The Effects of Poor Maternal Nutrition on Postnatal Growth and Development of Lambs

Kristen N. Peck
kristen.peck@uconn.edu

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The Effects of Poor Maternal Nutrition on Postnatal Growth and Development of Lambs

Kristen Nicole Peck

B.S., DeSales University, 2011

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The Effects of Poor Maternal Nutrition on Postnatal Growth and Development of Lambs

Presented by
Kristen Nicole Peck, B.S.

Major Advisor
Steven A. Zinn

Associate Advisor
Kristen E. Govoni

Associate Advisor
Thomas A. Hoagland

University of Connecticut
2013
DEDICATION

I would like to dedicate this thesis to my family and friends, who were my support system throughout this whole process. I would like to thank my parents, George and Cheryl Peck, for their never ending support and love. Thank you for always believing in me and providing me with words of encouragement. I am forever grateful for all the help and guidance you have given me throughout my academic career. I would also like to thank my brother, Jason Peck, and my friends who listened to me talk about my research and showed genuine curiosity in my work.
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synchronize the ewes for our study.
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### ABBREVIATIONS

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<td>ADAM</td>
<td>adamalysin</td>
</tr>
<tr>
<td>ADG</td>
<td>average daily gain</td>
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<td>Akt/PKB</td>
<td>protein kinase B</td>
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<td>ALS</td>
<td>acid labile subunit</td>
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<td>ARC</td>
<td>arcuate</td>
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<tr>
<td>AgRP</td>
<td>agouti-related peptide</td>
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<tr>
<td>AU</td>
<td>arbitrary units</td>
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<tr>
<td>AUC</td>
<td>area under the curve</td>
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<tr>
<td>BCS</td>
<td>body condition score</td>
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<td>BMSC</td>
<td>bone marrow stromal cells</td>
</tr>
<tr>
<td>BW</td>
<td>body weight</td>
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<tr>
<td>Ca²⁺</td>
<td>calcium</td>
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<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CAP</td>
<td>Cbl associated protein</td>
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<td>CREB</td>
<td>cAMP response element-binding protein</td>
</tr>
<tr>
<td>CRL</td>
<td>crown rump length</td>
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<tr>
<td>d</td>
<td>day</td>
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<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>ERK</td>
<td>extracellular signal-regulated kinase</td>
</tr>
<tr>
<td>FAK</td>
<td>focal adhesion kinase</td>
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<tr>
<td>FFA</td>
<td>free fatty acids</td>
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<td>GH</td>
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<td>GHBP</td>
<td>growth hormone binding protein</td>
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<td>GHR</td>
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<td>GHRH</td>
<td>growth hormone releasing hormone</td>
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<tr>
<td>GHRH-R</td>
<td>growth hormone releasing hormone receptor</td>
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<tr>
<td>GLUT</td>
<td>glucose transporter</td>
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<td>Grb2</td>
<td>growth factor receptor-bound protein 2</td>
</tr>
<tr>
<td>Go</td>
<td>stimulatory G protein</td>
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<td>HG</td>
<td>heart girth</td>
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<td>IGFBP</td>
<td>insulin-like growth factor binding proteins</td>
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<td>IM</td>
<td>intramuscular</td>
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<td>IRS</td>
<td>insulin receptor substrate</td>
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<td>IUGR</td>
<td>intrauterine growth retardation</td>
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<td>IVGTT</td>
<td>intravenous glucose tolerance test</td>
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<tr>
<td>JAK</td>
<td>janus kinase</td>
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<tr>
<td>K⁺</td>
<td>potassium</td>
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<td>LEA</td>
<td>loin eye area</td>
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<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metalloproteinases</td>
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<tr>
<td>mo</td>
<td>month</td>
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<tr>
<td>Na⁺</td>
<td>sodium</td>
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<td>NPY</td>
<td>neuropeptide Y</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
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<tr>
<td>NRC</td>
<td>National research council</td>
</tr>
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<td>Ob-R</td>
<td>leptin receptor</td>
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<td>PAPP-A</td>
<td>pregnancy-associated plasma protein A</td>
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<td>PDK-1</td>
<td>phosphoinositide-dependent kinase-1</td>
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<tr>
<td>PEPCK</td>
<td>phosphoenolpyruvate carboxykinase</td>
</tr>
<tr>
<td>PG</td>
<td>prostaglandin</td>
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<tr>
<td>PI3K</td>
<td>phosphatidylinositol 3-kinase</td>
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<tr>
<td>PIP3</td>
<td>phosphatidylinositol 3,4,5-triphosphate</td>
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<tr>
<td>PIT-1</td>
<td>pituitary-specific transcription factor 1</td>
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<td>PK</td>
<td>protein kinase</td>
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<td>pro-opiomelanocortin</td>
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<td>SOCS-3</td>
<td>suppressor of cytokine signaling 3</td>
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<td>SOS</td>
<td>son of Sevenless</td>
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<tr>
<td>SRIF</td>
<td>somatostatin</td>
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<tr>
<td>STAT</td>
<td>signal transducer and activator of transcription</td>
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<td>TBS</td>
<td>tris-buffered saline</td>
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<td>TC</td>
<td>total cholesterol</td>
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<td>TG</td>
<td>triglycerides</td>
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<td>VFA</td>
<td>volatile fatty acids</td>
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<td>week</td>
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<tr>
<td>α-MSH</td>
<td>alpha-melanocyte-stimulating hormone</td>
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LITERATURE REVIEW

EFFECTS OF POOR MATERNAL NUTRITION

The concept of fetal programming is explained in the developmental origins of adult disease theory. This theory suggests that alterations in fetal nutrition and endocrine status may result in developmental adaptations that permanently change the structure, physiology, and metabolism of the offspring. Therefore, poor nutrition during fetal life can predispose offspring to metabolic, endocrine, and cardiovascular disease (Barker and Clark, 1997). Programming involves resetting fetal development to accommodate the environment into which the fetus expects to be born, similar to the maternal and placental endocrine, and metabolic environment (Gluckman and Harding, 1997). Programming due to an inadequate environment in-utero allows the fetus short-term survival. However, these permanent changes can be harmful if the post-natal environment does not match that of the intrauterine environment, and can lead to disease in adult life (Kanaka-Gantenbein, 2010). Intrauterine growth retardation (IUGR) can result from changes in fetal programming due to poor maternal nutrition and can be detrimental to post-natal growth (Wu et al., 2006).

Intrauterine growth retardation is defined as the impaired growth and development of the mammalian embryo/fetus or its organs during pregnancy (Wu et al., 2006). It is associated with many negative consequences, permanently affecting growth and development of the offspring (Wu et al., 2006). This can result in impaired growth, decreased milk production, and decreased feed efficiency. Offspring with IUGR tend to have decreased birth weight and decreased organ weight due to the changes in energy allocation as a result of programming (Barker, 2006). Development of permanent lifetime health problems such as altered insulin secretion, insulin resistance, and cardiovascular disease in adult life is a concern for IUGR offspring (Da Silva et
As IUGR progeny reach reproductive age, they tend to have poor reproductive performance and delayed onset of puberty (Da Silva et al., 2002). These long-term effects can be very costly for animal producers since there is a high mortality rate of IUGR animals.

Intrauterine growth retardation is a problem in the agriculture industry and is considered an obstacle in achieving the main goals of animal production such as, enhancing efficiency of feed/forage utilization, and improving quality of meats, eggs, milk, and fiber (Wu et al., 2006). Body composition and growth rate are altered in IUGR animals, influencing the tenderness and palatability of meat. Fast growing animals tend to have a greater number of muscle fibers with smaller cross sectional areas, hence produce better quality meat (Gondret et al., 2005). For example, the concentration of glycogen and rate of glycolysis in muscle affects the production of lactic acid, meat pH and its water holding capacity, and the amount of intramuscular fat determines the rate of lipid peroxidation, which can cause changes in meat color and taste (Fang et al., 2002; Wu et al., 2006). The slower growth rate associated with IUGR animals, leads to an increase in fat and decrease in protein accretion.

Intrauterine growth retardation can be caused by many factors including, genetics, environmental stress, and poor maternal nutrition, due to under or over feeding, which will be the focus of this literature review (Wu et al., 2006). The intrauterine environment is an important determinant of fetal growth since it is dependent upon placental transfer from the mother (Wilson, 2002). Changes to maternal nutritional status can hinder fetal growth, since the fetus is fully dependent on transfer of oxygen and nutrients across the placenta for substrate supply (Gluckman and Harding, 1997). Poor maternal nutrition causes substrate limitation that can result in impaired fetal development leading to health problems in adulthood (Gluckman and Harding, 1997). Wallace et al. (2004) demonstrated that overfeeding, like underfeeding, once
pregnancy is established, retards fetal growth in sheep. Therefore, both maternal under-nutrition and over-nutrition can result in permanent negative consequences in the offspring (Wu et al., 2006).

For example, ruminants, such as cattle and sheep, graze in pastures and rangelands consuming forages. The quality of forage on rangeland can be poor, especially during the winter and periods of drought, and is insufficient for optimal nutrition of growing, gestating, and lactating animals (Fontaneli et al., 2005). This is a problem since the gestating period for ewes is normally during winter when forage quality and quantity are declining (Hoaglund et al., 1992). Therefore, under-nutrition at the fetal level can occur, resulting in decreased fetal growth (Mellor, 1993; Vonnahme et al., 2003; Wu et al., 2006). It has been shown that nutrient uptake of grazing ewes in the western United States is usually less than 50% of the National Research Council (NRC, 1985) recommendations and is considered nutrient-restriction (Thomas and Kott, 1995).

Maternal over-nutrition is just as detrimental to the fetus as under-nutrition. Over-nutrition is an increase in either protein or energy, or a combination of both. Long et al. (2010) produced a maternal obese sheep model where ewes were fed 150% NRC recommendations from 60 d before conception until parturition, resulting in a 30% body weight (BW) increase by breeding and a 52% BW increase by 135 d of gestation. An energy rich environment during fetal programming such as these has been reported to lead to the development of excess abdominal fat and insulin resistance, demonstrating that similar to under-nutrition, over-nutrition can also predispose offspring to developing diseases in adult life (Kanaka-Gantenbein, 2010).
Flushing, the practice of increasing energy intake to enhance the rate of ovulation in animals so that the number of embryos/fetuses is greater, is one case where management to increase the number of offspring can result in programming changes during fetal life (Cole, 1990). Multi-fetal pregnancies increase total placental weight, but placental mass per fetus is reduced, affecting fetal growth (Wootton et al., 1983; Redmer et al., 2004). High prolificacy in sheep is desirable because it increases the profitability of lamb production, but an increased number of fetuses in-utero results in decreased birth weight (Gootwine et al., 2006). This can result in negative consequences, that have permanent effects in post-natal life (Wallace et al., 2004). Overfeeding, during part or even all of gestation, can have negative pregnancy outcomes (Han et al., 2000; Nissen et al., 2003). Thus, the management scheme to increase the number of offspring can result in reduced birth weight and cause post-natal problems (Gootwine et al., 2006).

Poor maternal nutrition results in many negative consequences, but the timing of insult during gestation can have different effects on offspring post-natal growth. Many studies have determined the effects of poor maternal nutrition during specific periods of gestation including pre-gestation, early gestation, mid-gestation, late gestation, or a combination of these periods. The first half of gestation in sheep and humans is critical for placental growth and vascularization, and fetal organogenesis (Stegeman, 1974; Reynolds and Redmer, 1995; Schneider, 1996). Nutrient-restriction only during mid-gestation can result in variable placental and fetal growth, but if extended throughout gestation, fetal growth can be compromised (Redmer et al., 2004). Gardner et al. (2005) reported that under-nutrition, confined to early gestation might have an impact on adult cardiovascular function in sheep. Functional development of the pancreas, in sheep, takes place during mid to late gestation, so under-
nutrition during late gestation affects both the development and function of the pancreas. This can impair glucose tolerance later in life (Wallace and Bassett, 1966). Ford et al. (2009) reported increased pancreatic weight at mid-gestation in offspring born to ewes overfed starting 60 d before conception. Ford et al. (2007) demonstrated hyperglycemia and altered insulin secretion after a glucose tolerance test in lambs born to ewes nutrient-restricted starting at d 28 of gestation. Over-nutrition, beginning before conception and lasting throughout gestation, can decrease fetal pancreatic weight by late gestation and increase leptin concentrations of offspring (Long et al., 2010; Zhang et al., 2011). Gardner et al. (2005) reported that under-nutrition confined to late gestation alters glucose-insulin homeostasis of the adolescent, providing evidence of post-natal effects due to poor maternal nutrition during gestation, such as glucose intolerance and insulin resistance. These authors also demonstrated reduced expression of glucose transporter (GLUT) 4, in these animals, causing reduced glucose uptake in adipose tissue (Gardner et al., 2005). Overall, these reports demonstrate that nutritional insult at any time during gestation can negatively influence post-natal growth and metabolism of the offspring.

