

efficiency during milk feeding, but not completely catch up in size to their littermates as adults. Since piglets were being fed ad libitum throughout the study we expected runt piglets to deposit fat once the period of compensatory growth finished. Sow-fed controls were used as a reference group of normal growth and development. We expected large littermates to display similar growth patterns to that of sow-fed control piglets.

**Milk Feeding Phase**

Since compensatory growth typically occurs in early postnatal life, our results were divided into "milk feeding phase" and "chow feeding phase" and analyzed separately. During milk feeding, runts grew in weight, length and abdominal circumference at a significantly slower rate than other piglets, despite being fed ad libitum. Similar growth response in domestic runt piglets fed milk replacer ad libitum has been observed in other studies (Ritacco, Wolter 2002). Poore and Fowden (2004) found that lower birth weight (not runts) domestic piglets grew at a significantly slower rate than higher birth weight piglets during the first month of life. Although, the slower growth rate in our runts was expected, the reason is unknown. It is possibly due to reduced absorptive capacity, meaning the runt's maximal absorptive capacity is proportionately lower than their larger littermates. It could also have been due to reduced muscle fibres in runts. The longissimus dorsi muscle and gastrocnemius muscle of the pigs were obtained at necropsy as adults. Measuring the number of muscle fibres in these muscles could be useful to better understand early postnatal growth patterns in miniature pigs.

The growth rates (milk feeding) of runts and sow-fed controls relative to their birth weights were similar, whereas, larger littermates grew more slowly relative to their birth weight. A faster relative rate of growth indicates catch up growth. Poore and Fowden (2004) also found that low birth weight pigs had a significantly quicker relative rate of growth compared to high birth weight pigs during the first month of life, indicative of compensatory growth. The indication of catch-up growth in sow-fed piglets was surprising, but might be due to the fact that these piglets had greater access to more milk from the sow after the runt and large littermates were removed. Litters in miniature pigs are smaller (i.e. 5-7 piglets) than those in domestic pigs (10-15) and so removal of two piglets would lead to greater milk availability for the remaining piglets. Indeed, some litters were relatively small (3-5) and in other litters, some of the remaining piglets were taken for other studies, leaving only the sow-fed control piglet with the mother. This late removal of piglets could lead to a catch-up growth scenario for the remaining sow-fed control in this study.

Absolute weekly body weights, lengths and abdominal circumferences were not different between large littermates and sow-fed controls. Runt piglets remained significantly smaller than other piglets in terms of length and weight throughout the milk feeding period. This observation was expected, as runts do not usually completely catch up in body size to their littermates. At 4 weeks of age, there was no significant difference in abdominal circumference between groups, suggesting that runt piglets were using excess nutrients for fat deposition. In order to characterize growth more accurately, more sophisticated in vivo measures should be made. For example, dual energy X-ray absorptiometry (DEXA) could be used to determine the percentage of fat and lean mass

of the animals in vivo over time. Future studies using serial slaughter and subsequent carcass composition analysis could also be employed to more accurately determine the composition of growth in these animals over time.

During milk feeding, runt piglets experienced a period of increased feed efficiency during the third week of milk feeding, characteristic of compensatory growth. Ritacco and colleagues (1997) observed a similar period of nutrient efficiency in domestic piglets during the first two weeks of milk feeding. Establishing compensatory growth is important in this model, because in epidemiological studies, humans who are born small and experience compensatory growth are at an even higher risk for the development of chronic diseases than infants who are born of low birth weight alone.

**Chow Feeding Phase**

During the chow feeding phase, we expected pigs to grow linearly throughout 'childhood' and then to level off towards the end of the study once the pigs were past sexual maturity. Few studies have examined the growth of runt pigs into adulthood, as there are few economic benefits to doing so in swine production. Of the few studies that have examined growth to adulthood in domestic swine, runt piglets do not completely catch up in body size to their littermates (Powell et al, 1980, Wolter et al 2002). Therefore, we expected runt piglets to remain somewhat smaller than their littermates throughout the study.

Growth continued linearly in two phases throughout the entire study, with no differences between groups. Body length and abdominal circumference during the chow feeding phase was rapid until approximately 3.5 months of age and then slowed from 4 months until the end of the study (9 months). Feed efficiency steadily declined with age.

As expected, large littermates and sow-fed controls displayed similar growth patterns. The growth pattern displayed by these pigs is similar to that of humans. As the animals aged, their feed efficiency worsened, they stopped growing in length and continued gaining weight, likely as fat. In order to better describe the growth pattern, determining the composition of gain is necessary. It would have been useful to take DEXA measurements throughout the chow feeding phase to characterize the composition of growth throughout this period. At necropsy the viscera and carcasses were collected separately for composition analysis. Once these results have been compiled, the percentage lean mass, fat mass and bone ash will be identified, allowing us to confirm the composition of gain for the entire protocol.

By the end of the study, there were no significant differences in body size between runts and their littermates. This finding was unexpected, as runts typically do not completely catch up in body size to their littermates, but rather usually maintain a growth trajectory based on their weaning size. Wolter and colleagues (2002) examined the growth patterns of low and high birth weight domestic pigs fed ad libitum from 3 days of age until market weight (110 kg). They found that low birth weight pigs took significantly more days to reach market weight than high birth weight pigs. However, unlike the Wolter study (2002) we intentionally removed the runts from the sow to allow ad libitum feeding and catch up growth. When runts are left on the sow they usually cannot compete for a teat. Moreover, market weight was achieved at approximately 5 months of age, much younger than the pigs used in our study. Poore and Fowden (2004) compared the growth patterns of feed-restricted high and low birth weight pigs and found no difference in body weight between groups at one year of age. The pigs in their study

were at a similar stage of development (past sexual maturity) as our pigs, making their results more comparable. It is likely that the reason for complete catch up growth in our pigs was due to a combination of ad libitum feeding, decreased feed efficiency and increased fat deposition with age.

It is difficult to draw direct comparisons between the Yucatan miniature pigs used in this study to domestic breeds of pigs. Domestic breeds have been genetically selected for fast growth rates and protein deposition, whereas, miniature swine have been developed as a research model with a demonstrated propensity to obesity. Few studies carefully detailing the characteristics and composition of growth in miniature pigs are available. However, the results from this study support the use of the miniature pig as a model for human growth.

Epidemiological studies have shown that low birth weight infants who experience compensatory growth are more likely to develop obesity later in life (Taylor and Poston 2007). In pigs at 9 months of age (i.e. young adulthood), there were no differences in abdominal circumference between runts and larger pigs. Females had a significantly higher abdominal circumference than males. However, abdominal circumference is a rough estimate of obesity much like waist circumference in humans. In order to determine if our pigs developed obesity, carcass composition analysis will be completed. These results will reveal the degree of visceral obesity, which is considered a major risk factor for diabetes and cardiovascular disease in humans.

Studies in sheep and rats have suggested that appetite may be programmed in utero leading to hyperphagia and obesity in later life (Langley-Evans et al 2005). We measured feed intake throughout the entire study but the results were difficult to interpret.

From adaptation to chow (5 weeks of age) until roughly 4 months of age (sexual maturity), feed intake increased linearly with no differences between groups. After sexual maturity, feed intake results were difficult to interpret, as they were highly variable. Feed consumption in females was greatly influenced by their ovulation cycles. When in heat, females did not consume as much feed as when they were not in heat. Male pigs consumed roughly the same amount of feed daily once past sexual maturity. However, feed intake was influenced by social behaviours, such as fighting for status among the group. Stress also influenced feed intake in the pigs. Animals subjected to stress, for example during blood sampling, did not consume as much food after the procedure. These are simply observations; a more systematic method of quantifying the factors affecting feed intake should be developed. Another factor affecting feed intake in swine is temperature. In cold temperatures, pigs tended to consume more feed than in warm temperatures. Since the housing facility did not have tightly controlled temperature environment, feed intake results were likely affected by environment. Based on our results, runt piglets did not appear to be hyperphagic as observed in other animal models, although our data were probably too variable to detect modest differences. We found no evidence to support that appetite was programmed in utero given our measurement protocols.

**Conclusion**

It is not surprising that the Yucatan miniature pig proved to be a successful model for studying compensatory growth, as the domestic pig is already a well-characterized and established model of compensatory growth in the field of animal science (Foxcroft et al., 2006, Mitchell, 2007). Fetal programming has brought about a resurgence of interest

in compensatory growth in the human population. Low birth weight infants who experience compensatory growth appear to be at an even higher risk for later disease development than if they were born of low birth weight alone. In animal models of fetal programming such as the rat and pig, accelerated growth postnatally correlates to markers of diabetes (Langley-Evans et al 2005). The following chapter explains the relationships between birth weight and postnatal growth rates and markers of type 2 diabetes Yucatan miniature pigs.

## Chapter 4.0 Diabetes

### 4.1 Introduction

Diabetes Mellitus is a metabolic disease characterized by a dysregulation in glucose, insulin and lipid metabolism. The most prominent types of diabetes are insulin dependent diabetes mellitus (type 1 diabetes) and non-insulin dependent diabetes mellitus (type 2 diabetes). Type 1 diabetes is an autoimmune disease causing destruction of the pancreatic beta cells. Patients with type 1 diabetes are unable to produce insulin resulting in hyperglycemia. These patients require insulin therapy to keep them in euglycemia. Ninety percent of people suffering from diabetes have type 2 diabetes (World Health Organization). The metabolic defect causing type 2 diabetes is unknown but insulin secretory capacity and mass of pancreatic beta cells are often reduced (Donath and Halban 2004). Insulin resistance typically precedes type 2 diabetes.

Insulin resistance refers to the impaired glucose uptake by peripheral tissues. When insulin resistant, peripheral tissues are no longer sensitive to insulin causing impaired glucose uptake and utilization, leaving cells starved for glucose. The pancreas compensates by increasing insulin production and secretion causing hyperinsulinemia. The pancreas cannot keep up with the insulin demand and insulin production is impaired, resulting in hyperglycemia and type 2 diabetes. The cause of insulin resistance is unknown. Insulin resistance is considered a risk factor for diabetes development and is one of the main components of the metabolic syndrome.

The metabolic syndrome was first defined in 1988 by Reaven to be a dysregulation in glucose, insulin and lipid metabolism combined with hypertension.

Since then, the definition has been expanded to include obesity (Desroches and Lamarche 2007). Extensive research into the metabolic syndrome has identified it as a major risk factor for diabetes and cardiovascular disease, gaining prevalence worldwide (Ardern and Janssen 2007). Although the epidemiology of the metabolic syndrome is well characterized, the metabolic mechanisms are unclear. Currently there are several definitions for diagnosing of the metabolic syndrome that all include varying combinations of hyperinsulinemia, hyperglycemia, hypertriglyceridemia, obesity and hypertension. Identifying components of the metabolic syndrome is useful for the prediction of diabetes and cardiovascular disease development.

There are several tests to measure symptoms of diabetes and the metabolic syndrome. Fasting plasma analysis of glucose, insulin and lipids is a simple, quick and inexpensive method of identifying symptoms such as hyperglycemia, hyperinsulinemia and dyslipidemia. The findings from fasting plasma analysis may warrant the use of more sophisticated in vivo measurements of glucose and lipid metabolism such as glucose tolerance tests and insulin sensitivity tests.

Glucose tolerance tests measure the pancreatic beta cell's responsiveness to glucose. The oral glucose tolerance test is used to diagnose diabetes in humans. The individual consumes a glucose solution and a glucose concentration in the plasma is measured over a 2-hour period. If plasma glucose is still elevated (>11 mmol/L) at 2 hours, then the individual is considered diabetic (World Health Organization, 2006). Oral glucose tolerance tests can be impractical to perform in animals because animals may not voluntarily consume the glucose dose orally. Therefore, the intravenous glucose test has been used. A glucose bolus is given intravenously and glucose clearance from the plasma

is measured. The outcomes in this test respond much more quickly as there are no effects of gastric emptying. The rate of glucose clearance is a measure of how responsive the pancreatic beta cells are to glucose. Slower rates of glucose clearance indicate impaired glucose tolerance.

Insulin sensitivity tests measure insulin-stimulated glucose uptake by peripheral tissues, which is a measure of insulin sensitivity (Cobelli et al 2007). A large bolus of insulin is given and glucose clearance from the plasma is measured. The rate of glucose clearance from the plasma is a measure of insulin-stimulated glucose uptake. Slower rates of clearance suggest the individual is insulin sensitive which indicates insulin resistance. The gold standard method for assessing glycemic status is the hyperinsulinemic eugylcemic clamp method. In this method, somatostatin is infused to inhibit endogenous insulin secretion, insulin is continuously infused to create hyperinsulinemia, and glucose is infused and frequently and rapidly measured in the blood in order to keep the individual in euglycemia (Cobelli et al 2007). The rate of glucose infusion needed to maintain steady state is a direct measurement of whole body glucose uptake, which describes the individual's insulin sensitivity. The clamp method has been used successfully in several species including humans, rats and pigs (Cobelli et al 2007). However, this method is very expensive and requires trained personnel.

Several animal models have been used to study various aspects of diabetes. Rats and mice are the most extensively used animal models. Type 1 diabetic rat and mice models include spontaneous diabetic strains, chemically induced diabetes and transgenic models. Transgenic and knockout models have provided great insight into the genetic causes of type 1 diabetes (Gannon 2001). Type 2 diabetic rat and mice models are well

characterized and include transgenic models, chemical induction, high fat feeding, overfeeding, and fetal programming. Rat and mice models are advantageous for studying type 2 diabetes as they have a shorter life span, are small and easy to handle and are a well-established model for studying human physiology. However, rodents have different feeding behaviours, nutrient requirements and pancreatic development than humans.

The miniature pig is a more recent model for studying diabetes (Bellinger et al. 2006). The pig makes an excellent model for studying the metabolic mechanisms of diabetes because of their similar physiology, feeding habits (omnivores) and nutrient requirements to humans. Also, the pig's pancreas resembles the human's in size, shape, position and function (Larsen and Rolin 2004). The majority of studies using miniature pigs focus on therapeutic treatments for type 1 diabetes (Larsen and Rolin 2004) and chemical induction of type 1 diabetes is the most common technique. Type 2 diabetic pig models are not as common. Unlike rat strains, there are few reported cases of spontaneous diabetes in pigs. This finding is likely due to the expense and resources associated with housing adult pigs. Recently, researchers have used high fat feeding and overfeeding to induce obesity in miniature pigs, accelerating diabetes development (Sébert et al. 2005). Poore and Fowden (2002) observed impaired glucose tolerance at 1 year of age in low birth weight pigs. Their findings suggest that low birth weight may accelerate diabetes development in pigs. The potential use of the pig as a model for type 2 diabetes warrants further research.

## 4.2 Chapter Objectives

1. Validate the use of the miniature pig as a model for type 2 diabetes.
2. Determine the effects of birth weight and postnatal growth rate on the development of type 2 diabetes
3. Determine the impact of early postnatal nutrition and mode of feeding on the susceptibility to type 2 diabetes.

## 4.3. Methods

**Animals and housing.**

For detailed information on animals and housing see Chapter 3: Compensatory Growth.

**Oral Glucose Tolerance Test (OGTT)**

At approximately six months of age, an oral glucose tolerance test was performed after an overnight fast. Ear pricks were made with a lancet and blood glucose was measured instantly with an Ascensia Contour blood glucose meter (Bayer, Toronto ON). Two fasting measurements were made before administering 2 g/kg D-Glucose (Sigma) dissolved in 100 ml of tap water via gavage. Ear prick glucose measurements were made every 10-15 minutes for roughly 2.5 hours until blood glucose values returned to fasting.

**Surgical Procedures.**

At approximately eight months of age, animals were transported to the Health Sciences Center at least one day before surgery and were fasted overnight. Pigs were anaesthetized using an induction dose of 20 mg/kg ketamine hydrochloride (Ketalean, Bimeda-MTC Cambridge ON) and 2 mg/kg xylazine (Rompum, Bayer Toronto ON) and

maintained with 0.5 – 1.5 % halothane gas and 3/2 oxygen/nitrous oxide. A small incision was made on the inside of the left leg in order to isolate the femoral vessels. Two 2.4 meter tygon catheters, internal and outer diameters of 0.040 and 0.070 (Norton Performance Plastics, Akron Ohio), respectively, were inserted into the femoral vein and advanced to the inferior vena cava. Catheters were tunnelled under the skin and exteriorized by a small incision on the animal's back between the shoulder blades. The catheter of a TA11PA-D70 radiotelemeter implant (cat # 270 0044 835, Data Sciences International, St. Paul MN) was inserted into the femoral artery and advanced to the femoral artery. The telemeter body was implanted under the skin between the peritoneum and inner thigh. Before surgery and two days immediately following surgery, 0.067 mL/kg trimethoprim sulfadoxine (Borgal, Intervet Canada Ltd. Witby ON) was given. 300 μg buprenorphrine hydrochloride (Temgesic, Schering-Plough Ltd. UK) was given immediately after surgery and again 24 hours later to alleviate pain.