Altered hormonal sensitivity is a common feature of the pathophysiology of IUGR, which could cause the long-term consequences associated with IGUR (Gluckman and Harding, 1997). Many hormones regulate fetal growth including insulin-like growth factors (IGF)-I and -II, and insulin. Placental glucose transfer is a major pathway in the regulation of fetal growth since it is responsible for regulating insulin release from the pancreas (Gluckman and Harding, 1997). Insulin, in turn regulates fetal IGF-I release (Gluckman and Harding, 1997). The role of IGF-II is more prominent in embryonic growth, whereas IGF-I is prominent in late gestational and post-natal growth (Gluckman and Harding, 1997). Studies have demonstrated that maternal nutritional status before and in early pregnancy influences not only fetal growth rate, but also
development of the fetal glucose-insulin axis, and thus, post-natal glucose tolerance and insulin sensitivity (Bloomfield et al., 2013). Given that programming affects insulin sensitivity, it may explain the origin of the association between IUGR and diabetes in humans (Gluckman and Harding, 1997). The release of leptin from adipose tissue is regulated by insulin, and leptin regulates energy expenditure and food intake, making it an important hormone in the growth process. Both insulin and leptin are critical hormones in nutrient storage, especially related to adiposity. Therefore, programming due to poor maternal nutrition can lead to changes in hormones of the somatotropic axis as well as the insulin-glucose axis and leptin concentrations, causing permanent changes in offspring development and potentiating disease later in life.
SOMATOTROPIC AXIS

Overview

The somatotropic axis is a multilevel hormonal system consisting of neuropeptides from the hypothalamus, including growth hormone releasing hormone (GHRH) and somatostatin (SRIF); growth hormone (GH) produced from the anterior pituitary gland; IGF-I and -II and their binding proteins (IGFBP), produced from the liver, and peripheral tissues including adipose, skeletal muscle, and bone (Skaar et al., 1994; Renaville et al., 2002). This axis is important in the regulation of growth, development, energy, and protein metabolism (Gerrard and Grant, 2006). Measures of the somatotropic axis can be predictive of nutritional status and growth rate of individual animals (Rausch et al., 2002; Govoni et al., 2011).

Somatotrope cells, in the anterior pituitary gland, produce and secrete GH (Tuggle and Trenkle, 1996). Growth hormone is regulated by two hypothalamic hormones, GHRH and SRIF (Kazmer et al., 2000; Sosa et al., 2009). The arcuate (ARC) nucleus of the hypothalamus releases GHRH, allowing it to bind to its receptor on the somatotrope cells of the anterior pituitary gland and stimulate GH release. Somatostatin, produced by the neurons of the anterior periventricular nucleus, inhibits the release of GH from the somatotrope cells.

Upon secretion, GH is released into circulation where it can bind to GH binding protein (GHBP), which can then bind to hepatic GH receptors (GHR; Baumann, 1994). Growth hormone can also act on other tissues such as adipose, bone, and muscle, by binding to specific cell surface receptors. It is a major regulator of metabolism and post-natal longitudinal growth by acting in both indirect and direct manners (Breier, 1999). Growth hormone stimulates
synthesis of IGF in tissues, and in turn, IGF (-I and –II) mediate many actions of GH (Renaville et al., 2002)

Insulin-like growth factor-I and -II act in an endocrine manner in circulation as well as an autocrine/paracrine manner locally. Once released from the liver into circulation, IGF can bind to one of six IGFBP. These binding proteins act as carriers of IGF and increase IGF half-life (Renaville et al., 2002). Most IGF binds to IGFBP-3 and an acid labile subunit (ALS), forming a tertiary complex. Over the years, our laboratory has demonstrated how IGF-I, IGFBP-2, and IGFBP-3 respond to changes in growth, development and nutritional status (Rausch et al., 2002; Govoni et al., 2003, 2004).

Locally produced IGF can exhibit autocrine and paracrine effects on bone, muscle, and adipose tissue through interactions of cell surface IGF receptors (Jones and Clemmons, 1995). Insulin-like growth factors have a major role in skeletal muscle regeneration and hypertrophy as well as mammary gland cell proliferation, survival during pregnancy and lactation (Hadsell, 2004; Clemmons, 2009). Growth hormone has insulin-antagonistic properties and fluxes of GH can cause insulin resistance (Holt, 2003). In contrast, IGF-I has insulin-like effects and increases insulin sensitivity (Annunziata et al., 2011).

These hormones of the somatotropic axis all contribute to the overall growth rate and metabolism of animals, making the study and knowledge of this axis essential to the understanding of post-natal growth and development. It is also important to understand how factors such as age and nutrition can influence the somatotropic axis, therefore affecting growth.
Growth hormone

Growth hormone is a 22 to 23 kDa protein that exhibits structural homology among species with prolactin and placental lactogen (Goffin et al., 1996). Growth hormone and the GHR belong to a large family of cytokine peptides and their receptors (Bazan, 1990). Most GH circulates in the blood as a monomer, but other variant forms arise from alternate splicing during transcription (Baumann, 1999). Growth hormone is composed of four anti-parallel helical bundles connected by loops of varying length (Frank and Messina, 2002). The human, rat, bovine, and ovine GH gene sequences are similar, all containing 2.6 to 3.0 kilobase pairs and five exons and four introns (Barta et al., 1981; DeNoto et al., 1981; Tuggle and Trenkle, 1996). The ovine gene has been determined to be more similar to the bovine gene than other species. In most species such as cattle and pigs, there is one GH gene, but in sheep, multiple copies of the GH gene have been found (Gootwine et al., 1998). Sheep have two alleles at the ovine gene locus: the GH1 allele with a single GH copy and the GH2 allele that has two GH copies of the GH coding region (GH2-N and GH2-Z; Valinsky et al., 1990; Gootwine et al., 1993). These two copies of GH2 are almost identical in nucleotide sequence, but only GH2-N is found in the anterior pituitary gland (Gootwine et al., 1998). Ewes with an extra copy of the GH gene have been shown to have a greater ovulation rate and increased plasma GH, but also developed more health problems (Adams and Briegel, 2005).

Growth hormone receptor is a type I glycoprotein, cytokine receptor that is known to utilize the Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway (Bazan, 1990; Brooks et al., 2008). The full length GHR of various species are in the range of 600 amino acids in length and contain large cytoplasmic domains (Frank and Messina, 2002). Alternatively spliced forms of the GHR gene exist and encode receptors different from
the full-length GHR (Frank and Messina, 2002). One of these isoforms circulates as a high affinity GHBP (Frank and Messina, 2002).

In humans, about 50% of circulating GH is associated with GHBP (Baumann et al., 1988). Two independent mechanisms derive GHBP. Growth hormone binding protein is composed of a soluble form of the GHR extracellular domain (Frank and Messina, 2002) and can stimulate and inhibit GH action (Baumann, 2001). Most GHBP exists in a 1:1 ratio of GH:GHBP (Frank and Messina, 2002). In circulation, GHBP increases the half-life of bound GH and acts to stabilize GH bioavailability (Baumann, 2001).

Both GHR and GHBP are derived from the same gene either by alternate mRNA splicing or by proteolytic cleavage of the GHR protein (Baumann, 1994). The extracellular domain of the receptor consists of two fibronectin type III β sandwich domains connected by a helical transmembrane linker (Brooks et al., 2008). The intracellular domain is composed of two motifs, Box I and Box 2. These motifs bind the tyrosine kinase, JAK2 and several tyrosine residues that are substrates for phosphorylation by JAK2 (Brooks et al., 2008). Box 1 is a proline rich sequence found in the cytoplasmic domain of the GHR membrane and Box 2 is a short acidic section, 30 residues long (Murakami et al., 1991; Frank and Messina, 2002). Growth hormone receptor is N-glycosylated and spans the membrane surface. The GH molecule has two binding sites for the extracellular domain of the GHR and forms a 1GH:2GHR complex by sequential homodimerization (Breier, 1999). Site 1 has a higher affinity for GH binding than site 2 and interacts with the first receptor, leaving the second site to interact with the second receptor (Cunningham et al., 1991). The GHR-GHR dimerized receptor is the activated form of GHR and is required for stabilization of the GH complex (Frank and Messina, 2002).
Growth hormone binding to the GHR activates a number of intra-cellular pathways including mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3K) cascade (Figure 1). Growth hormone receptor association with JAK2 is critical to GH action (Frank and Messina, 2002). Other tyrosine kinases are activated in response to GH including focal adhesion kinase (FAK; Zhu et al., 1998). Growth hormone receptor signaling results in activation of STAT molecules by recruiting the STAT molecule to the tyrosine phosphorylated receptor or JAK, allowing tyrosine phosphorylation of the STAT src homology 2 (SH2) domain (Davey et al., 1999). Growth hormone promotes the tyrosine phosphorylation and DNA-binding capacity of STAT1, 3, 5a, and 5b (Campbell and Scanes, 1995). Activation of JAK2 by GH is also required for the MAPK cascade, through recruiting adaptor protein Shc, which then binds growth factor receptor-bound protein 2 (Grb2), and son of Sevenless (SOS) activating Ras, Raf and in turn, Mek. The PI3K cascade is activated by adaptor protein insulin-receptor substrate (IRS), leading to activation of Akt. These pathways lead to cell proliferation and gene transcription (Kelly et al., 2001).

There has been detection of GHR mRNA and immunoreactivity in most post-natal tissues with the greatest abundance in liver and adipose tissue (Breier, 1999). Pituitary-specific transcription factor-1 (PIT-1) binding directs cell specific gene expression in somatotrope cells (Dasen and Rosenfeld, 2001). Pituitary specific transcription factor-1 is required for the development and maintenance of somatotrope cells and regulation of GH by activating transcription of GH1. This leads to increased GH1 mRNA and GH protein, replenishing GH stores (Lin-Su and Wajnrajch, 2002). Within the anterior pituitary gland, the GHRH receptor (GHRH-R) is co-expressed with PIT-1 (Mayo et al., 2000). Transcription of GHRH-R gene can be stimulated by PIT-1, increasing receptor concentration on somatotropes (Mayo et al., 2000).
Regulation of growth hormone secretion

The regulation of GH secretion and its action on peripheral tissues is thought to be the most fundamental determinant of body size (Butler and Le Roith, 2001). Growth hormone is regulated by nutrition and by the hormonal and genetic milieu that controls the timing and rate of growth (Butler and Le Roith, 2001). Growth hormone secretion from somatotrope cells in the anterior pituitary gland occurs under control of a complex neuroendocrine system involving two hypothalamic hormones from the median eminence (Tuggle and Trenkle, 1996; Sosa et al., 2009). Growth hormone releasing hormone stimulates GH release and SRIF inhibits it (Tuggle and Trenkle, 1996; Sosa et al., 2009). The secretory pattern of GH is pulsatile in most species and is dependent on the interaction between GHRH and SRIF at the somatotrope level (Muller et al., 1999). This pattern of GH release is important in determining growth rate in mammals (Butler and Le Roith, 2001). Neurons in the ARC nucleus and anterior periventricular nucleus produce GHRH and SRIF, respectively (Devesa et al., 1992).

Growth hormone releasing hormone was originally isolated from a human pancreatic tumor and identified as a hypothalamic peptide that stimulates the release of GH from the pituitary (Guillemin et al., 1982). It stimulates GH synthesis through increasing intracellular cyclic adenosine monophosphate (cAMP) concentrations, activating protein kinase (PK) A (Mayo et al., 2000). Growth hormone releasing hormone also has mitogenic effects on somatotropes and is required for their normal proliferation during development (Cella et al., 1994), most likely through activation of MAPK (Mayo et al., 2000). Growth hormone releasing hormone receptor is a class II G protein coupled receptor mainly found on pituitary somatotrope cells (Mayo, 1992; Gaylinn et al., 1993). It is 423 amino acids long and consists of seven transmembrane domains. After GHRH is released from the ARC nucleus of the hypothalamus, it
Growth hormone binds to growth hormone receptor (GHR) activating the phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways. Binding activates Janus kinase (JAK) 2, causing phosphorylation of other substrates such as signal transducers and activators of transcription (STAT) molecules, focal adhesion kinase (FAK), insulin receptor substrate (IRS) -1/2, and Shc. Phosphorylation of IRS-1/2 leads to the PI3K pathway and activation of Akt. The MAPK pathway is activated by phosphorylation of Shc, which recruits growth factor receptor-bound protein 2 (Grb2) and son of Sevenless (SOS), activating Ras, then Raf and Mek, leading to proliferation. Tyrosine phosphorylation of STAT molecules promotes their DNA-binding capacity (Modified and reprinted from Postel-Vinay and Kelly, 1996 with permission from Elsevier.)
binds to the GHRH-R located on somatotrope cells. Binding of GHRH activates a stimulatory G-protein (G_s) by catalyzing binding of GTP to the \( \alpha \) subunit (Lin-Su and Wajnrajch, 2002). Adenylyl cyclase is stimulated and increases intracellular cAMP. A sodium (Na\(^+\)) channel in the somatotrope opens in response to receptor activation, causing depolarization to take place. As a result, a voltage-gated calcium (Ca\(^{2+}\)) channel opens and the influx of Ca\(^{2+}\) causes release of GH from secretory granules (Petersenn and Schulte, 2000; Lin-Su and Wajnrajch, 2002).

Not only does the binding of GHRH to its receptor stimulate GH secretion, but GH synthesis is stimulated as well. This occurs in response to the increase in cAMP stimulating PKA to phosphorylate and activate cAMP response element-binding protein (CREB; Petersenn and Schulte, 2000). Growth hormone production increases due to an increase in transcription of the GH1 gene and GHRH receptor gene (Mayo et al., 1995; Muller et al., 1999; Petersenn and Schulte, 2000).

Somatostatin acts in opposition to GHRH by inhibiting GH release. Somatostatin is synthesized as a 116 amino acid precursor, cleaved by endopeptidases, resulting in two forms secreted from the hypothalamus: SRIF-28 and SRIF-14 (Krantic et al., 2004). These two forms are produced in different proportions by neurons and secretory cells through the differential processing of a common pro-SRIF precursor (Patel and O’Neil, 1988). Both SRIF-14 and SRIF-28 are found in the periphery and central nervous system, with SRIF-14 as the predominant form (Krantic et al., 2004). Both monomers and dimers of SRIF-14 exist in circulation (Devesa et al., 1992). The two forms of SRIF have overlapping physiological functions. The main function of SRIF is to inhibit hormone secretion, particularly GH secretion. Administration of SRIF-28 in cattle has been reported to effectively inhibit GHRH induced GH release, proving SRIF involvement in regulation of GH (Kazmer et al., 2000). Somatostatin regulates potassium (K\(^+\))
channels causing hyperpolarization of the plasma membrane, decreasing Ca\(^{2+}\) influx (Benali et al., 2000). This reduces intracellular Ca\(^{2+}\), thus inhibits GH release (Benali et al., 2000). The actions of SRIF are mediated by the coupling of G high affinity plasma membrane receptors to adenyl cyclase, K\(^+\), Ca\(^{2+}\) channels, and protein tyrosine phosphatase, with the help of G proteins (Koch and Schonbrunn, 1988).

Once GH is released from the anterior pituitary gland, it circulates in the blood and exerts many biological functions. Growth hormone can act on many tissues including liver, muscle, adipose, and bone, to stimulate production of IGF-1 locally (Isaksson et al., 1987). Circulating IGF acts to mediate the local autocrine/paracrine effects of GH (Butler and Le Roith, 2001). Growth hormone also acts directly on adipose tissue by decreasing lipogenesis and insulin sensitivity (Renaville et al., 2002). Many of the effects of GH on growth and metabolism are mediated indirectly via control of the synthesis of other growth factors, such as IGF (Butler and Le Roith, 2001). This makes GH the main regulator of growth via the production of systemic and local IGF-I production (Monzavi, 2002).

Insulin-like growth factors

Insulin-like growth factor-I and -II are single-chain homologous polypeptides, similar in structure to insulin, that regulate proliferation and differentiation of many different cell types and exert insulin-like metabolic effects (Cohick and Clemons, 1993; Hwa et al., 1999). Most tissues of the body produce IGF, with the liver being the predominant source of production (Cohick and Clemons, 1993). The human IGF-I gene is located on the long arm of chromosome 12 (Brissenden et al., 1984; Tricoli et al., 1984). It has multiple promoters and contains six exons, four of which can be alternatively spliced (Rotwein et al., 1986; Smith et al., 2002). The mature IGF-I is a single 7.5 kDa chain, 70 amino acids in length with three disulfide
bridges (Rotwein et al., 1986). Insulin-like growth factor-I has four domains: A, B, C, and D. The A and B domains are structurally homologous to insulin A and B chains and C is similar to the connecting C peptide in pro-insulin (Humbel, 1989). The amino acid sequence of the mature form of ovine IGF-I is highly homologous to the human, mouse, and rat sequences (Dickson et al., 1991), only differing from human and porcine sequences at a single amino acid residue (Tavakkol et al., 1988). Dickson et al. (1991) found that the DNA sequence of the ovine gene is highly homologous to that of human IGF-I, with the nucleotide sequence of the coding regions exhibiting 89 to 100% homology, and the exon-intron boundaries showing co-incident, splice consensus sequences. Bovine IGF-I was found to be identical to human IGF-I by Honegger and Humbel (1986). Insulin-like growth factor-II is similar to IGF-I in structure, but only has 67 amino acids (Rotwein et al., 1986). The IGF-II gene is about 30 kilobases long and has three promoters. The liver, as well as other tissues, produce IGF-II, but it is not regulated by GH (Clemmons, 2006; Kaplan and Cohen, 2007). Insulin-like growth factor-II has an important role in embryonic and fetal growth, but less of a role in post-natal life, when the role of IGF-I is more prominent (Annunziata et al., 2011). Both IGF-I and –II are conserved among species.

The IGF can interact with type I and type II IGF receptors, and insulin receptors. The type I IGF receptor is a heterotetrameric glycoprotein, tyrosine kinase receptor that spans the cell membrane and mediates the effects of IGF-I and -II (Le Roith, 1996; Baserga et al., 1997). Insulin-like growth factor-I binds with high affinity to the type I IGF receptor. Insulin and IGF-II can also bind to this receptor, but with lower affinity (Le Roith, 2003; Himpe and Kooijman, 2009). The type I IGF receptor is homologous to the insulin receptor and is a disulfide-linked dimer composed of two α and two β subunits in each half (Le Roith, 1996). The precursor peptide of the type I IGF receptor is 1,367 amino acids and when cleaved produces the subunits
(Le Roith, 1996). The α subunits are extracellular and modified by N-glycosylation and the β subunits anchor the receptor in the membrane and contain a tyrosine kinase domain in their cytoplasmic portion (Le Roith, 1996).

The type II IGF receptor is structurally distinct from the type I IGF and insulin receptors in that it contains a short cytoplasmic region that lacks tyrosine kinase activity (Le Roith, 1996). It is a monomeric, 250 kDa, transmembrane protein. Insulin-like growth factor-II binds with high affinity to the type II IGF receptor, IGF-I binds with less affinity, and insulin does not bind to this receptor (Shimasaki and Ling, 1991). In addition to binding IGF-II, it can also bind mannose-6-phosphate-containing ligands, and interact with G protein pathways (Nissley and Lopaczynski, 1991; Ikezu et al., 1995). Within each of the 15 contiguous extracellular repeats of the receptor, there are eight conserved cysteine residues with similar disulfide bonding (Le Roith, 1996). This receptor has anti-proliferative and pro-apoptotic activities by sequestering IGF-II and reducing its interaction with the type II IGF receptor (Hankins et al., 1996; O’Gorman et al., 2002; Pollak, 2008). Hybrid receptors can also be formed due to the close similarity between the insulin receptor and type I IGF receptor and can bind both IGF-I and IGF-II with high affinity, and insulin with lower affinity (Soos et al., 1993).

Upon IGF-I binding to the α subunit of the type I IGF receptor, activation of the intrinsic tyrosine kinases of the β subunit and three tyrosine residues that act as docking sites for signaling proteins, occurs (Himpe and Kooijman, 2009). The signaling proteins then recruit other substrates leading to activation of different signaling cascades, including the PI3K pathway and the MAPK pathway, that stimulate cell proliferation and survival (Figure 2; Le Roith, 2003; Himpe and Kooijman, 2009). There are two main substrates involved in signal transduction
Insulin-like growth factor (IGF) -I or -II binds to the type I IGF receptor (IGFIR), autophosphorylating the β subunit tyrosine kinases. This activates two major pathways: phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK). Tyrosine residues act as docking sites for signaling proteins. The PI3K pathway is activated by phosphorylation of insulin-receptor substrate (IRS) -I, resulting in an increase of phosphatidylinositol 3, 4, 5-triphosphate (PIP3), which binds to Akt. The MAPK pathway is activated by binding of Shc to the receptor and recruitment of growth factor receptor-bound protein 2 (Grb2) and son of Sevenless (SOS), activating Ras then Raf and Mek, eventuating in the activation of extracellular signal-regulated kinase (ERK) -1/2. Proliferation can then be induced (Modified and reprinted with permission from: Bikle, 2008).
of type I and insulin receptors: IRS-1 and Shc (Le Roith, 1996). Activation of the PI3K pathway occurs by binding of the SH2 domain of its regulatory subunit to IRS-1, which IGF type I receptor phosphorylates before binding (Vincent and Feldman, 2002). The concentrations of phosphatidylinositol 3, 4, 5-triphosphate (PIP3) are increased and bind to the pleckstrin homology domain of protein kinase B (AKT/PKB) and phosphoinositide-dependent kinase-1 (PDK-1). This kinase phosphorylates residue Thr308 on AKT/PKB as well as other proteins, including p70 ribosomal protein S6 kinase, PKC and PKA.

Another pathway involves isoforms of the MAPK family, extracellular signal-regulated kinase (ERK) -1 and -2. This pathway is activated by binding of Shc and recruitment of Grb2, which binds SOS, a guanine nucleotide exchange factor, that activates Ras (Himpe and Kooijman, 2009). Then Ras activates Raf, in turn activating Mek-1/2, then activating ERK-1 and ERK-2 (Himpe and Kooijman, 2009). This pathway causes cell differentiation and migration to occur (Yoshida et al., 2006).

**Insulin-like growth factor binding proteins**

Insulin-like growth factor binding proteins maintain IGF in circulation and transport them to target tissues where they can promote cell growth, proliferation, differentiation, and cell survival through binding to the type I IGF receptor (Denley et al., 2005). These proteins increase the half life of IGF and mediate their metabolic clearance rate (Firth and Baxter, 2002). There are six mammalian IGFBP (IGFBP-1 to 6) derived from distinct genes (Firth and Baxter, 2002). The precursor forms of all six IGFBP have secretory signal peptides, 20 to 39 amino acids in length and the mature proteins are extracellular (Firth and Baxter, 2002). In their soluble form, the IGFBP have greater affinity for the IGF than the type I receptor does, potentially inhibiting
the biological effects of the hormone (Denley et al., 2005). Insulin-like growth factor binding protein-1 through -5 bind both IGF-I and IGF-II with similar affinities, but IGFBP-6 has a higher affinity for IGF-II than IGF-I (Headey et al., 2004; Siwanowicz et al., 2005).

The IGFBP family is classified as cysteine rich proteins (16 to 20 cysteines in the pre-peptides) with similar amino acid sequences. The cysteines are located at the N-terminal third and at the C-terminal third of the proteins. There are three domains, of approximately equal length, that make up the IGFBP; a conserved N-terminal domain, a variable linking domain (L domain), and a conserved C-terminal domain (Bach et al., 2005). The N and C-terminal domains are both necessary in IGF binding and they are connected by the L domain that is not involved in binding (Bach et al., 2005).

The N-terminus contains 80 to 93 amino acid residues after the signal peptides and has 58% similarity among IGFBP. Of the 16 to 20 cysteines in the pre-peptides, this domain consists of 12 cysteins (Duan, 2002). There are intra-domain disulfide bonds in the N-terminus, but no inter-domain disulfide bonds due to an even number of cysteine residues (Bach et al., 2005). Sub-domains form between cysteines that are close together in the primary sequence, two of these sub-domains are within the N-terminus domain. The first sub-domain consists of the first six cysteines and the second sub-domain contains the next four cysteines (Hwa et al., 1999). The local motif (GCGCCxxC) in the N-terminal domain is well conserved, except in IGFBP-6 where a GCAEAEGC sequence is substituted, but is still able to maintain high affinity for IGF (Hwa et al., 1999). This motif is considered important in high affinity IGF binding, which is the only function of this domain (Hwa et al., 1999; Bach et al., 2005).
The second domain, the L domain, separates the N-terminal domain from the C-terminal domain. This domain is not conserved and is not involved directly with IGF binding (Bach et al., 2005). Post-translational modifications, such as glycosylation and phosphorylation of IGFBP, occur in this mid-region (Firth and Baxter, 2002). Insulin-like growth factor binding protein -3 has three potential sites of N-glycosylation at an asparagine residue that is part of the consensus sequence Asn-X-Ser/Thr, located at Asn$^{89}$, Asn$^{109}$, and Asn$^{172}$ (Firth and Baxter, 1999). Insulin-like growth factor binding protein-5 and -6 are O-glycosylated, but there is no consensus sequence for predicting sites for O-glycosylation (Neumann and Bach, 1999). Glycosylation does not affect IGFBP high-affinity binding to IGF, but modifies other properties such as stability and susceptibility to proteolysis and circulating half-life (Marinaro et al., 2000; Firth and Baxter, 2002). Post-translational modification by phosphorylation occurs in IGFBP-1, -3, and -5 (Coverley and Baxter, 1997). Phosphorylation allows cells to regulate the activities of numerous intracellular proteins, such as proteins involved in the cell cycle, signal transduction pathways and in gene expression (Jones et al., 1991). Phosphorylation occurs predominantly at serine residues in the mid-region of the proteins. Phosphorylation of IGFBP-3 may affect interactions with ALS or cell surface (Coverley, 2000).

The C-terminal domain is highly conserved and 34% homologous among IGFBP. This domain consists of six cysteines and studies indicate intra-domain disulfide bond formation among the residues (Duan et al., 2002). Due to the level of homogeneity between the last five cysteines in the C-terminal, the tertiary structure of this domain should be almost identical in all IGFBP. The C-terminal domain in IGFBP-3 and -5 contains an 18 amino acid region where ALS can bind (Firth and Baxter, 2002). The C-domain has a wide range of functions including IGF-independent actions such as growth inhibition, promotion of apoptosis, and modulation of
cell migration (Bach et al., 2005). This domain of IGFBP-1 and -2 contain RGD motifs that bind integrins, leading to altered cell migration (Wang et al., 2006).