**Experimental Design**

Animals were allowed to recover for 4-5 days after surgery before any in vivo testing began. The in vivo testing period included several other tests not considered in this thesis including blood pressure by telemetry and fat tolerance tests and lasted approximately one month. During this period catheters were flushed daily with 5 mL of 0.2% heparinized saline. Body temperature was also measured daily with a digital ear thermometer and Hibitane antibiotic/antifungal cream (Ayerst, Guelph ON) was rubbed on all wound sites to monitor and prevent infection. Antibiotics were only given if the

animal presented a temperature of greater than 40°C. The five-hour daily feeding regime was re-established the day immediately following surgery.

Five days after surgery, provided the animal had no fever, an intravenous glucose tolerance test (IVGTT) was performed after an overnight fast. Two fasting 4 mL blood samples were taken in EDTA tubes before intravenous administration of 0.5 g/kg body weight 50% glucose solution. Blood was sampled from the other catheter every five minutes and blood glucose was measured using an Ascensia Contour blood glucose meter (Bayer, Toronto ON). The test was stopped when blood glucose returned to fasting levels. Blood samples were centrifuged for 10 minutes at 4000 x g at 4 °C. The plasma was obtained and stored at –20 °C for later analysis of plasma glucose and insulin.

Six days after surgery an insulin sensitivity test (IST) was performed after an overnight fast. A fasting 4 mL blood sample was taken in EDTA at time –10 min. Next, somatostatin was administered intravenously (4μg/kg) at time –5 min to inhibit the endogenous release of insulin from the pancreas. At time 0, a 0.5 g/kg 50% glucose solution was given intravenously. Blood samples were taken every 3 minutes and whole blood glucose was measured instantly with an Ascensia Contour glucose meter. Somatostatin has a short half life and therefore, a maintenance dose was given after each blood sample. When blood glucose concentrations stabilized, Humulin R insulin (Eli Lilly, Toronto ON), 0.5 U/kg body weight was given intravenously. Blood samples were then taken every 5 minutes until blood glucose concentrations returned to fasting levels. Blood was centrifuged for 10 minutes at 4000 x g at 4 °C. The plasma was obtained and stored at –20 °C for later analysis of plasma glucose, insulin and C-peptide.

Two days following the IST, a fat tolerance test was performed after an overnight fast. Two days after the fat tolerance test, continuous 48-hour baseline blood pressure recordings began. Only one pig could be recorded at a time, therefore, pigs were rotated into and out of the recording pen. Immediately following baseline measurements, pigs were fed a high salt diet for 8 days. Blood pressure was recorded continuously for the last 48 hours of high salt feeding. Once high salt feeding ceased, pigs were fed their regular chow for at least three days before being killed. Since my primary objective was to look for the development of type 2 diabetes in these pigs, I will only discuss the results of the IVGTT and IST.

**Necropsy**

Pigs were anaesthetized with 105 mg/kg sodium pentobarbital (Euthanyl, Biomeda-MTC Cambridge ON) and ventilated and maintained with 0.5 – 1 % halothane gas mixed with oxygen. Organs were removed from anaesthetized animals and samples stored in 10% neutral buffered formalin and/or liquid nitrogen for later analyses. Animals died by exsanguination. Carcasses and visceral organs were homogenized separately and frozen (-20°C) for later composition analysis.

**Biochemical Analyses**

Fasting plasma glucose and insulin were measured at 6 months of age and in the initial fasting sample of the IVGTT (approximately 8.5 months). Plasma glucose and insulin were measured in all samples during the IVGTT and IST. In order to verify that endogenous insulin secretion was suppressed during the IST, C-peptide was measured in plasma. Plasma glucose was measured using a Rapid Lab blood biochemistry analyzer (Bayer Diagnostics, Toronto ON). Plasma insulin was measured using a porcine insulin

radioimmuoassay kit (Linco Research, St. Charles Missouri). Briefly, a known concentration of radiolabelled insulin and an unknown amount of insulin in plasma compete for a fixed amount of binding sites on anti-insulin antibody. The radiolabelled insulin and plasma insulin reach equilibrium such that the amount of radiolabelled insulin bound decreases as the amount of plasma insulin increases. A standard curve is constructed with fixed concentrations of insulin and radiolabelled insulin. Radioactivity is counted and plasma insulin concentrations can be determined using the standard curve. C-peptide was also measured using a porcine insulin radioimmunoassay kit (Linco Research, St. Charles Missouri) using similar principles.

**Statistical Analysis**

All statistical analyses were performed using Graph Pad Prism 4 software (GraphPad Software, Inc. San Diego, CA). Area under the curve (AUC) was calculated when glucose versus time plots were made using Graph Pad Prism 4 software. Comparisons between groups of pigs and gender were made by non-repeated measures 2 way ANOVA using Graph Pad Prism 4. This test could not compare litter effect because sow fed piglets were not the same sex for two groups (one for each gender); as a result the non-repeated measures approach was used to compare overall group effect and overall gender effect (which was balanced). Table 4.1 illustrates the gender imbalance. Comparisons between runt and littermate (i.e. the litter effect) and gender were made using repeated measures 2 way ANOVA; this test was possible because all pairs of runt-littermates were same-sex. When gender tested non-significant, paired students t tests were used to make runt and littermate comparisons. Linear regression was used to

determine correlations. Statistical significance was declared if $p < 0.05$. All data are expressed as mean, plus or minus standard deviation.

Table 4.1: Representation of gender inequality. Similar numbers represent siblings, for example, Runt 1 is sibling of Littermate 1 and Sow-fed 1 (all females). Sow-fed control pigs from litters 3 and 6 are of different gender than the runts and littermates of litters 3 and 6.

| | Runt | Littermate | Sow-fed |
|---|---|---|---|
| Female | 1 2 3 | 1 2 3 | 1 2 **6** |
| Male | 4 5 6 | 4 5 6 | 4 5 **3** |

## 4.4 Results

### Pilot Data from OGTT

Results from the OGTT were highly variable (Table 4.2). Fasting glucose was not different between animals. The areas under the curve for glucose were extremely variable, with littermates experiencing the most variance. Time to baseline was not different between animals.

Table 4.2: Pilot results from the oral glucose tolerance test (OGTT) performed on runt, littermate and sow-fed pigs at 6 months of age.

| | Runt (N=4) | Littermate (N=3) | Sow-fed (N=4) |
|---|---|---|---|
| Body Weight (kg) | 39.8 ± 5.7 | 44.0 ± 4.4 | 44.5 ± 8.4 |
| Glucose Given (g) | 79.50 ± 11.36 | 88.00 ± 8.72 | 89.00 ± 16.69 |
| Fasting Glucose (mmol/l) | 2.5 ± 0.3 | 2.9 ± 0.5 | 2.8 ± 0.4 |
| AUC | 106.5 ± 40.6 | 173.1 ± 130.5 | 105.2 ± 48.8 |
| Time to Baseline (min) | 114 ± 19 | 144 ± 7 | 103 ± 29 |

**Fasting Plasma Analyses**

Fasting plasma glucose and insulin were examined at 6 and 8.5 months of age. At six months of age there were no differences in plasma glucose, insulin or glucose: insulin between runt, littermate and sow-fed (Table 4.3). Sow-fed pigs were then excluded from the analysis and repeated measures 2 way ANOVAs were performed to examine differences between runts, littermates and gender. Plasma glucose and glucose: insulin was still not different between runts and littermates. Larger littermates had significantly higher fasting insulin than runts ($p=0.045$).

Table 4.3. Fasting plasma concentrations of glucose (mmol/L) and insulin (μU/mL) and glucose: insulin ratio in runt, larger littermate and sow-fed pigs at 6 months of age.

| | Runt | Littermate | Sow-fed |
|---|---|---|---|
| Plasma glucose (mmol/L) | 6.4 ± 2.5 | 5.3 ± 0.79 | 5.5 ± 1.0 |
| Plasma insulin (μU/mL) | 21.60 ± 14.37 [a] | 36.55 ± 12.60 [b] | 29.93 ± 12.42 |
| Glucose: Insulin | 0.41 ± 0.30 | 0.17 ± 0.09 | 0.23 ± 0.15 |

At 8.5 months of age there were no differences in plasma glucose, insulin or glucose: insulin between runt, littermate and sow-fed (Table 4.4). When sow-fed pigs were removed from the analysis and repeated measures two way ANOVAs were performed, there were still no differences between runts and littermates.

Table 4.4: Fasting plasma concentrations of glucose (mmol/l) and insulin (μU/ml) and glucose: insulin ratio in runt, larger littermate and sow-fed pigs at 8.5 months of age.

| | Runt | Littermate | Sow-fed |
|---|---|---|---|
| Plasma glucose (mmol/l) | 5.4 ± 0.2 | 5.3 ± 0.4 | 5.3 ± 0.2 |
| Plasma insulin (μU/ml) | 14.47 ± 4.92 | 18.18 ± 8.67 | 13.37 ± 3.23 |
| Glucose: Insulin | 0.41 ± 0.1 | 0.36 ± 0.18 | 0.41 ± 0.09 |

Fasting glucose did not change over time and remained in the normal non-diabetic range for plasma glucose. Insulin decreased significantly from 6 months to 8.5 months.