The most abundant IGFBP in circulation is IGFBP-3. Insulin-like binding protein-3 has a very high affinity for both IGF-I and IGF-II (Cohick and Clemmons, 1993). Insulin-like growth factor binding protein-3 is 46 to 53 kDa when glycosylated and 29 kDa just from its amino acid sequence (Yamada and Lee, 2009). There are three potential N-glycosylated sites (Asn-X-Ser/Thr) located in the non-conserved mid-region of the IGFBP-3 sequence (Asn\textsuperscript{89}, Asn\textsuperscript{109}, Asn\textsuperscript{172}-sites 1, 2, 3 respectively; Firth and Baxter, 1999). In circulation, this binding protein is in its glycosylated form. The glycosaminoglycan binding domains of IGFBP-3 allow localization to the extracellular matrix and cell surfaces, decreasing the affinity of IGFBP-3 for IGF-I, thus enhancing IGF action (Yamada and Lee, 2009). Two major phosphorylation sites of IGFBP-3 exist and dephosphorylation of the protein appears to enhance its binding to ALS and cell surfaces. Acid labile subunit is a glycoprotein, ~ 85 kDa that contains several leucine rich domains, known to facilitate protein-protein interactions and binding of IGFBP-3 (Boisclair et al., 2001). It is capable of sequestering IGF from IGF type I receptor since it has greater affinity for IGF than its receptor. This decreases IGF bioavailability and action (Yamada and Lee, 2009). In mammals, IGFBP-3 carries most circulating IGF-I and -II (> 90%) in a 150 kDa ternary complex also made up of ALS (Boisclair et al., 2001). Like IGF, the liver is the main source of IGFBP-3 and ALS. Growth hormone stimulates production of all three subunits of this complex and regulates their concentrations in circulation (Kelly et al., 2002).

The second most abundant binding protein in circulation is IGFBP-2 (Wheatcroft and Kearney, 2009). It is a ~36kDa protein and is not glycosylated or phosphorylated (Shimasaki and Ling, 1991; Wheatcroft and Kearney, 2009). The distribution of cysteines in IGFBP-2 is
similar to IGFBP-1 (Shimasaki and Ling, 1991). The N-terminal of IGFBP-2 is 128 amino acids in length and includes the first 12 cysteines. Exon 4 contains the coding region for the C-terminal and the 3’ non-coding region. Insulin-like growth factor binding protein-2 inhibits IGF by hindering cell growth and proliferation (Jones and Clemmons, 1995). Insulin-like growth factor, IGFBP-3, and IGFBP-2 concentrations in serum are age dependent. Concentrations of IGF and IGFBP-3 are low at birth, peak at puberty and decrease thereafter, while IGFBP-2 concentrations are increased in infancy and decreased in adulthood (Cohen and Rosenfeld, 1994; Govoni et al., 2003, 2004).

The other IGFBP bind with IGF in a smaller fraction. The liver and kidney express IGFBP-1, which is a non-glycosylated 30 kDa protein (Shimasaki and Ling, 1991). Expression of IGFBP-1 is regulated by insulin and glucocorticoids. Insulin inhibits IGFBP-1 expression and glucocorticoids stimulate it (Kim et al., 1997). Concentrations of IGFBP-1 are dependent on metabolic status (Collett-Solberg and Cohen, 1996). There is an increase in IGFBP-1 in the fetal compartment. This along with the reduced IGFBP-3 in maternal circulation creates a gradient that IGF can move down toward fetal circulation (Kim et al., 1997). Insulin-like growth factor stimulated cell proliferation is inhibited by IGFBP-4 (Mohan et al., 1989). Bone cells mainly express IGFBP-4, which has a role in bone growth (Kim et al., 1997). It acts to decrease proliferation of bone cells (Mohan et al., 1989). The main role of IGFBP-5 is in ovarian function (Kim et al., 1997). Insulin-like growth factor binding protein-5 is a 29 kDa phosphorylated and glycosylated protein that is secreted by many cell types (Firth and Baxter, 2002). Mammary gland development and other embryonic developmental processes involve IGFBP-5 (Flint et al., 2000). Production of IGFBP-5 by mammary epithelial cells increases during involution of the mammary gland in rodents. Insulin-like growth factor binding protein-6 is different than the
other five binding proteins in that it is O-glycosylated and has greater affinity for IGF-II than IGF-I. This binding protein antagonizes follicular development.

Insulin-like growth factor binding protein proteases

Three mechanisms can release IGF from their binding proteins so they can bind to receptors and exert their mitogenic and metabolic effects (Bunn and Fowlkes, 2003). These mechanisms include binding of IGFBP to extracellular matrix (ECM) molecules, phosphorylation of IGFBP, and proteolytic degradation of IGFBP (Bunn and Fowlkes, 2003). Proteolytic cleavage is the most predominant mechanism (Bunn and Fowlkes, 2003). Protease activity regulates IGFBP, generating fragments of IGFBP that alter or decrease their bioavailability. The IGFBP proteases can be serine proteases, metalloproteinases, or cathepsins (Conover and De Leon, 1994).

Cathepsins are lysosomal proteinases that are active at acidic pH 4 to 5.5 and cleave IGFBP-1 and -5 (Conover and De Leon, 1994). Cathepsin D is an acid IGFBP protease and regulates extracellular IGFBP. Zwad et al. (2002) showed that Cathepsin L degrades IGFBP-3. Serine proteinases are the proteases responsible for cleavage of IGFBP-5 (Collett-Solberg and Cohen, 1996). The metalloproteinases include the matrixins (MMP), the adamalysins, and the pappalysins (Bunn and Fowlkes, 2003). The MMP are peptide hydrolases that require a metal ion for their catalytic activity (Collett-Solberg and Cohen, 1996). This family of proteases includes collagenases such as interstitial collagenase (MMP-1), gelatinase A (MMP-2), stromolysis 1 (MMP-3), and gelatinase B (MMP-9; Collett-Solberg and Cohen, 1996). Metalloproteinases may have a role in regulating cellular growth and proliferation through the degradation of IGFBP-3 to enhance IGF bioavailability (Collett-Solberg and Cohen, 1996). The
ADAM 12-S protein, a part of the adamalysin class of proteases, binds and degrades IGFBP-3 (Shi et al., 2000). It also can degrade IGFBP-5, suggesting that it requires the interaction with the positively charged heparin-binding domain only found on these two binding proteins for proteolysis to occur (Bunn and Fowlkes, 2003). Pregnancy-associated plasma protein A (PAPP-A) is the IGF-dependent, IGFBP-4 protease and facilitates IGF release from this binding protein (Bunn and Fowlkes, 2003). These IGFBP proteases are important for IGF action to be possible since IGF cannot bind to receptors when their affinity for IGFBP is greater.

Effect of poor nutrition on the somatotropic axis

In normal growing animals, the concentration of GH declines with age and concentration of IGF-I increases with age (Govoni et al., 2003, 2004). Concentrations of IGFBP-3 increase with age and growth rate and IGFBP-2 concentrations vary depending upon species, but normally decrease with age and growth rate (Rausch et al., 2002; Govoni et al., 2003, 2004). Due to the association of IGFBP-2 and IGFBP-3 with changes in nutrition, these binding proteins can be used as predictors of nutritional status in animals (Rausch et al., 2002). For these reasons, we focused on studying changes in IGFBP-2 and IGFBP-3 concentrations.

Nutritional status plays a major role in determining concentrations of GH, IGF-I, IGFBP-2 and -3. Fasting, nutrient-restriction, and over-nutrition can all alter the concentrations of somatotropic axis hormones. These nutrition-induced changes in hormone concentrations in the mother during gestation can cause inadequate nutrition for the fetus; leading to fetal programming changes. These permanent metabolic changes that take place in-utero can lead to disease later in life (Wu et al., 2006).
The primary adaptation of the fetus to nutrient-restriction late in gestation is to reduce growth by altering its endocrine environment, particularly by reducing endocrine and paracrine IGF-I, which is associated with a reduction of blood flow to protect vital organs (Gluckman and Harding, 1997). Prolonged periods of maternal under-nutrition have been reported to lead to an irreversible, permanent slowing of fetal growth rate in late gestation, which persists after birth, as well as a degree of IGF-I, insulin, and GH resistance (Gluckman and Harding, 1997). Shorter periods of poor maternal nutrition lead to a transient slowing of growth rate followed by catch-up growth, suggesting that catch-up growth may be dependent on the length and severity of substrate limitation in-utero (Mellor, 1979). Ewes undernourished between 60 d before and 30 d after conception carry fetuses that grow slower in mid to late gestation and at 110 d of gestation have abnormal regulation of IGFBP (Gluckman and Harding, 1997). This is compatible with the development of insulin resistance (Gluckman and Harding, 1997). Maternal nutrient-restriction and IUGR have been shown to alter insulin secretion and homeostasis (Hales and Barker, 2001; Lobelo, 2005).

Growing animals, fed a restricted diet over an extended period of time have reduced growth rate, reduced IGF-I and IGFBP-3, and increased GH and IGFBP-2 (Rausch et al., 2002). The increase in GH associated with periods of under-nutrition, reflects stress, hypoglycemia, and low serum free fatty acids (FFA; Breier, 1999). This is due to reduced metabolic clearance rate of GH, facilitated by impaired GH mediated feedback due to altered properties of GHR (Breier, 1999). Growth hormone receptor concentrations positively correlate with nutritional status, suggesting that under-nutrition induces a degree of GH resistance (Breier, 1999). Nutrition is also an important regulator of IGF-I synthesis, and IGF-I has a major role in growth (Straus, 1994). There is a decrease in circulating IGF-I concentration as a result of both fasting and
under-nutrition (Clemmons and Underwood, 1991). Reduced IGF-I during fasting is a result of decreased binding of GH to its receptor (Cohick and Clemmons, 1993). There is also a decrease in hepatic IGF-I and GHR mRNA (Bornfeldt et al., 1989; Straus and Takemoto, 1990). Reduced IGF-I due to protein restriction is more likely in response to GH resistance (Clemmons and Underwood, 1991). Nutritional restriction and post-partum negative energy balance generally result in a decrease in the concentration of both circulating IGF-I and IGFBP-3, due to their lower hepatic synthesis and an up-regulation of IGFBP-2, decreasing the bioavailability of IGF-I for peripheral tissues (Breier, 1999). These changes exhibited in GH, IGF-I, IGFBP-2 and -3 results in a decrease in overall growth rate of an animal.

Over-nutrition, leading to obesity, normally leads to opposite hormonal changes than under-nutrition. Obesity causes an increase in growth rate and increase in adipose deposition. Growth hormone secretion is negatively and independently associated with age and the amount of adiposity in normal animals (Iranmanesh et al., 1991). Increased adiposity causes a blunting effect of GH secretion and the metabolic clearance rate of GH is accelerated (Veldhuis et al., 1991). Insulin-like growth factor-I concentrations in obesity are variable. Different studies have shown increased, normal, or decreased concentrations (Rudman et al., 1981; Marin et al., 1993; Frystyk et al., 1995). Free IGF-I tend to be increased in obesity since IGF-I synthesis and secretion depend on peripheral GH sensitivity, reflected by increased GHR and GHBP concentrations (Frystyk et al., 1995). Concentrations of IGFBP-1 are reduced due to increased peripheral sensitivity to GH as a result of hyperinsulinism (Brismar et al., 1994). Insulin inhibits IGFBP-2 in obesity and IGFBP-3 concentrations are normal or increased in circulation (Argente et al., 1997; Nam et al., 1997). Long et al. (2010) established maternal obesity, due to over-nutrition, before gestation in sheep and the offspring of these obese ewes were insulin resistant.
and gained more adipose tissue than control offspring later in life. The offspring from these overfed ewes also had increased feed intake compared with controls. Both under-nutrition and over-nutrition can have major metabolic and physiological effects. Therefore, poor nutrition in general, whether under-nutrition or over-nutrition can have severe effects on the growth and survival of animals.

INSULIN-GLUCOSE HOMEOSTASIS

Overview

The regulation of feed intake and whole-body energy balance is important in livestock to optimize animal reproduction, growth, lactation, and overall health. Therefore, altering body composition by repartitioning of nutrients to favor lean protein accretion and improve production efficiency is a major goal in animal agriculture (Houseknecht et al., 1998). For these reasons, a clear understanding of energy metabolism, adipose accretion, and feed intake is important to improve animal health and decrease the probability of obesity (Houseknecht et al., 1998). Glucose homeostasis is required to regulate whole-body energy balance. For this to be maintained, insulin must stimulate glucose uptake and storage in adipose and muscle tissue, and stimulate glucose storage and inhibit gluconeogenesis in the liver. Disruptions in homeostasis, due to factors such as changes in nutrient intake, can lead to insulin resistance, causing disorders such as obesity and type 2 diabetes to develop in humans (Saltiel and Kahn, 2001).

Glucose homeostasis in embryonic and early fetal life depends on placental glucose transfer and tissue glucose utilization (Mena et al., 2001). Understanding the changes in glucose and insulin regulation associated with nutrient changes and IUGR is important to determine how these changes to glucose homeostasis in fetal life can have lasting negative effects in post-natal
life. In animal models of IUGR, early developmental shifts in fetal metabolism include increased hepatic gluconeogenic gene expression, glucose production, and decreased pancreatic insulin secretion (Limesand et al., 2006; Thorn et al., 2011). These changes can continue into post-natal life, causing offspring to have increased hepatic glucose production, insulin resistance, and altered insulin secretion (Owens et al., 2007; Park et al., 2008). The activation of gluconeogenesis in IUGR fetuses may be a beneficial adaptive response to maintain glucose supply to vital organs as placental glucose diminishes, but can cause permanent negative consequences post-natally by promoting glucose production in excess of utilization capacity, contributing to hyperglycemia (Thorn et al., 2011).

Blood glucose concentrations must be carefully maintained since mammalian cells depend on a steady supply of glucose as a source of energy. The liver has a major role in blood glucose homeostasis by maintaining the balance between uptake and storage of glucose through glycogenesis and the release of glucose via glycogenolysis and gluconeogenesis (Nordlie et al., 1999). In order for glucose homeostasis to be maintained, plasma glucose concentration is regulated by actions of the pancreatic hormones, insulin and glucagon. These hormones control glucose production and utilization in multiple tissues and organs (Bell and Bauman, 1997).

Insulin is the main metabolic regulator in ruminants, similar to other mammalian species (Sasaki, 2002). The actions of insulin affect carbohydrate, lipid, and protein metabolism. Insulin acts on a variety of tissues including muscle, adipose, liver, and pancreas by altering processes including membrane translocation and enzyme activity. These actions make insulin the major mediator of glucose homeostasis (Sasaki, 2002).

In muscle and adipose tissue, insulin signaling causes translocation of GLUT 4 and increases glucose uptake into the tissues. Insulin acts on hepatocytes to stimulate the utilization
and storage of glucose as lipid and glycogen as well as inhibit glucose production and release (Saltiel and Kahn, 2001). Once plasma glucose concentrations decrease, glucagon release from pancreatic α cells is stimulated (Layden et al., 2010). Glucagon acts in opposition to insulin and stimulates hepatic gluconeogenesis to increase glucose concentrations in plasma (Layden et al., 2010).

Glucose

Placental glucose transfer is important to fetal growth, and is required for regulation of fetal insulin release, which then regulates fetal IGF-I (Gluckman and Harding, 1997). Therefore, the somatotropic axis and the glucose-insulin axis are important in regulating fetal growth and furthermore, post-natal growth. Altered glucose concentrations, due to IUGR, can have a permanent effect on offspring. Glucose is an important carbohydrate required to meet the energy requirements of the body. The main sources of glucose come from the diet and the liver. Ruminants are different from monogastrics in that they depend mostly on hepatic glucose production, whereas monogastrics can absorb glucose from their diet. From birth to about three weeks of age, lambs are considered pre-ruminants and the reticular groove located in the esophagus allows for milk to be transported directly to the abomasum, similar to a monogastric animal (Parish et al., 2009). As these animals grow, rumen development occurs and instead of the abomasum making up most of the stomach in the pre-ruminant, the reticulorumen makes up a greater percentage of the stomach in the mature ruminant (Parish et al., 2009). Around two to three months (mo) of age lambs are considered functional ruminants, as the reticular groove reflux is lost (Parish et al., 2009). The change in dietary nutrient pattern and supply, due to adaptation from pre-ruminant to functional ruminant, causes alterations in hepatic function and energy requiring processes such as glucose synthesis (Seal and Reynolds, 1993). The fetal/pre-
Ruminant lamb is able to utilize glucose as an energy source similar to a monogastric, but due to the microbial fermentation of carbohydrates that occurs in the reticulorumen, mature ruminants must use volatile fatty acids (VFA) that are produced in the rumen and converted to glucose via hepatic gluconeogenesis (Bell and Bauman, 1997). Therefore, in the recently fed, post-absorptive state, ruminants must depend on gluconeogenesis in the liver and on the kidneys for their tissue glucose requirements.

Propionate, one of the major VFA byproducts of pre-gastric fermentation, is the principle precursor for hepatic gluconeogenesis in well-fed ruminants. Propionate is absorbed via the ruminal epithelium into portal venous blood and removed by the liver (Elliot, 1980; Brockman, 1993). The production rate of ruminal propionate and other VFA is directly related to dietary intake of fermentable substrate, suggesting ruminant whole-body glucose production is highly correlated with digestible energy intake (Elliot, 1980). During meals, propionate is rapidly taken up by the liver and converted into intermediates of the citric acid cycle (Allen et al., 2009). Oxidation of propionate within a meal increases the energy state of hepatocytes more than normal, generating a satiety signal to stop eating (Allen et al., 2009). Once gluconeogenesis depletes the hepatic ATP pool, hunger is stimulated (Allen et al., 2009).

The importance of lactate, amino acids, and glycerol increases when the supply of propionate decreases (Brockman, 1993). Insulin stimulates the utilization of these substrates more than propionate (Brockman, 1993). Other VFA, such as acetate and butyrate, can be substituted for glucose as a respiratory fuel or lipogenic substrate in adipose and muscle tissue (Bergman et al., 1989). Insulin-responsive tissues, such as adipose and muscle tissues, are dependent on insulin for promotion of glucose transport and metabolism (Jarrett et al., 1974).
Glucose is a hydrophilic compound so it cannot pass through the lipid bilayer by simple diffusion and requires specific carrier proteins to mediate its transport into the cytosol. All mammalian cells contain one or more members of the GLUT gene family (Wood and Trayhurn, 2003). The function of GLUT proteins is to regulate the movement of glucose between the extracellular and intracellular compartments maintaining a constant supply of glucose for metabolism (Wood and Trayhurn, 2003). There are five main isoforms of GLUT (GLUT 1 to 5; Sasaki, 2002). The isoform, GLUT 4, is considered the insulin-responsive glucose transporter that is mainly found in muscle and adipose tissue and is responsible for most of the insulin-stimulated glucose transport in adipose and muscle (Kasanicki and Pilch, 1990). It is also found in hepatic tissue, but to a smaller degree. Insulin regulates glucose uptake in tissues through GLUT 4 and GLUT4 is present in all insulin-responsive tissues in cattle (Abe et al., 1994).

Insulin

The protein insulin contains two polypeptide chains linked to each other by two disulfide bridges (Mayer et al., 2007). These chains are homologous to the A and B chains of IGF-I and -II (Mayer et al., 2007). Synthesis of insulin occurs in the β-cells of the islets of Langerhans in the pancreas and is stored in β-granules of these cells (Mayer et al., 2007). Glucose concentration in circulation regulates insulin secretion from β-granules. Glucose can be taken up by pancreatic β-cells via GLUT 2, where it is metabolized, leading to an increase in ATP (Layden et al., 2010). This increase results in K⁺ channels closing and the depolarization of the cell. Calcium channels open and allow a flux of Ca²⁺ into the cell and this accumulation of Ca²⁺ causes insulin secretion to occur. Calcium has an essential role in insulin secretion since glucose does not stimulate insulin secretion in the absence of extracellular Ca²⁺ (Grodsky and Bennett,
Secretion of insulin from β-cell granules occurs via exocytosis, expelling insulin into the extracellular space.

In circulation, insulin acts on tissues, such as the liver, adipose, and muscle. Insulin binds to an insulin receptor on the plasma membrane of these tissues and stimulates intracellular signaling pathways. The insulin receptor has two purposes: to bind insulin with high affinity and to transmit a transmembrane signal resulting in changes to intracellular metabolic pathways. The insulin receptors are oligotetramers that consist of two α subunits, with a weight of 130 kDa, and two β subunits that are each 90 kDa (White and Kahn, 1994). Each type of subunit is responsible for one function of the receptor. The α subunit is the insulin binding subunit and is extracellular, whereas the β subunit is a transmembrane protein and possesses insulin-sensitive tyrosine kinase activity (Kahn, 1985). The insulin receptor is highly conserved among mammalian cell types and species (Muggeo et al., 1979).

Once insulin binds to the α subunit of its specific cell surface receptor a signal is induced, leading to rapid stimulation of tyrosine kinase activity of the β subunit (Patti and Kahn, 1998). Autophosphorylation occurs, increasing the kinase activity of the receptor (Patti and Kahn, 1998). This results in tyrosine phosphorylation of cytosolic substrates such as members of the IRS family, Shc and Cbl, an oncogene. These proteins interact with signaling molecules through SH2 domains, which results in different signaling pathways including PI3K, Cbl/CAP (Cbl associated protein) cascade, and the MAPK cascade, all acting to coordinate the regulation of glucose metabolism and homeostasis (Saltiel and Kahn, 2001; Figure 3).

Insulin receptor substrate-I, is shown to mediate insulin-stimulated glucose transport in related insulin target tissues (Kahn and White, 1988; Qunon et al., 1994). Tyrosine
Insulin binds to the insulin receptor, causing tyrosine autophosphorylation, resulting in tyrosine phosphorylation of insulin receptor substrate (IRS)-1 to 4, activating 3 different signal transduction pathways: phosphatidylinositol 3-kinase (PI3K), CAP/Cbl, and mitogen activated protein kinase (MAPK). The PI3K pathway mediated glucose/lipid/protein metabolism and insulin stimulated glucose uptake through activation of protein kinase B (PKB)/Akt by phosphoinositide-dependent kinase 1 (PDK-1). The CAP/Cbl pathway, also required for glucose transporter (GLUT) 4 translocation, is activated by tyrosine phosphorylation of the Cbl gene and CAP binding as well as the CrkII and C3G complex activating TC10 to signal GLUT4. The third pathway, MAPK, results in cell proliferation/differentiation and is activated by binding growth factor receptor-bound protein 2 (Grb2) and Shc, which binds son of Sevenless (SOS), activating Ras then Mek (Modified and reprinted with permission from: Mlinar et al., 2006).
phosphorylation of IRS-I induces its association with several protein containing SH2 domains, including Grb2 and p85 regulatory subunit of PI3K. This leads to translocation of GLUT 4 from intracellular vesicles to the plasma membrane (Greenfield and Campbell, 2004). The PI3K inhibitors can block the stimulation of glucose transport and lipogenesis by insulin, suggesting that PI3K may be necessary for these actions of the hormone (Evans et al., 2005). Insulin activates PKB/AKT through phosphorylation of Thr$^{308}$ and Ser$^{473}$ by PDK-1 (Anderson et al., 1998). Autophosphorylation of tyrosine residues of insulin receptor β subunit and phosphorylation of IRS-1, PI3K, and PKB/Akt play a key role to regulate intracellular glucose metabolism. The Cbl/CAP pathway involves tyrosine phosphorylation of the Cbl protooncogene (Ribon et al., 1997). The adaptor protein, CAP, binds to the proline-rich sequences in Cbl through its carboxyl-terminal SH3 domain (Ribon et al., 1998). Expression of CAP in insulin sensitive tissues is induced by adipocyte differentiation (Ribon et al., 1998). The protein, CAP contains three SH3 domains and once phosphorylation occurs, the Cbl-CAP complex translocates to lipid raft domains in the plasma membrane. Translocation of phosphorylated Cbl recruits the adaptor protein CrkII via its SH2 domain (Chiang et al., 2001). A complex is formed between CrkII and guanyl nucleotide-exchange protein, C3G, which catalyzes the exchange of GTP for GDP with the G protein TC10, activating it (Chiang et al., 2001). Once activated by insulin, TC10 provides a second signal to GLUT 4 for translocation to occur.

The MAPK pathway involves the phosphorylation of IRS or Shc proteins that interact with the adaptor protein Grb2 and recruit the SOS exchange protein to the plasma membrane, activating Ras. Activation of Ras also requires stimulation of the tyrosine phosphatase SHP2, through its interaction with receptor substrates such as Gab-1 or IRS-1/2. Activated Ras stimulates a serine kinase cascade through stepwise activation of Raf, MEK, and ERK. The
insulin signal transduction pathways mainly act to increase uptake and storage of glucose in insulin sensitive tissues in order to maintain glucose homeostasis.

Glucose utilization

Glucose is needed in many tissues of the body for survival. In sheep, the brain accounts for more than ten to fifteen percent of glucose utilization (Hocquette and Abe, 2000). Skeletal muscle is a major consumer of glucose through oxidation and storage. Eighty percent of glucose is stored as glycogen in sheep (Pethick, 1984). Adipose tissue in ruminants only accounts for one percent of total glucose (Pethick, 1984). Glucose is taken up by muscle and adipose tissues via the GLUT 4 transporter. Glucose transporter 4 is mainly found in the basal state inside the cell and is translocated to the plasma membrane for insulin stimulation to increase the extraction rate of glucose (Hocquette and Abe, 2000). Glucose oxidation and storage in muscles and lipogenesis in adipose is also stimulated by insulin. Adipocytes are one of the most insulin-responsive cell types (Kahn and Flier, 2000). The role of insulin is to promote adipocyte triglyceride storage by differentiation of pre-adipocytes, stimulating glucose transport and lipogenesis, and inhibiting lipolysis (Kahn and Flier, 2000). In addition, insulin also increases the uptake of fatty acids derived from circulating lipoproteins by stimulating lipoprotein lipase activity in adipose tissue (Kahn and Flier, 2000). Skeletal muscle accounts for up to 75% of insulin-dependent glucose disposal, where it is either stored as glycogen or oxidized (Saltiel and Kahn, 2001).

Consequences of altered glucose homeostasis

A number of factors including insulin resistance can cause alterations in glucose homeostasis. Insulin resistance is resistance to the effects of insulin on glucose uptake,
metabolism, or storage. This is caused by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle as well as impaired suppression of hepatic glucose output (Kahn and Flier, 2000). These defects may be caused by impaired insulin signaling in target tissues as well as down regulation of GLUT 4 in adipocytes. Expression of GLUT 4 is normal in skeletal muscle, but translocation and docking to the plasma membrane is impaired (Kahn and Flier, 2000). There is a reduction in insulin binding to its receptor, receptor phosphorylation and tyrosine kinase activity, and phosphorylation of IRS in both muscle and adipocytes (Kahn and Flier, 2000). Reduced insulin binding and action leads to insulin resistance, causing an increase in adiposity.