The ratio of fasting plasma glucose to insulin increased significantly from 6 to 8.5 months in littermates only (Figure 4.1). Fasting insulin was hypothesized to increase, not decrease over time.

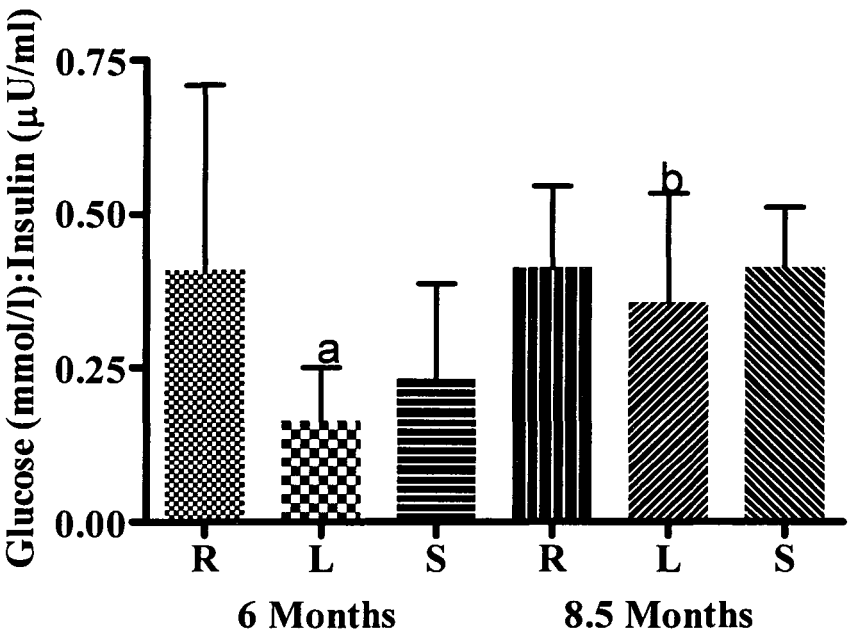


Figure 4.1: The ratio of fasting plasma glucose (mmol/l): fasting plasma insulin (μU/ml) at 6 and 8.5 months of age in runt, littermate and sow-fed control pigs. The ratio was significantly higher at 8.5 months than 6 months in littermates only.

Runt

Littermate

Sow-fed

**Intravenous Glucose Tolerance Test (IVGTT)**

Time for glucose and insulin to return to baseline, area under the curve (AUC) for glucose and insulin (calculated from initial baseline measurement to the final plasma sample), the ratio of AUC of glucose to AUC insulin, peak glucose and insulin, time to peak glucose and insulin and the rate of glucose clearance were also determined (Table 4.5). All of these measurements were later correlated to fasting insulin and fasting glucose (Table 4.6). There were no differences in any measurement from the intravenous glucose tolerance test between groups of pigs. Sow-fed pigs were removed from the statistical analysis and repeated measures two way ANOVAs were done to look for differences between runt, littermate and gender. However, there were still no differences between runts and littermates. Gender had an effect on glucose area under the curve (AUC), peak glucose (mmol/l) and the ratio of insulin AUC to glucose AUC (Table 4.7). Females had a higher AUC for glucose ($p=0.0349$) and peak glucose than males ($p=0.0030$). The ratio of insulin AUC to glucose AUC was higher in males than females ($p=0.0172$). In 5 pigs, insulin did not return to baseline before the test was stopped (Table 4.8). The test was stopped when glucose concentrations returned to baseline. These five animals were still included into the statistical analyses. Therefore, the insulin area under the curve was underestimated in these animals.

Table 4.5: Measurements of glucose tolerance derived from intravenous glucose tolerance tests (IVGTT) performed in runt, littermate and sow-fed pigs at 8.5 months of age.

| | Runt | Littermate | Sow-fed |
|---|---|---|---|
| Glucose AUC | 461.97 ± 227.48 | 461.35 ± 165.09 | 510.78 ± 142.61 |
| Insulin AUC | 4287 ± 1152 | 4125 ± 716.3 | 4338 ± 2000 |
| Insulin AUC: Glucose AUC | 11.08 ± 6.13 | 9.73 ± 3.17 | 8.98 ± 0.52 |
| Peak glucose (mmol/l) | 30.9 ± 6.0 | 32.1 ± 7.8 | 32.5 ± 2.2 |
| Time to peak glucose (min) | 3.0 ± 1.3 | 3.0 ± 1.3 | 3.1 ± 1.7 |
| Peak insulin (μU/ml) | 204.30 ± 43.64 | 185.75 ± 7.58 | 194.05 ± 98.04 |
| Time to peak insulin (min) | 16.4 ± 7.6 | 13.7 ± 9.8 | 12.6 ± 3.2 |
| Slope ln bG | -0.048 ± 0.014 | -0.051 ± 0.017 | -0.051 ± 0.015 |
| Glucose time to baseline (min) | 41.5 ± 16.3 | 37.3 ± 11.5 | 38.3 ± 10.0 |

Table 4.6: Measurements of glucose tolerance derived from the intravenous glucose tolerance test (IVGTT) were correlated to fasting plasma glucose (mmol/l) and fasting plasma insulin (μU/ml) at 8.5 months of age.

| | Fasting Insulin (μU/ml) | Fasting Glucose (mmol/l) |
|---|---|---|
| Fasting plasma glucose (mmol/l) at 6 months | -- | Sow-fed<br>$R^2$=0.77<br>p=0.02 |
| Fasting insulin (μU/ml) at 6 months | -- | -- |
| Glucose area under the curve (AUC) | -- | Runt<br>$R^2$= -0.85<br>p=0.01 |
| Insulin area under the curve (AUC) | -- | -- |
| Insulin AUC: Glucose AUC | -- | -- |
| Peak glucose (mmol/l) | -- | All pigs<br>$R^2$= -0.28<br>p=0.02 |
| Time to peak glucose (min) | Littermate<br>$R^2$= -0.93<br>p=0.002 | -- |

| | | |
|---|---|---|
| Peak insulin (μU/ml) | -- | -- |
| Time to peak insulin (min) | Sow-fed (+)<br>$R^2$=0.83<br>p=0.01 | Runt<br>$R^2$= -0.67<br>p=0.05 |
| Rate of glucose clearance | -- | Runt (+) Littermate<br>$R^2$=0.66 $R^2$= -0.72<br>p=0.049 p=0.03 |
| Glucose time to baseline (min) | -- | Runt<br>$R^2$= -0.92<br>p=0.002 |

-- indicates no significant correlation

Table 4.7: Gender differences in measurements of glucose tolerance derived from intravenous glucose tolerance test performed at 8.5 months of age

| | Female | Male | P value |
|---|---|---|---|
| Glucose AUC | 578.9 ± 26.9 | 377.1 ± 73.0 | 0.0349 |
| Peak glucose (mmol/l) | 36.2 ± 2.4 | 27.5 ± 2.8 | 0.0030 |
| Insulin AUC: Glucose AUC | 7.21 ± 0.66 | 15.41 ± 5.57 | 0.0172 |

Table 4.8: Final insulin concentrations (μU/ml) were elevated above fasting concentrations before the intravenous glucose tolerance test was stopped in 1 runt, 2

larger littermates and 2 sow-fed control pigs. The difference in insulin concentration (μU/ml) from initial concentrations and when the test was stopped.

| | Glucose Time to Baseline (min) | Final Insulin (μU/ml) | Fasting Insulin (μU/ml) | Difference (μU/ml) |
|---|---|---|---|---|
| Runt (male) | 32 | 37.22 | 15.09 | 22.13 |
| Littermate (female) | 32 | 66.50 | 7.80 | 58.70 |
| Littermate (male) | 29 | 59.83 | 34.69 | 25.14 |
| Sow-fed (male) | 32 | 23.71 | 10.23 | 13.48 |
| Sow-fed (female) | 57 | 19.24 | 6.556 | 12.68 |

When looking at all pigs there were no significant correlations between any measurement made during the IVGTT and fasting insulin (Table 4.6). Therefore, correlations were sought in runts, littermates and sow-fed controls separately. As fasting insulin increased in littermates the time to reach glucose decreased ($r^2$=0.93, p=0.002). In sow fed controls, the time to reach peak insulin increased with increasing fasting insulin concentrations ($r^2$=0.83, p=0.01). All measurements made during the IVGTT were next correlated to fasting plasma glucose. The time to peak plasma glucose was negatively correlated to fasting plasma glucose ($r^2$=0.28, p=0.02). There were no other significant correlations when looking at all the pigs together. In runts, glucose area under the curve, time to peak insulin and glucose time to baseline were negatively correlated to fasting glucose. The rate of glucose clearance was positively correlated to fasting glucose in runts, but negatively correlated to fasting glucose in littermates. In sow-fed controls

fasting glucose at six months was positively correlated to fasting glucose before the IVGTT.

**Insulin Sensitivity Test (IST)**

C-peptide concentration (μg/ml) remained constant throughout the insulin sensitivity test (Figure 4.2). The rate of glucose clearance was determined by plotting the natural log of blood glucose values against their respective t time point. The slope after the insulin administration was considered the rate of glucose clearance in response to insulin. Female sow-fed control pigs had a significantly higher rate of glucose clearance than sow-fed male pigs ($p=0.04$). There were no differences between runts and littermates (Figure 4.3). The rate of glucose clearance did not correlate to fasting insulin or glucose.

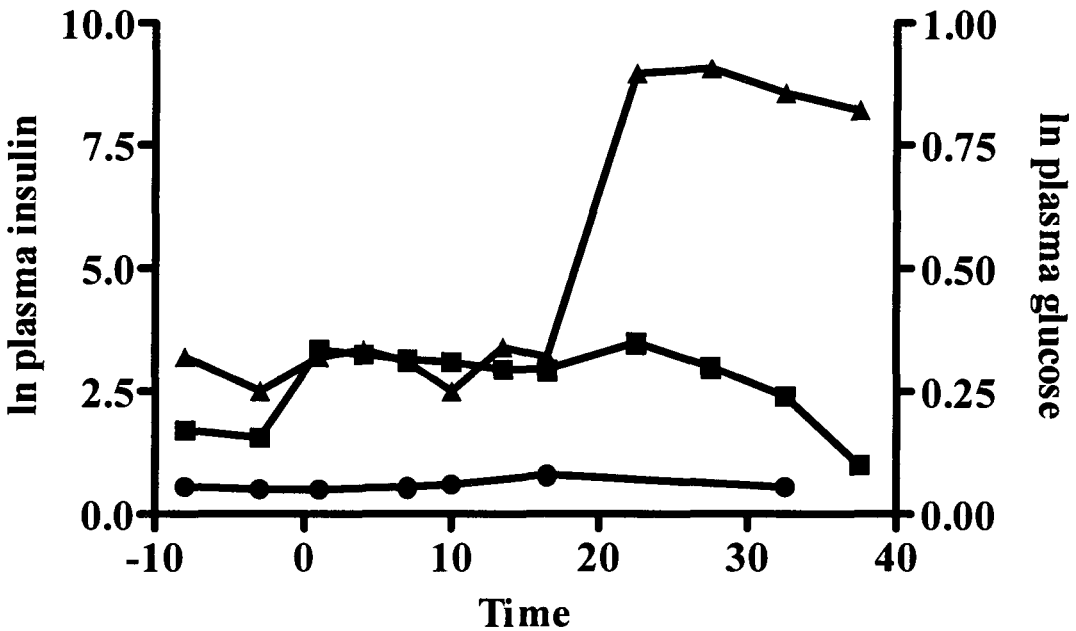


Figure 4.2: Natural logarithm (ln)of plasma glucose and insulin and plasma c-peptide (μg/ml) in an individual pig during an intravenous glucose tolerance test.

ln Plasma Glucose

ln Plasma Insulin

Plasma C-peptide (μg/ml)

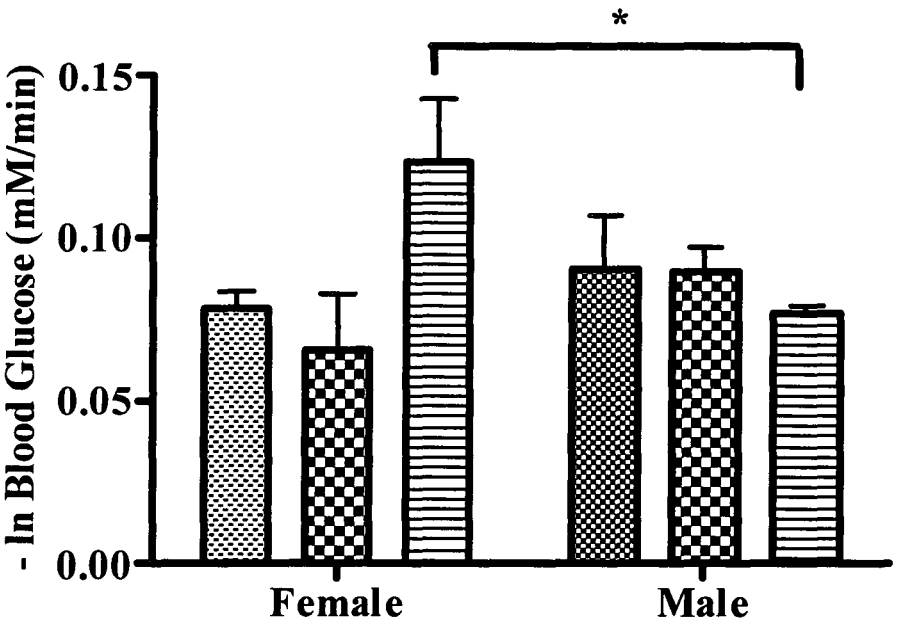


Figure 4.3. The average rate of glucose clearance during an insulin sensitivity test (IST) in runt, littermate and sow-fed control pig. Female sow-fed pigs had a statistically significantly higher rate of glucose clearance than male sow-fed pigs (p=0.04)

Runt

Littermate

Sow-fed

**Correlations**

In order to determine the relationships of birth weight and postnatal growth rate to symptoms of type 2 diabetes, birth weight and postnatal growth rates were correlated to all measurements of type 2 diabetes including fasting plasma glucose, insulin and all measurements from the IVGTT and IST (Table 4.9). When looking at all pigs there were

no significant correlations found between any measurement of diabetes and birth weight, growth rate or abdominal growth rate.

Table 4.9. Correlations were made between measurements of glucose tolerance and birth weight (kg), postnatal growth rate (kg/d) during milk feeding, and abdominal circumference growth rate (cm/d) during milk feeding.

| | Birth weight (kg) | Growth rate during milk (g/d) | Abdominal circumference growth rate (cm/d) |
|---|---|---|---|
| Insulin 8.5 months (μU/ml) | -- | -- | -- |
| Glucose AUC | -- | -- | -- |
| Insulin AUC | -- | Littermate $R^2$=0.74 p=0.03 | -- |
| Insulin AUC: Glucose AUC | -- | -- | -- |
| Peak glucose (mmol/l) | -- | -- | -- |
| Peak insulin (μU/ml) | -- | -- | Runt $R^2$= -0.09 p=0.006 |
| Time to peak insulin (min) | Littermate $R^2$=0.76 | -- | -- |

| | | | |
|---|---|---|---|
| | p=0.02 | | |
| Glucose clearance (IVGTT) | -- | -- | Runt<br>$R^2$= -0.81<br>p=0.02 |
| Glucose clearance (IST) | -- | -- | -- |

-- means no significant correlation

Correlations were then made between all measurements of diabetes and current weight, current abdominal circumference and current body mass index (Table 4.10). Again when looking at all pigs there were no significant correlations observed.

Table 4.10. Correlations were made between measurements of glucose tolerance and body weight (kg), abdominal circumference (cm) and body mass index ($kg/m^2$) at 8.5 months of age.

| | Current weight (kg) | Current abdominal circumference (cm) | Current BMI ($kg/m^2$) |
|---|---|---|---|
| Insulin 8.5 months (μU/ml) | -- | Runts<br>$R^2$= -0.81<br>p=0.01 | -- |
| Glucose AUC | Littermate<br>$R^2$= -0.69<br>p=0.04 | -- | Littermate<br>$R^2$=0.72<br>p=0.03 |
| Insulin AUC | -- | -- | -- |
| Insulin AUC: Glucose AUC | -- | -- | Littermate<br>$R^2$= -0.75<br>p=0.03 |
| Peak glucose (mmol/l) | -- | Sow-fed<br>$R^2$=0.66<br>p=0.05 | -- |
| Peak insulin (μU/ml) | -- | Littermate<br>$R^2$=0.72<br>p=0.03 | -- |

| | | | |
|---|---|---|---|
| Time to peak insulin (min) | -- | -- | -- |
| Glucose clearance (IVGTT) | -- | -- | -- |
| Glucose clearance (IST) | Littermate $R^2$=0.83 p=0.01 | -- | Sow-fed $R^2$=0.79 p=0.02 |

-- means no significant correlation

## 4.5. Discussion

### Validating the miniature pig as a model for type 2 diabetes

When an individual is type 2 diabetic, glucose is not readily transferred into cells because there is a lack of response to insulin. Initially the pancreas overcompensates by producing excess insulin in an attempt to drive glucose into cells and to keep the individual in euglycemia. At this point the individual is considered insulin resistant, characterized by high fasting plasma insulin, normal fasting plasma glucose and a low ratio of glucose to insulin. An individual can remain insulin resistant for years before developing diabetes. Eventually the pancreas cannot keep up with the insulin demand and decompensates insulin production. Since cells are no longer normally responsive to insulin, there is reduced glucose uptake resulting in hyperglycemia and type 2 diabetes. The liver responds by increasing gluconeogenesis adding to the hyperglycemia and cells begin using fatty acids as an energy source. Diabetic individuals present dyslipidemia, high concentrations of fasting glucose, low concentrations of plasma insulin and a high ratio of glucose to insulin. In humans, fasting plasma analysis of insulin, glucose and lipids is the first step in assessing an individual's glycemic status.