Increased adipose energy storage in obesity results in increased triglyceride storage in these tissues, which promote insulin resistance and other adverse effects causing lipotoxicity (Kahn and Flier, 2000). Increased plasma FFA impair the ability of insulin to suppress hepatic glucose output, stimulate glucose uptake into skeletal muscle, and inhibit insulin secretion from pancreatic β-cells. Normally functioning β-cells in the pancreas respond to insulin resistance by secreting more insulin, causing hyperinsulinemia. Type 2 diabetes can result from insulin resistance normally caused by obesity (Layden et al., 2010). In the case of type 2 diabetes, the body is unable to increase insulin secretion to overcome the insulin resistance due to pancreatic β-cell dysfunction and causes hyperglycemia (Layden et al., 2010). Glucagon concentrations increase in individuals with type 2 diabetes, causing hyperglycemia to get worse since glucagon increases hepatic glucose production (Layden et al., 2010). Hepatic lipogenesis and lipid storage in adipose tissue could possibly be increased while other insulin effects of glucose homeostasis are impaired (Kahn and Flier, 2000). In IUGR animals, changes in homeostasis can lead to permanent consequences for the offspring in post-natal life such as insulin resistance and obesity.
Overall, insulin acts to increase glucose uptake in muscle and adipose tissue through stimulation of GLUT 4 translocation, increase glucogenesis in the liver, and inhibit glucose production. Glucose stimulates insulin secretion from the pancreas in order for these actions to occur. When plasma glucose concentrations are too low glucagon is released and increases hepatic glucose production to increase circulating glucose concentrations (Layden et al., 2010). Therefore, insulin and glucagon work together to tightly regulate plasma glucose concentrations to insure glucose homeostasis is maintained.

**LEPTIN**

Leptin is another hormone required to maintain glucose homeostasis and energy balance. Plasma concentrations of leptin are correlated with fat mass in humans and ruminants and are also positively correlated to adipocyte volume (Chilliard et al., 2001; Delavaud et al., 2002). Blache et al. (2000) found that plasma leptin concentration was highly correlated with backfat thickness in sheep, Tokuda and Yano (2001), Tokuda (2003), and Altmann (2005) reported similar findings. Concentrations of leptin are also positively correlated with insulin concentrations (Tokuda and Yano, 2001). Leptin acts both in central and peripheral manners, regulating central endocrinology and physiological processes as well as peripheral tissue activity (Moran and Phillip, 2003). This shows that leptin is a major regulator of adipose deposition in the body, demonstrating a relationship with insulin in regulating energy stores and maintaining glucose homeostasis.

Leptin, a 16 kDa peptide hormone, is the product of the Ob gene and consists of 146 amino acids (Zhang et al., 1994). It is part of the type I helical cytokine family and is related to prolactin, GH, and the interleukins (Huising et al., 2006). Leptin is encoded by two exons and...
forms a four-helix bundle conformation. Leptin has a pair of conserved cysteine residues that form a disulfide bridge required for full biological activity (Crespi and Denver, 2006). There are three identified receptor binding sites with conserved amino acid sequences among species. The leptin gene is expressed mainly in white adipose tissue and in smaller amounts in placental and fetal tissues, mammary gland, stomach, muscles, and brown adipose tissue (Andrews, 1998). The leptin mRNA abundance in adipose tissue has been shown to decrease significantly by food deprivation or under-nutrition, and increase by re-feeding in sheep (Kumar et al., 1998).

Leptin is mainly secreted from white adipose tissue and the synthesis of leptin is stimulated by insulin. Once in circulation, leptin can cross the blood-brain barrier and act on receptors located in the ARC nucleus of the hypothalamus (Friedman and Halaas, 1998). Neurons within the ARC nucleus are associated with obesity and respond directly to a variety of hormonal and nutrient signals including leptin, insulin, and glucose (Elmquist, 2005). The neuropeptide Y (NPY)/agouti-related protein (AgRP) neurons promote feeding and are activated by appetite stimulating signals. The ARC/pro-opiomelanocortin (POMC) neurons promote BW loss through expression of alpha melanocyte-stimulating hormone (α-MSH), which acts at melanocortin receptors to suppress food intake (Schwartz, 2001). Leptin acts to up regulate POMC expression within the ARC nucleus, thereby limiting energy intake (Schwartz, 2001). Leptin can also act directly on peripheral tissues by binding to receptors in the pancreas, liver, skeletal muscle, or adipose tissue (Muoio and Dohm, 2002). The actions of leptin all work to increase fatty acid oxidation and decrease triglyceride storage, to decrease adipose tissue mass.

The leptin receptor (Ob-R) is present in the hypothalamus and choroid plexus, as well as various peripheral tissues and is encoded by the db gene (Tartaglia et al., 1995). It is similar in function and structure to the class I cytokine receptors and exists in multiple isoforms; their
intracellular domains varying in length due to alternative splicing of the db gene (Lee et al., 1996; Bjorbaek et al., 1997). The isoforms of leptin have an identical extracellular ligand-binding domain at the N-terminus, but differ at the C-terminus.

The long form (OB-R\textsubscript{b}), responsible for the central actions of leptin, contains a 302 amino acid intracellular domain and is abundantly expressed in specific nuclei of the hypothalamus (Tartaglia et al., 1995; Mercer et al., 1996; Elmquist et al., 1997). The short forms (OB-R\textsubscript{a}, \textsubscript{c}, \textsubscript{d}, and \textsubscript{e}), contain 32 to 97 amino acids and are distributed throughout peripheral tissues, but little is known about the physiological activity of this form (Wang et al. 1997; Szanto and Kahn, 2000). The extracellular domain of both the long and short forms of the leptin receptor contain two cytokine domains, each containing a single copy of the typical Trp-Ser-X-Trp-Ser motif and a fibronectin III domain (White and Tartaglia, 1996).

Since leptin is considered a type I cytokine receptor, signal transduction is dependent on ligand-induced phosphorylation of receptor tyrosine kinases in the JAK family, specifically JAK2 (Huang and Cia, 2000). These kinases then phosphorylate tyrosine residues on the receptor that serve as a docking site for SH2 domain proteins (Banks et al., 2000). Binding initiates signal transduction and activated JAK2 phosphorylates tyrosine residues, Tyr\textsuperscript{985} and Tyr\textsuperscript{1138} of the leptin receptor (Darnell, 1997; Banks et al., 2000). Once Tyr\textsuperscript{1138} is phosphorylated, it serves as a binding site for STAT proteins, specifically STAT3 (Bendinelli et al., 2000). These recruited STAT proteins become tyrosine-phosphorylated by JAK, causing dissociation from the receptor to form homo- or heterodimers (Heim, 1996). Then STAT dimers translocate into the nucleus and act as transcription factors by binding specific response elements (Bendinelli et al., 2000). Leptin binds to an Ob-R homodimer with high affinity, causing activation of JAK2. The
Ob-Rb is present in the hypothalamus and its actions are mediated by STAT3 and NPY (Schwartz et al., 1996).

Another signaling pathway stimulated by leptin is the MAPK pathway. This pathway can be stimulated by either the long or short isoforms (Banks et al., 2000; Figure 4.). The Tyr^{985} residue of the long leptin receptor form can induce full Erk activation, by becoming phosphorylated and recruiting JAK2 (Banks et al., 2000). It provides a docking site for the SH2 domain containing protein tyrosine phosphatase, SHP-2. After binding to this specific tyrosine residue, SHP-2 is phosphorylated at the C-terminus and with its adaptor molecule Grb2, activates downstream signaling effects (Banks et al., 2000). The short form of the leptin receptor can also activate this pathway by association of JAK2 with the SH2 domain-containing adaptor protein Grb2 and SHP-2. This complex then activates further signaling steps (Banks et al., 2000).

Central actions

Leptin acts centrally through the nervous system to control food intake and energy expenditure (Houseknecht et al., 1998). The major goal of leptin is to increase energy expenditure, decrease food intake, and increase GH secretion, overall decreasing adipose deposition in the body. Leptin has also been shown to regulate bone formation and resorption indirectly through the hypothalamus (Cirmanova et al., 2008). Neuropeptide Y is considered a major target of leptin action and acts as a strong stimulator of food intake and also inhibits brown fat thermogenesis, increasing plasma insulin and steroid concentrations (Moran and Phillip, 2003). Leptin acts to inhibit NPY by binding to the leptin receptor of the NPY/AgRP neurons in the ARC nucleus of the hypothalamus (Cusin et al., 1996; Bates et al., 2003). Suppression of NPY release from the hypothalamus, results in inhibition of food intake, increasing
Figure 4. Leptin signal transduction pathway activated through leptin binding with the long form leptin receptor (Ob-Rb).

After leptin binds to the long form leptin receptor (Ob-Rb), intracellular signaling pathways are activated, mainly through stimulation of tyrosine phosphorylation and activation of janus kinase (JAK) 2. JAK2 then phosphorylates tyrosines within SHB2 and signal transducers and activators of transcription (STAT) 3 binding sites. Signal transducer and activator of transcription-3 can be transported into the nucleus, where it is involved in transcription of suppressor of cytokine signaling 3 (SOCS-3). Once SHB2 is recruited, it binds to growth factor receptor-bound protein 2 (Grb2) and the extracellular signal-regulated kinase (ERK) 1/2 cascade is activated. JAK2 can also activate insulin receptor substrate (IRS) and then phosphatidylinositol 3-kinase (PI3K) and Akt, stimulating growth and survival. Leptin can also bind to short forms of its receptor, mostly in peripheral tissues, which also activates intracellular pathways through the activation of JAK (Modified and reprinted with permission from: Garofalo and Surmacz, 2006).
thermogenesis and reducing adipose tissue mass (Stephens et al., 1995). Leptin can bind to
ARC/ POMC neurons and activate secretion of α-MSH, an anorexic neuropeptide (Bates et al.,
2003). This activation by leptin reduces food intake and increases energy expenditure
(Niswender and Schwartz, 2002).

Another central role of leptin is stimulating GH secretion from somatotrope cells of the
anterior pituitary gland, through stimulation of GHRH and inhibition of SRIF (Breier et al.,
2001; Moran and Phillip, 2003). Since actions of the somatotropic axis are known to be
inhibited in IUGR animals, the action of leptin on growth and organ weight may be mediated by
stimulation of the somatotrophic axis (Djiane et al., 2008). The effect of leptin on GH secretion
may also involve NPY, since leptin suppresses NPY expression, and infusion of NPY is known
to suppress GH secretion (Kamegai et al., 1996). Increasing GH secretion increases GH
inhibitory action on adipose tissue, ultimately decreasing adipose tissue mass.

Peripheral actions

In addition to the central effects of leptin, leptin has peripheral effects on the pancreas,
skeletal muscle, liver, and possibly bone, and feeds back to inhibit excess leptin gene expression
in adipocytes. The peripheral actions of leptin occur mainly via the interaction of leptin with the
short forms of the leptin receptor l.found outside of the central nervous system, which activate
JAK signaling pathways (Tartaglia et al., 1995).

In muscle, leptin can increase fatty acid oxidation and decrease triglyceride storage,
promoting an increase in energy expenditure and insulin sensitivity (Muoio and Dohm, 2002).
Leptin also exhibits direct actions on insulin secretion, since both the long and short forms of the
leptin receptor are present in pancreatic β-cells (Kullkarni et al., 1997). Leptin inhibits insulin
biosynthesis and secretion by inhibiting glucose transport to the pancreas via GLUT 2,
contributing to peripheral insulin resistance (Kieffer et al., 1997). Insulin stimulates leptin secretion from adipose tissue, creating a hormonal regulatory feedback loop. In adipose, leptin increases fatty acid oxidation and decreases triglyceride storage.

Leptin has been shown to have both insulin-like and insulin-antagonistic effects on the liver, specifically hepatic glucose metabolism (Moran and Phillip, 2003). Leptin has been shown to have a direct effect on glucose metabolism through leptin receptors identified in hepatocytes (Zhao et al., 2000). Leptin, either alone or in combination with insulin, reduces hepatic glucose production by decreasing the synthesis of the key enzyme of gluconeogenesis, phosphoenolpyruvate carboxykinase (PEPCK; Moran and Phillip, 2003). The effects leptin has on mediating insulin effects in the liver is not quite clear, especially in ruminants, and more studies need to be done to determine the exact effects leptin has on the liver.

Bone marrow adipocytes can serve as a direct source of leptin and can stimulate differentiation of bone marrow stromal cells (BMSC). Leptin is thought to stimulate BMSC differentiation into osteoclasts and inhibit differentiation into adipocytes, but if leptin concentrations are too great they can lead to BMSC apoptosis (Cirmanova et al., 2008). Normal concentrations of leptin directly affect proliferation, differentiation, and mineralization of osteoblasts by inhibiting apoptosis (Gordeladze et al., 2002). In addition to the interaction between central and peripheral pathways, the overall effect of leptin on bone seems to be modulated by serum concentration, where an increase in leptin stimulates bone formation. However, when concentrations of serum leptin are too great, they can inhibit bone formation (Cirmanova et al., 2008). Plasma leptin concentrations have also been shown to be associated with bone mass. If leptin concentrations in plasma are reduced, then bone mass is suggested to
be decreased (Hamrick, 2004). Yamauchi et al. (2001) reported that plasma leptin concentrations were positively correlated with bone mineral density.