Fasting plasma insulin and glucose concentrations were measured in our pigs at 6 (juvenile) and 8.5 months of age (young adult). Since insulin resistance generally precedes type 2 diabetes, we expected to see indicators of insulin resistance such as high fasting plasma insulin and a low ratio of glucose to insulin before seeing evidence of type 2 diabetes. Type 2 diabetes would be suspected if fasting glucose was higher than normative values found in the literature combined with a high ratio of glucose to insulin. Hematological and serum biochemistry values of healthy sexually mature Yucatan pigs

were determined by Radin and colleagues (1986). They found serum glucose ranged from 2.40 to 7.40 mM ($\mu$ = 3.71 mM) and was not different between males and females at 80 weeks of age. A normal range of 3.0–5.0 mM for glucose and 5 – 16 $\mu$U/mL for insulin has been observed in recent studies using the Yucatan miniature pig as a model for diabetes (Xi et al 2004, Larsen et al 2002, Otis et al 2003). When diabetes was induced in other wise healthy Yucatan swine by using streptozotocin treatment, fasting plasma glucose was markedly higher, 15-20 mM, while insulin remained normal (Larsen et al 2002, Otis et al 2003). The pigs in our study had fasting glucose of approximately 5.7 mM at 6 months and 5.3 mM at 8.5 months. The observed glucose concentrations were within the normal range for glucose found in the literature. Fasting glucose over the course of the study (3 months – 8 months) did not change significantly and was not different between groups of pigs. At 6 months of age, fasting insulin was 31.2 $\mu$U/mL, much higher than the values of healthy pigs used in other studies (Larsen, et al 2002, Otis, et al 2003). Plasma insulin was within the normal range for miniature swine 15.3 $\mu$U/ml at 8.5 months of age. When examining the ratios of glucose and insulin at 6 and 8 months, the results were unexpected. At 6 months of age, the ratio of glucose to insulin was very low (0.18), suggesting the pigs were insulin resistant. However, insulin resistance was not present at 8.5 months of age. The brief period of insulin resistance was surprising, as insulin resistance was expected to develop into diabetes.

At 6 months of age the majority of the pigs were coming into sexual maturity (puberty). Sebert and colleagues (2005) observed a similar spike in plasma insulin in healthy non-castrated male Yucatan miniature pigs undergoing sexual maturity (N=5). Plasma glucose, insulin and lipids were measured at 4, 10 and 16 months of age. At 4

months, plasma insulin and glucose were within the normative values found in the literature. However, at sexual maturity plasma insulin was elevated to 28.8 μU/ml ± 7.82, while glucose remained approximately 4.5 mM. Extensive research has been performed investigating hormonal changes affecting growth during puberty in swine. However, Sebert and colleagues are the only researchers, to my knowledge, to examine insulin sensitivity during puberty in swine from a diabetes perspective. Insulin resistance during puberty is thought to be influenced by growth hormone, insulin-like growth factor-1 (IGF-1) and changes in body fat distribution. Moran and colleagues (1999) performed the hyperinsulinemic euglycemic clamp method (the gold standard method for assessing insulin sensitivity) in 357 children. Puberty was associated with high fasting serum insulin concentrations and insulin resistance, irrespective of body weight. A recent longitudinal study by Hannon and colleagues (2006) found significant differences in glucose homeostasis in prepubescence compared to pubescence using the hyperinsulinemic euglycemic clamp method. Puberty was associated with a 50% decrease in insulin sensitivity compensated by a doubling in insulin secretion. Similar studies in pig models of diabetes are needed to better understand the mechanisms of insulin resistance during puberty. Also, the onset of sexual maturity in miniature pigs needs to be defined. In future studies, careful notes should be taken as to when signs of sexual maturity are occurring and when they subside. Identifying when sexual maturity is occurring may help to understand and explain unanticipated results. To further enhance our study, additional biochemical analyses should be conducted at several time points throughout the study to better characterize the observed episode of insulin resistance. In addition, fasting plasma IGF-1, and lipids including cholesterol and triglycerides should

be analyzed throughout the study to understand changes in metabolism and identify indicators of disease development.

In humans, fasting plasma analysis is a quick and cost-effective screening method for chronic disease development. However, it only provides a snap shot of an animal's glycemic status. Therefore, in vivo tests are executed to better assess glucose tolerance and insulin sensitivity. Two practical in vivo methods for determining glycemic status were successfully performed in our pigs: the intravenous glucose tolerance test (IVGTT) and insulin sensitivity test (IST). In contrast, the oral glucose tolerance test proved to be impractical, stressful and ultimately unsuccessful in our pigs. An oral glucose tolerance test (OGTT) measures the pancreas' responsiveness to glucose and is the most common test used to assess glucose tolerance and subsequently diagnose diabetes in humans. During our OGTT, the oro-gastric gavage technique was used to administer glucose with limited success, as it was too difficult to restrain the animals and accurately determine the amount of glucose consumed by the animal. Also, the gavage technique was extremely stressful, as was the ear prick glucose measurements made throughout the test. Glucocorticoids from such stress stimulate gluconeogenesis in the liver. As a result, blood glucose concentrations rise above normal within minutes of being subjected to the stressor. Since the animals were extremely stressed during the initial dosing phase, it was not possible to interpret the results from the oral glucose tolerance test. A variation of the oral glucose tolerance test termed the mixed meal has been used successfully in Gottingen miniature pigs (Kjems, et al 2001, Larsen, et al 2002, Xi et al 2004). After an 18-24 hour fast, animals were offered a mixed meal of 2 g/kg glucose and 25 g standard pig chow and blood glucose was monitored over a 2 hour period. Typically during the mixed meal

glucose tolerance test, the animals are surgically fitted with a venous catheter for easy, non-strenuous blood sampling. The mixed meal method was attempted in our pigs after an overnight fast at 6 months of age and later at 8.5 months of age when the pigs were surgically fitted with catheters. However, the animals did not consume the mixed meal even when the fast was extended to 24 hours. Therefore, the mixed meal test was abandoned. An alternative to the oral glucose tolerance is the intravenous glucose tolerance test (IVGTT).

Like the OGTT, the IVGTT measures the pancreatic beta cell responsiveness to glucose. During an IVGTT, glucose is infused intravenously causing a rapid rise in plasma glucose followed by an incremental decrease in plasma glucose typically over a one hour period. Plasma insulin displays a similar curve to that of plasma glucose throughout the test. The IVGTT is much quicker than OGTT as there are no effects of gastric emptying rates and intestinal absorption. In our animals, the IVGTT provided a quick, non-stressful method for determining glucose tolerance. The IVGTT generated many results, the most significant of which being glucose and insulin time to baseline, glucose and insulin area under the curve (AUC), and the rate of glucose clearance. When an animal is glucose intolerant, insulin secretion and function is impaired leading to impaired glucose uptake. When glucose is infused, a glucose intolerant animal will be unable to or will take longer to clear glucose from the plasma resulting in a slow rate of glucose clearance, an inability for glucose to return to baseline, and a large glucose AUC.

All individual pigs in this study displayed normal glucose curves and glucose returned to fasting levels within one hour after infusion. The rate of glucose clearance was similar to that of non-diabetic control animals used by Otis and colleagues (2003). It

is difficult to compare glucose AUC values found in our study to those found in literature due to differences in study methodology. The glucose AUC is affected by the concentration of glucose infused and how the AUC is calculated. Johansen and colleagues studied 9-10 month old female Gottingen miniature pigs fed either a low fat (13% fat) or a high fat (55%) diet for 5 weeks. At the end of the 5 weeks an IVGTT was performed using a 0.3 g/kg glucose bolus. The glucose AUC was measured over a 120 minute period after glucose infusion. Pigs fed the high fat diet had a higher AUC for glucose than pigs fed the low fat diet, 636 ±.26 and 578 ± 10, respectively. The authors did not explain how AUC was calculated. Poore and Fowden (2004[2]) performed an IVGTT (0.5 g/kg) in low and high birth weight domestic pigs at 3 and 12 months of age. The glucose AUC was calculated from the mean of the pre-infusion fasting glucose values until 120 minutes after glucose infusion. At 12 months of age low birth weight pigs had significantly higher glucose AUC than high birth weight pigs, approximately 280 and 200, respectively. The overall average glucose AUC of all our pigs was 478 ± 172. Females had a statistically significantly higher AUC than males ($p<0.05$), 578.94 ± 26.86 and 377.12 ± 72.96, respectively. Our methodology was most similar to that of Poore and Fowden (2004), yet our pigs experienced much higher glucose AUC. However, there is not enough evidence to define the glucose tolerance of our animals. Insulin AUC in our pigs was difficult to interpret. In the majority of our animals, plasma insulin followed the expected decay curve and returned to fasting levels within one hour. However, five of the pigs had plasma insulin concentrations still elevated when the test was stopped, even though plasma glucose had returned to fasting levels. The ratio of glucose to insulin when the test was stopped was low, suggesting these pigs were

somewhat insulin resistant. In future studies, the test should be extended after glucose has returned to fasting levels to better assess insulin sensitivity.

Insulin sensitivity tests (IST) are different from glucose tolerance tests in that they measure the insulin-stimulated glucose uptake by the muscle. In most insulin sensitivity tests, a large bolus of insulin is given and glucose clearance in response to that insulin is examined. The rate of glucose clearance from the plasma represents glucose uptake by peripheral tissues. Otis and colleagues (2003) administered several doses of insulin (0, 0.05 and 0.10 U/kg) to diabetic and non-diabetic control, young adult Yucatan miniature pigs. They found that 0.10 U/kg insulin induced comas and seizures in non-diabetic animals due to severe hypoglycemia. This observation led them to develop a novel alternative insulin sensitivity test where non-diabetic pigs were made hyperglycemic before giving insulin. Since our animals had normal fasting plasma glucose values, we did not suspect the animals to be overtly diabetic. Therefore, we used this alternative insulin sensitivity test in our study. First, somatostatin was given intravenously to inhibit endogenous insulin secretion. Somatostatin has a short half life and therefore, a maintenance dose was given after each blood sample. Glucose was infused next making the pigs hyperglycemic which is necessary to avoid symptoms of severe hypoglycemia in response to the large insulin bolus subsequently administered. This bolus dose of insulin was given and the rate of glucose clearance from the plasma was determined with serial blood sampling. The test was successful in that all pigs followed a similar pattern of plasma glucose and insulin clearance. Also, somatostatin successfully inhibited endogenous insulin secretion, as shown by the unchanging C-peptide concentrations in response to glucose. Furthermore, none of the pigs exhibited any symptoms of

hypoglycemia. The rate of glucose clearance in our pigs was similar to that of the non-diabetic control animals of Otis and colleagues (2003) indicating that the pigs were not insulin resistant. This novel adaptation of the traditional insulin sensitivity test was successful in our pigs. More studies using this novel technique are needed to determine the reproducibility of results.

Table 4.11 summarizes the expected outcomes and observed outcomes.

Table 4.11: Summary of expected and observed results of fasting plasma analysis, oral glucose tolerance test, intravenous glucose tolerance test and insulin sensitivity test

| | Expected Results | Observed Results |
|---|---|---|
| Fasting Plasma Analysis | If an animal is diabetic hyperglycemia and a high ratio of glucose: insulin would be observed. Hyperinsulinemia and a low ratio of glucose: insulin indicates insulin resistance. | Pigs did not exhibit hyperglycemia or hyperinsulinemia. |
| Oral Glucose Tolerance Test | If an animal is glucose intolerant they will exhibit a high glucose area under the curve and plasma glucose will not return to fasting levels. Area under the curve for glucose will be positively correlated to fasting glucose. | The OGTT was unsuccessful and yielded no reliable results. |
| Intravenous Glucose Tolerance Test | If an animal is glucose intolerant high glucose area under the curve (AUC), slow glucose clearance and inability for glucose to return to baseline would be observed. The AUC and time to return of baseline | The animal's AUC, rate of glucose clearance and time for glucose to return to baseline were similar to non-diabetic pigs. There were no correlations between fasting glucose and any outcomes |

| | | |
|---|---|---|
| | would be positively correlated to fasting glucose, whereas, the rate of glucose clearance would be inversely correlated to fasting glucose | of the IVGTT when looking at all pigs. In large littermates there was a negative correlation between the rate of glucose clearance and fasting glucose. |
| Insulin Sensitivity Test | If an animal was insulin resistant then the rate of glucose clearance would be slow and negatively correlated to fasting glucose | The rate of glucose clearance was similar to that of non-diabetic control animals used by Otis et al (2003). There was no correlation between the rate of glucose clearance and fasting glucose. |

**Determining the effects of birth weight and postnatal growth rate on the development of type 2 diabetes**

In order to determine the effect of birth weight on glucose intolerance and insulin sensitivity, comparisons between runt and larger littermates were made. We expected runts to develop symptoms of diabetes more readily than their larger littermates, as observed in other models (Poore and Fowden 2004[2]). However there were no differences in any measurements of glucose tolerance or insulin sensitivity between runts and larger

littermates at 8.5 months of age. Also, there were no significant correlations between birth weight and any measurement of type 2 diabetes. These findings differ from those of Poore and Fowden (2004[2]) who found that lower birth weight pigs had significantly higher fasting glucose and insulin area under the curve during an intravenous glucose tolerance test than higher birth weight animals at 12 months of age. However, low birth weight rats have been shown to have better glucose tolerance in young adulthood (12 weeks) compared to later in life (17 months). An age-dependent decline in glucose tolerance has been observed in humans (Preuss 1997), rats (Reavan et al 1983, Muzumdar et al 2004) and pigs (Larsen, et al 2001), irrespective of birth weight. Therefore, it is possible that the pigs in this study were too young to develop glucose intolerance or insulin sensitivity. In future studies, pigs should be tested for symptoms of diabetes in mid-to-late adulthood.

Epidemiological studies out of Helsinki, Finland showed that low birth weight infants that experienced catch up growth had higher rates of type 2 diabetes than children that did not experience compensatory growth (Forsen, et al 2000). Their findings suggest that low birth weight combined with compensatory growth puts individuals at an even higher risk for developing chronic diseases in adulthood than low birth weight alone. As a result of those studies, we expected postnatal growth rate to be positively correlated to fasting glucose and insulin and glucose and insulin area under the curve. The rate of glucose clearance during the IVGTT and IST were expected to be negatively correlated to post natal growth. However, overall postnatal growth rate (g/d) during milk feeding in our pigs was not correlated to any measurement of glucose tolerance or insulin sensitivity. Again, these results differ from those of Poore and Fowden (2002), who found that

glucose AUC during an IVGTT was significantly positively correlated to growth rate from birth to one month. Since runt piglets were growing rapidly in abdominal circumference during milk feeding, correlations between abdominal growth rate and measurements of type 2 diabetes were also performed. Combining all pigs into the analysis, there were no significant correlations observed. However, within runts, the rate of glucose clearance was negatively correlated to abdominal circumference growth rate. Although this observation was expected, it was not enough evidence to suggest that postnatal abdominal growth rate significantly impacted later glucose tolerance. Since symptoms of glucose intolerance and insulin resistance were not related to birth weight or postnatal growth rate, the effects of current body weight, current abdominal circumference and current body mass index were determined. In humans, type 2 diabetes is strongly associated with obesity, particularly visceral obesity (Keller 2006). Therefore, we hypothesized a positive correlation between abdominal circumference and various measurements of type 2 diabetes. However, no significant relationships were found between any measurement of type 2 diabetes and current weight, abdominal circumference or body mass index. Presently, visceral fat is being measured and will be correlated with our measures of type 2 diabetes.

**Determining the impact of early postnatal nutrition and mode of feeding on the susceptibility to type 2 diabetes.**

The major goals of this study were to determine the effects of birth weight and postnatal growth rate on development of later chronic disease. Birth weight is primarily influenced by fetal nutrition, whereas, postnatal growth rate is affected by infant nutrition. Human studies have shown that infants that are breastfed grow more slowly than formula

fed infants (Dewey, 1998) and may be less likely to develop chronic diseases in adulthood. Therefore, we also wanted to determine the impact of early postnatal nutrition on the development of type 2 diabetes. In this study, the sow-fed group served as a breastfed control group. Where possible, sow-fed pigs were the same sex as their littermates and were closest in birth weight to the larger littermate. Therefore, the major difference between sow-fed pigs and large littermates was early postnatal nutrition and mode of feeding. If breastfeeding protects against later disease development, then sow-fed controls should experience better glucose tolerance and insulin sensitivity than large littermates. However, in this study, there were no differences in any of the measurements of type 2 diabetes between sow-fed controls and formula-fed littermates. Therefore, infant nutrition did not impact glucose tolerance or insulin sensitivity at 8.5 months of age. Considering neither formula-fed group of pigs showed any signs of early development of insulin resistance and glucose intolerance, it is not surprising that the sow-fed group did not offer any additional protection.