Overall, leptin centrally regulates whole-body energy homeostasis and mediates peripheral metabolic processes such as controlling cellular lipid balance by promoting β-oxidation and lipolysis as well as inhibiting synthesis of triacylglycerol. Leptin also plays a part in regulation of insulin and glucose homeostasis. Leptin action in muscle, adipose, liver, and pancreas tissues, requires signaling through the IRS-PI3K pathway, the same pathway insulin uses for signal transduction in these tissues. Therefore, leptin may act in a synergistic manner with insulin (Sivitz et al., 1997). Animals exhibiting leptin resistance or deficiency store inappropriately increased concentrations of triacylglycerides in non-adipocyte cells, not adapted for storing excess FA. These animals have been shown to have complications defined by metabolic syndrome (Unger and Orci, 2001).

Influence of nutrition on leptin concentrations

Leptin response is stimulated by nutritional status, relating plasma leptin concentrations to adipose deposition. In adult animals, including ruminants, plasma leptin concentrations are shown to increase with the plane of nutrition (Ehrhardt et al., 2000; Ingvartsen and Boisclair, 2001). Energy expenditure and food intake are regulated by plasma leptin concentrations, allowing for changes in leptin concentrations based on the amount of stored nutrients allocated for energy (Morton, 2007).

In response to reduced nutrition, the brain initiates responses to promote positive energy balance to restore and maintain energy and glucose homeostasis (Morton, 2007). In periods of nutrient-restriction or starvation, there is a decrease in plasma leptin concentrations signaling to increase feed intake and decrease energy expenditure to increase energy stores (Friedman, 2002).
Reduced concentrations of leptin in circulation indicate an inadequate amount of fat, causing an adaptive response to replenish these energy stores (Friedman, 2002). Insulin is the major stimulator of glucose storage in peripheral tissues. Decreased leptin concentrations allow insulin to stimulate glucose uptake in these tissues, increasing energy stores.

It has been demonstrated that leptin concentration increases at the end of fetal life, but at birth leptin concentrations are reduced in IUGR progeny (Djiane et al., 2008). In animals with IUGR, active cell proliferation may continue after birth, more so than in normal situations, suggesting that excess proliferation of fat cells in IUGR may be designed to improve long term capacity for lipid storage to adapt in a restricted-food environment during fetal life. However, if nutrients become abundant in post-natal life, this adaptation could promote obesity (Djiane et al., 2008). Nutrition as well as adiposity regulates plasma leptin in early post-natal life of lambs (Ehrhardt et al., 2003).

In instances of over-nutrition and excess energy storage, negative energy balance is promoted. Impaired responses or resistance to hormonal nutrient signals, such as leptin could lead to obesity and insulin resistance (Morton, 2007). Increased adiposity leads to increased leptin concentrations, causing an increase in energy expenditure and decrease in food intake to reduce adipose deposition. Overfeeding sheep throughout pregnancy has been determined to increase maternal leptin concentrations (Thomas et al., 2001). In lambs born to ewes overfed during late gestation, an increase in adipose tissue mass was reported to be associated with down regulation of the leptin receptor in the ARC nucleus. This would result in decreased leptin sensitivity (Muhlhausler et al., 2006). Furthermore, over-nutrition in pre-natal life may alter responses of the central neural network to an increase in fat mass in post-natal life (Muhlhausler et al., 2006). Long et al. (2010) observed that maternal over-nutrition can eliminate the leptin
peak in lambs, demonstrating that over-nutrition can alter development of appetitive centers. In obese individuals, increased leptin concentrations suggest leptin resistance and decreased concentrations suggest decreased leptin production (Friedman, 2002).

Leptin has been shown to have a role in the regulatory action of adipose tissue on total body sensitivity to insulin (Moran and Phillip, 2003). Along with insulin, leptin has been shown to regulate glucose metabolism. Leptin increases insulin signaling by reducing intracellular lipid concentrations. Hyperinsulinemia precedes the development of insulin resistance and obesity (Le Stunff and Bougneres, 1994; Ferrannini et al., 1997). Leptin resistance seen in obesity could be a result of inhibition of the JAK/STAT signaling pathway. Suppressor of cytokine signaling-3 (SOCS-3) can bind to the phosphorylated tyrosine residues of signaling molecules and mediate their degradation or inhibition, which inhibits leptin signal transduction (Hansen et al., 1999). In obese individuals, there may be a failure of STAT signaling, causing a disruption in energy homeostasis (Ghilardi et al., 1996).

Both leptin and insulin are critical for nutrient storage and maintenance of energy balance. Changes to either one of these hormones could potentially alter glucose homeostasis, adiposity, and whole-body energy balance. These changes could be caused by nutrient-restriction or over-nutrition and could lead to detrimental effects for offspring in post-natal life, such as the development of obesity, and insulin and leptin resistance. Therefore, the relationship between insulin and leptin and their involvement in regulating glucose homeostasis is important for maintaining energy balance and metabolism.
OBJECTIVES

The somatotropic axis is a known regulator of growth and development. This axis has been shown to be altered due to insults such as poor maternal nutrition, with nutrient-restriction and over-nutrition both leading to a similar outcome for the offspring. Given our knowledge of this axis along with the role of both leptin and insulin in nutrient storage and glucose homeostasis, we hypothesized that lambs born to nutrient-restricted or overfed ewes would have reduced body weight, reduced growth rate, and increased adiposity, which would be associated with changes in the somatotropic axis, leptin, and insulin-glucose homeostasis.

Variables including BW, body condition score (BCS), crown rump length (CRL), and heart girth (HG) are considered to be determinants of growth rate. Circulating concentrations of hormones involved in the somatotropic axis, including GH, IGF-I, and IGFBP-2 and -3, are known to respond to nutrition (Rausch et al., 2002; Govoni et al., 2003, 2004). Therefore, these hormones can be potentially used as predictors of nutritional status in animals. Of the six IGFBP, IGFBP-2 and IGFBP-3 are specifically involved in changes in growth rate and response to nutrient status (Govoni et al., 2003, 2004). The hormones insulin and leptin are critical for nutrient storage, especially related to adiposity, and maintaining glucose homeostasis. Thus, changes to either one of these hormones can lead to detrimental effects such as obesity, insulin and leptin resistance.

Therefore, our objective was to determine the effects of poor maternal nutrition on post-natal growth rate and metabolism of offspring by studying the changes of the somatotropic axis, insulin-glucose homeostasis, and leptin concentrations, as well as body composition of lambs. Analyzing these body variables and metabolic hormones will provide us with insight to how poor maternal nutrition can affect offspring in post-natal life.
MATERIALS AND METHODS

Animals

Forty multiparous ewes from the University of Connecticut flock were selected for synchronization of estrus during seasonal anestrus by intravaginally inserting a controlled internal drug release (CIDR) device (EAZI-BREED CIDR, Pfizer, New York, NY) containing 300 mg progesterone for 12 days (Naderipour et al., 2012). Upon removal of CIDR device, ewes were given a single 20 mg intramuscular (IM) injection of prostaglandin (PG) F\textsubscript{2α} (Knights et al., 2003). After breeding, pregnancy was confirmed by ultrasound and 36 (25 Dorsets, 7 Shropshires, and 4 Southdowns) of the 40 ewes were selected for the study. Ewes were individually housed in pens and acclimated to a control diet for one week. At d 31 ± 1.3 of gestation, ewes were randomly assigned 1 of 3 treatment diets: control (CON, 100%), restricted (RES, 60%), or overfed (OVER, 140%) of NRC (National Research Council, 1985) requirements until the last four weeks of gestation when protein and energy intake were increased to maintain restriction relative to the needs of the late gestating ewe (NRC, 1985; Table 1). Treatments were balanced by breed (CON, 9 Dorsets, 2 Shropshires, 1 Southdown; RES, 8 Dorsets, 3 Shropshires, 1 Southdown; OVER, 8 Dorsets, 2 Shropshires, 2 Southdowns). Ewes were given a complete pelleted feed diet (Table 2), which was offered each day based on BW of the ewe. Leftover feed was removed and weighed to determine daily feed intake. Ewes had free access to water and straw (Table 2) was provided in mornings. Body weight and BCS (Russel, 1991) of each ewe was determined weekly and feed was adjusted accordingly.
Table 1. Amount of complete pelleted diet in kilograms given to each ewe treatment group on study based on NRC requirements for gestating sheep.

<table>
<thead>
<tr>
<th>Amount fed during gestation</th>
<th>Treatment</th>
<th>Control</th>
<th>Restricted</th>
<th>Overfed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acclimation week, kg</td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>During gestation, kg</td>
<td></td>
<td>1.0</td>
<td>0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Last 4 weeks of gestation, kg</td>
<td></td>
<td>1.6</td>
<td>1.2</td>
<td>2.0</td>
</tr>
</tbody>
</table>

1 Amount of feed was calculated for each individual ewe based on body weight.
2 Treatments: Control = 100% NRC; Restricted = 60% NRC; Overfed = 140% NRC.
3 1 week prior to treatment, d 23 to 30 of gestation.
4 d 31 of gestation until d 119 of gestation.
5 d 120 of gestation until parturition.

Table 2. Feed analysis of diet fed to ewes.

<table>
<thead>
<tr>
<th>Components</th>
<th>Complete Pelleted Diet</th>
<th>Straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>12.80</td>
<td>15.60</td>
</tr>
<tr>
<td>Acid detergent fiber, %</td>
<td>31.10</td>
<td>43.80</td>
</tr>
<tr>
<td>Neutral detergent fiber, %</td>
<td>42.10</td>
<td>66.70</td>
</tr>
<tr>
<td>TDN1, %</td>
<td>74.00</td>
<td>53.00</td>
</tr>
<tr>
<td>NEI2, mcal/kg</td>
<td>1.74</td>
<td>0.26</td>
</tr>
<tr>
<td>NEm3, mcal/kg</td>
<td>1.74</td>
<td>0.26</td>
</tr>
<tr>
<td>NEg4, mcal/kg</td>
<td>1.12</td>
<td>0.05</td>
</tr>
</tbody>
</table>

1 Total digestable nutrients.
2 Net energy lactation.
3 Net energy for maintenance.
4 Net energy for growth.
Following parturition, lambs remained with the ewes for up to 24 h to ensure sufficient colostrum consumption then one lamb was removed from each ewe, for the study. There were a total of 35 lambs selected for the study (one dorset, restricted ewe had stillborn lambs and was not used). The largest male was selected if there were two males, the male was selected if there was a male and female, or the largest female was selected if there were two females. We selected males since males are usually marketed for meat, whereas ewes are kept as flock for reproduction. This resulted in 6 rams and 6 ewes in the control group, 8 rams and 3 ewes in the restricted group, and 7 rams and 5 ewes in the overfed group. Eighteen lambs [CON, n = 6 (3 rams, 3 ewes); RES, n = 6 (3 rams, 3 ewes); OVER, n = 6 (4 rams, 2 ewes)] were euthanized with an intravenous injection of Beuthanasia-D solution within 24 h of birth. The remaining 17 lambs were fed a control diet until 3 mo of age and then euthanized. Birth and 3 mo necropsy groups were balanced by breed and birth status. The 17 lambs were group housed (4 to 5/pen) until 3 mo of age [CON, n = 6 (3 rams, 3 ewes); RES, n = 5 (all rams); OVER, n = 6 (3 rams, 3 ewes)]. The control diet consisted of milk replacer (Land O’Lakes Animal Milk Product Company; Shoreview, MN) fed at 1.7% of BW until weaning at 56 d of age, and ad libitum creep feed (Lamb BT, Blue Seal Feeds; Litchfield, CT) and second cutting hay at beginning of weaning [4 weeks (wk) of age] until 3 mo of age. Lambs also had free access to water. Lambs were weighed every 2 d to determine growth rate and milk replacer amounts were adjusted accordingly.

Sample Collection

Body weight and BCS of ewes were recorded once per week during gestation, until parturition. The average BW gain of each treatment group during gestation was: CON = 22.8 kg, RES = 10.3 kg, OVER = 29.3 kg, which demonstrates the effectiveness of the ewe diets. Lamb
BW was measured at birth and 3 times per wk until weaning then once a wk thereafter. Crown rump length and HG were measured at birth and once a wk until slaughter. Lamb BCS were determined once per week starting at 4 wk of age until slaughter. Ewe blood samples (20 mL) were collected once a week via jugular venipuncture using an 18 G needle with syringe. Lamb blood samples (10 mL) were collected at birth and one week of age; then 20 mL were collected once a week until slaughter. Blood was collected in serum BD (Franklin Lakes, NJ) vacutainer tubes and plasma heparin and EDTA BD vacutainer tubes. Serum tubes were kept at room temperature for 4 to 6 hours to allow blood to clot then stored overnight at 4°C and centrifuged the next morning. Plasma tubes were inverted several times after blood collection and kept on ice until centrifugation, which was done within one hour of collection. Blood was centrifuged for 30 minutes at 1,800 x g (Sorval RT7; Kendro Laboratory Products, Newtown, CT) at 4°C and serum and plasma were harvested and stored at -20°C until hormone and metabolite analyses were performed.