### 4.6. Summary

Although the in vivo tests for diagnosing diabetes were successful, none of the pigs were found to be insulin resistant or type 2 diabetic at 8.5 months of age. There were no relationships found between indicators of insulin resistance or diabetes and birth weight or postnatal growth rate.

Since the pigs were young adults it is not surprising that they did not develop overt diabetes. However, symptoms of insulin resistance and glucose intolerance were expected, with runts predicted to be worse than larger littermates and sow-fed controls

better than other animals. It is possible that consuming a healthy diet of standard pig chow protected the pigs from developing disease in early adulthood. Currently there are parallel groups of pigs consuming a high trans and saturated fat, high sugar and high salt diet. This poor diet may challenge the pigs to develop markers of disease earlier in life than when consuming chow. Desai and colleagues (2005) challenged male rats born from protein-restricted dams with a highly palatable high fat diet from weaning to young adulthood (12 weeks). They found that low birth weight rats developed insulin resistance, but not high birth weight animals, irrespective of diet. Low birth weight rats fed the high fat diet had worse insulin resistance than low birth weight animals consuming the control diet. Male Gottingen miniature pigs fed a high fat, high sugar diets have been shown to develop obesity, and have increased fasting glucose compared to pigs fed regular chow (Larsen, 2001). Similar findings were observed in Ossabaw pigs fed a high fat diet (Dyson, et al 2006). Feeding a poor diet to the pigs may allow for differences in glucose tolerance and insulin sensitivity to be observed between groups of animals.

In this study, there may have been very subtle differences in insulin sensitivity between groups of pigs that went undetected because the IVGTT and IST were not sensitive enough. The gold standard for assessing insulin sensitivity is the hyperinsulinemic euglycemic clamp method (Cobelli et al 2007). In this method somatostatin is infused to inhibit endogenous insulin secretion, insulin is continuously infused to create hyperinsulinemia, and glucose is infused and frequently and rapidly measured in the blood in order to keep the individual in euglycemia. The rate of glucose infusion needed to maintain steady state is a direct measurement of whole body glucose

uptake, which describes the individual's insulin sensitivity. In practice this method is very expensive, time consuming and requires trained individuals to complete safely and effectively. The clamp method is not used routinely in clinical practice and is used only in specialized research trials where steady state conditions are required. Therefore, it was not employed in this study, but should be considered in future studies to determine subtle differences in insulin sensitivity between groups of animals.

Bellinger and colleagues (2006) recently published an extensive review of all swine models of type 2 diabetes and insulin resistance. There are several breeds of pigs which have been used as models of diabetes including: Yucatan miniature pigs, Sinclair miniature pigs, Gottingen miniature pigs, Chinese Guizhow miniature pigs, Yorkshire (and Yorkshire crosses) and Ossabaw pigs. The Gottingen miniature pig is the most extensively used pig model for diabetes. The plasma glucose, insulin and lipoprotein concentrations have been well characterized. These pigs have primarily had chemically induced diabetes, but have also been shown to become type 2 diabetic in response to high fat feeding. The Ossabaw breed is found on the Ossabaw islands off the coast of Georgia. They have been living in genetic isolation for centuries and are considered obese animals. A recent study by Dyson and colleagues found that Ossabaw pigs fed a high fat diet had higher triglycerides, and blood pressure compared to pigs fed a low calorie diet. The Ossabaw pig model is in its infancy but it may prove to be a model for fetal programming in the future. The Yucatan pig has primarily been a type 1 diabetes model, but has also been shown to develop impaired insulin sensitivity in response to overfeeding. Until now, no studies have used Yucatans as a model for fetal programming.

Bellinger and colleagues (2006) recently formulated a step-wise reference guide to validate the pig as a model for studying type 2 diabetes (Table 2 of Bellinger et al. 2006). Using this reference guide, we have only begun to validate our Yucatan miniature pig model of diabetes and fetal programming. There are several measurements that still need to be performed on our animals in order to more clearly determine their glycemic status.

1. The major focus of this chapter has been defining Step 1. Fasting insulin, glucose and insulin sensitivity were measured using fasting plasma analysis, and in vivo tests of glucose tolerance and insulin sensitivity.
2. The next step will be to determine the lipid profile throughout the study. Plasma cholesterol and triglycerides will be determined. This step is important, as the majority of diabetic individuals suffer some form of dyslipidemia. The previous chapter on compensatory growth discussed the body fat measurements made throughout the study. In the future, proximate analysis will be completed to determine the percentage fat in viscera and whole body. These percentages will then be correlated to measurements of type 2 diabetes making stronger comparisons than with abdominal circumference.
3. The metabolic mechanisms and progression of diabetes is not completely understood. Many serum markers are thought to be related to diabetes including: C-reactive protein, leptin, TNFα, and resistan. Homocysteine is another serum marker that we did measure, but more markers should be measured in the future.
4. Diabetic individuals are often hypertensive. We have measured mean arterial pressure by radio-telemetry. These data should be correlated to plasma insulin to see if there is a positive relationship.

5. End-organ damage often observed in animal models of diabetes includes: reduction in beta cell mass, atherosclerosis and endothelial dysfunction. Pancreatic beta cell mass is typically reduced in type 2 diabetes which limits the capacity for insulin secretion. In the protein-restricted rat model, low birth weight pups are born with a reduction in beta cell number (Bertin et al 1999). This reduction is thought to be a direct consequence of poor fetal nutrition which (depending on infant nutrition) permanently alters beta cell function leading to glucose intolerance in later life. Beta cell mass will be measured by histological examination of the pancreas and total pancreatic insulin in our pigs. The aortic arch and the coronary artery will be examined for evidence of atherosclerosis. Endothelial dysfunction is also being measured.
6. The final step in the reference guide refers to future studies examining the reduction of the above mentioned criteria when treated animals are compared to controls.

It is obvious that validating an animal model of disease takes considerable time, effort and resources. This chapter is the initial step in identifying the Yucatan miniature pig as a model for type 2 diabetes and fetal programming. Completing the remaining analyses will help determine the impact of birth weight and postnatal growth rate on glucose tolerance and insulin sensitivity.

## Chapter 5.0 Conclusions

The overall goal of this research was to determine the effects of birth weight, postnatal growth rate and early postnatal nutrition on the development of type 2 diabetes in Yucatan miniature swine. In order to do so, we needed to demonstrate compensatory growth and validate the miniature pig as a model for type 2 diabetes.

Runt piglets experienced a period of increased nutrient efficiency during the second week of milk feeding, characteristic of compensatory growth. Although, the composition of growth is yet to be determined, runt piglets appeared to be depositing fat towards the end of milk feeding based on abdominal circumference measurements. Runts eventually caught up in body size to their littermates in early adulthood. It was not surprising the Yucatan miniature pig proved to be a successful model for studying compensatory growth, as the domestic pig is already a well-characterized and established model of compensatory growth in the field of animal science (Foxcroft et al., 2006, Mitchell 2007).

In order to determine the relationship between postnatal growth and the development of type 2 diabetes, we needed to validate the Yucatan miniature pig as a model for type 2 diabetes. We expected pigs to develop markers of insulin resistance and diabetes, with runts presenting markers more readily and severe than their littermates. Fasting plasma analyses and in vivo tests of glucose tolerance and insulin sensitivity were successfully developed in this model, but the results did not indicate diabetes development in any of the pigs or differences between groups of pigs. When measurements of insulin resistance and diabetes were correlated to birth weight and postnatal growth rate, no relationships were found.

Although the results from this study did not reveal any relationship between birth weight, postnatal growth rate or early postnatal nutrition and markers of type 2 diabetes, the miniature pig should not be ruled out as a model for fetal programming. Additional analyses including plasma lipid analysis, and beta cell mass are currently being completed to more accurately understand their metabolism. Risk factors of other diseases, including hypertension and cardiovascular disease, are also being performed to determine if this miniature pig model is valid for other diseases suspected to be programmed in utero. A parallel group of pigs fed a high fat, high sugar, and high salt diet is also currently being studied. It is possible that this poor diet may challenge the miniature pig to develop markers of disease more readily.

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