Animals were euthanized with intravenous injection of Beuthanasia-D solution containing 390 mg/mL sodium pentobarbital and 50 mg/mL phentoin calculated based on BW measured the day of euthanasia. Crown rump length and HG were measured and blood samples (20 mL) were collected. Organ weights were recorded from the heart, liver, kidneys, adipose, quadracep muscle and loin muscle. Loin eye area (LEA; cm²), the cross section of the longissimus dorsi muscle, was measured at birth and 3 mo using a grid. Backfat thickness (cm) was measured only at 3 mo since there was no measurable amount present at birth. Tissue samples were collected from the heart, liver, adipose, muscle, pancreas, and kidney and frozen in liquid nitrogen then stored at -80°C for later RNA extraction.
Intravenous Glucose Tolerance Test (IVGTT)

An IVGTT was performed on the lambs at 1 and 3 mo of age. Twelve hours before the test, all feed was removed. Lambs were provided water ad libitum. The neck areas of the lambs were shaved and cleaned with 70% ethanol and betadine. A catheter (Abbocath 18 g x 2.5 in long) was inserted into a jugular vein of lambs 1 hour before the challenge to allow lambs time to recover. The catheters were held in place by bandage tape and vet wrap (Johnson & Johnson; Arlington, TX). Blood samples (3 mL) were taken at -30, -15, 0, 2, 5, 10, 15, 30, 60, and 120 minutes relative to glucose infusion. Blood samples were immediately placed in BD vacutainer lithium heparinized plasma tubes for later insulin and glucose analysis. A single bolus injection of glucose (50% dextrose solution; Vedco, St. Joseph, MO) was given via the jugular catheter, which was calculated based on 0.25g/kg of BW (Ford et al., 2007).

Hormone and Metabolite Analyses

Serum IGF-I concentrations were determined by radioimmunoassay as described by Govoni et al. (2002). Insulin-like growth factor binding proteins were separated using a glycylglycine hydrochloric acid extraction method (Govoni et al., 2003). A standard curve was prepared by serial dilution of IGF-I standard (5 ng/mL) using assay buffer. All samples were run in duplicate. Anti-human IGF-1 rabbit antiserum (NIDDK, Parlow; Torrance, CA) was used at 1:100,000 dilution. Secondary antibody, goat anti-rabbit G-globulin (EMD Millipore, Billerica, MA), was used at a dilution of 1:4. The amount of $^{125}$I-IGF-I (Perkin Elmer, Shelton, CT) added was adjusted to 10,000 cpm per tube. Bound radioactivity was determined by a gamma counter (GMI Iso-Data 20/20 series). Intra-assay coefficients of variation averaged 8.9% for low and 7.4% for high pools, respectively. Inter-assay coefficients of variation averaged 26.6% for low
(52.9 ng/mL) and 16.7% for high (156.3 ng/mL). The large inter-assay coefficient for the low pool is due to few samples used (n = 3) and large standard deviation (20.1)..

Serum concentrations of GH were determined by radioimmunoassay as described by Kazmer et al. (1992). Preparation and purification of $^{125}$I-GH was performed using the Iodogen method (Pierce, Rockford, IL) as described by Cohick et al. (1989), using 5 µg ovine GH and 1 mCi $^{125}$I. The standard curve was prepared by serial dilution of GH standard (NIDDK Parlow; Torrance, CA) containing 40 ng/mL using assay buffer. Samples were run in duplicate. There was 20,000 cpm of $^{125}$I-GH added to each tube. Rabbit antiserum ovine GH (NIDDK-anti-oGH-2, Parlow, Torrance, CA) was used at a dilution of 1:20,000. The secondary antibody, goat anti-rabbit G-Globulin (EMD Millipore, Billerica, MA) was used at a dilution of 1:40. The bound radioactivity was determined using a gamma counter (GMI Iso-Data 20/20 series). Intra-assay and inter-assay coefficients of variation averaged 7.4 and 32.3% for low (0.88 ng/mL) and 9.5 and 13.4% for high (1.13 ng/mL) pools, respectively. Inter-assay coefficients of variation were high due to number of samples used (n = 4) and standard deviation of low (0.28) and high (0.15) pools.

Plasma leptin concentrations were determined by a multi-species leptin RIA kit (Millipore Corporation, Billerica, MA). This assay used $^{125}$I labeled human leptin and a multi-species leptin antiserum to determine the leptin concentrations in serum. The sensitivity of this assay is 0.801 ng/mL + 2 SD HE (100 µL sample size). Intra-assay and inter-assay coefficients of variation averaged 12.5 and 14.7% for Quality Control 1 (2.75 ng/mL) and 10.12 and 8.1% for Quality Control 2 (17.5 ng/mL), respectively.
Serum insulin concentrations were determined by an ovine insulin ELISA kit from ALPCO Diagnostics. The microplate (96-well) was coated with monoclonal antibody specific for insulin. A 5 parameter logistic (pl) fit was used to optimize the accuracy and precision of the sigmoidal curve. The sensitivity of this assay was 0.14 ng/mL. Inter-assay coefficients of variation averaged 3.24% for control 1 (3.22 ng/mL) and 5.72% for control 2 (0.88 ng/mL).

Plasma glucose concentrations from the IVGTT were determined by a glucose colorimetric assay kit (Cayman Chemical, Ann Arbor, MI). This glucose assay used the glucose oxidase-peroxide reaction for the determination of glucose concentrations. The range of this assay is 0 to 25 mg/dL. As stated in this assay kit, the intra-assay coefficient of variance was 4.6% and inter-assay coefficient of variance was 1.7%.

Concentrations of IGFBP-3 and -2 in serum were determined by Western ligand blot after gel electrophoresis as described by Freake et al. (2001) and Govoni et al. (2002). Proteins were separated by gel electrophoresis on a gradient SDS-polyacrylamide gel, made using 30% acrylamide. A bovine IGFBP-3 standard, human recombinant IGFBP-3 standard (Petrotech), and six serum samples (2 µL) were run in eight lanes on a Mini Protean II (BioRad, Richmond, CA) for 30 min at 100 V then 1 h at 150 V. Gels were then transferred to nitrocellulose membrane at 45 V for 1 h and 30 min in a transfer tank. Membranes were blocked with 3% Igepal (Sigma Aldrich Corp., St. Louis, MO) in Tris-buffered saline (TBS) then washed for 2 h with 1% Tween 20 (Fisher Scientific). Membranes were then incubated over night with 300,000 cpm of $^{125}$I -IGF-I (PerkinElmer, Shelton, CT) in 0.1% Tween 20. After 15 h incubation membranes were washed with Tween 20 and TBS to remove any unbound radiolabel. After washing, the membrane was exposed to a multipurpose phosphor screen (PerkinElmer) for 18 h and imaged with a Cyclone Storage Phosphor System (PerkinElmer). Both IGFBP-2 and -3
were quantified using OptiQuant acquisition and analysis Software (PerkinElmer). Each binding protein was measured as digital light units (DLU)/mm² and expressed as a percentage of the signal of a bovine standard IGFBP-3 to account for variation between gels. Each band was measured twice per gel and the four measurements were averaged together then expressed as arbitrary units (AU).

Serum total cholesterol (TC) was determined by enzymatic analysis using cholesterol reagents from Pointe Scientific (Canton, MI). Serum triglyceride (TG) concentration was determined using Wako Chemical USA triglyceride kit (Richmond, VA). This kit uses the L-type triglyceride M method, an enzymatic method, to analyze triglyceride concentrations (Kim et al., 2013).

**Statistical Analysis**

Statistical analysis was done using the mixed model analysis of variance procedure (SAS Inst. Inc., Cary, NC) to determine changes in age. Five covariant structures [CS, VC, AR (1), TOEP, Huynh-Feldt], were examined. A goodness of fit statistic was used to determine which covariate adequately fit the data. Variance components for all analyses were estimated comparing variance components and least square means. All data are expressed as least square means ± standard error of the mean.

The birth time point, for all variables, included measurements of all 35 lambs. The weekly time points after birth, until 3 mo of age, include measurements of the lambs that remained on study for 3 mo (n = 4 to 6/ treatment).

For all variables overtime; treatment, week, and treatment by week were included in the final model. The subject in the repeated statement was animal and the repeated variable was
week. For ADG, BW, CRL, HG, and BCS the covariate model used was VC, AR (1), AR (1), TOEP, and VC, respectively. For IGF-I, GH, leptin, insulin, IGFBP-2, and IGFBP-3 the covariate model used was TOEP, CS, CS, AR (1), VC, and CS, respectively. Pre-weaning (wk 0 to 8) and post-weaning (wk 9 to 12) were analyzed for all body variables and hormones using the covariate model previously mentioned for each variable. Total cholesterol and TG were analyzed by ANOVA at birth and 3 mo time points with treatment in the final model.

Analysis of a gender effect was performed for BW, CRL, HG, IGF-I, GH, IGFBP-2, IGFBP-3, TC, and TG at the birth time point by ANOVA with treatment, gender, and treatment by gender in the final model. Gender was not analyzed for variables at 3 mo since there was not enough experimental units of each gender per treatment. There were no differences in gender at birth for any variables measured.

For the IVGTT, glucose and insulin concentrations were analyzed overtime by TOEP and AR (1), respectively. Baseline concentrations of each were determined from blood samples collected at -30, -15 and 0 time points relative to glucose infusion. Area under the curve (AUC) was determined using the trapezoidal method (Weber et al., 2005). Peak, baseline, and AUC concentrations for glucose and insulin were analyzed by a variance components covariance structure. First phase insulin response was calculated as the sum of the glucose concentration at 2 and 5 minutes relative to glucose infusion divided by the average baseline glucose concentration (Ford et al. 2007). Insulin to glucose ratio was determined overtime and analyzed by variance component covariance structure using a between-within method, with time being the repeated measure.
For organ weights at birth and 3 mo, necropsy an analysis of covariance was done using the GLM procedure with the organ as the dependent variable and BW as the covariate. This analysis was done for the heart, kidney, liver, quadracep muscle, loin muscle, various fat depots, as well as measurements of loin eye area and backfat.
RESULTS

Body Variables

Body weight, averaged across all time points, from birth until 3 mo of age tended to be greater in lambs born to overfed ewes than lambs born to control \((P = 0.09)\) and restricted \((P = 0.05)\) ewes \((15.45 \pm 0.64, 15.07 \pm 0.64, 16.97 \pm 0.64 \text{ kg, control, restricted, overfed, respectively; Figure 5})\). At birth, overfed offspring were heavier \((P < 0.01)\) than control and restricted offspring and continued to maintain this heavier weight until 3 mo of age \((P \leq 0.05; \text{ Table 3})\). The greatest BW gain \((P \leq 0.05)\) in overfed offspring vs. control and restricted offspring was observed after weaning, from wk 9 to 12. There were no differences in BW of restricted lambs when compared with control lambs during the study \((P = 0.7)\).

Average daily gain was calculated for each lamb throughout the study. Lambs born to overfed ewes had greater \((P = 0.01)\) ADG, averaged across all time points from wk 1 until 3 mo of age, when compared with control and restricted offspring \((0.21 \pm 0.01, 0.21 \pm 0.01, 0.23 \pm 0.01 \text{ kg, control, restricted, overfed, respectively; Table 3})\). The greatest ADG in the overfed vs. control and restricted offspring was observed post-weaning from week 9 to 12 \((P \leq 0.004; \text{ Figure 6})\). There were no differences among treatment groups during the pre-weaning period \((wk 1 \text{ to } 8)\) when analyzed separately from post-weaning \((P = 0.9)\).

At birth, overfed offspring had 10% greater \((P = 0.01)\) CRL than control offspring and tended to have greater \((P = 0.07)\) CRL than restricted offspring \((46.46 \pm 1.20, 48.14 \pm 1.20, 51.27 \pm 1.20 \text{ cm, control, restricted, overfed, respectively})\). Averaged across time points, from birth until 3 mo of age, overfed offspring had greater \((P \leq 0.03)\) average CRL than control and restricted offspring, and restricted offspring tended to have reduced \((P = 0.08)\) average CRL than control offspring \((66.14 \pm 0.85, 64.02 \pm 0.85, 68.86 \pm 0.85 \text{ cm, control, restricted, overfed, respectively})\).
Figure 5. The effect of maternal diet on body weight (BW; kg) of lambs measured weekly from birth until three months of age.

Body weight of lambs born to ewes given 1 of 3 diets from 31d of gestation until parturition (Control, 100% NRC; Restricted, 60% NRC; Overfed, 140% NRC). All lambs were fed a control diet in post-natal life. Body weight at the birth time point was analyzed in all 35 lambs on study. After birth, BW was analyzed in lambs monitored for another 3 months (Control, n = 6; Restricted, n = 4 to 5; Overfed, n = 4 to 6/week). Body weight treatment differences between overfed and control at that specific time point; * $P \leq 0.05$, # $P \leq 0.1$. Birth SEM = 0.19, Week 1 to 12 largest SEM = 0.64.
**Table 3.** Body variables of lambs measured weekly from birth until 3 months (mo) of age from ewes given 1 of 3 diets.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Restricted</th>
<th>Overfed</th>
<th>SEM²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Body weight, kg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>4.82ᵃ</td>
<td>4.65ᵃ</td>
<td>5.78ᵇ</td>
<td>0.19</td>
</tr>
<tr>
<td>3 mo</td>
<td>29.67ᵃ</td>
<td>28.41ᵃ</td>
<td>33.51ᵇ</td>
<td>1.37</td>
</tr>
<tr>
<td>Pre-weaning³</td>
<td>10.73</td>
<td>10.35</td>
<td>11.71</td>
<td>0.69</td>
</tr>
<tr>
<td>Post-weaning⁴,⁵</td>
<td>26.07ᵃ</td>
<td>25.58ᵃ</td>
<td>29.37ᵇ</td>
<td>1.13</td>
</tr>
<tr>
<td><strong>Average from birth to 3 mo⁵,⁶</strong></td>
<td>15.45</td>
<td>15.07</td>
<td>16.97</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>Average daily gain, kg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-weaning³</td>
<td>0.18</td>
<td>0.17</td>
<td>0.18</td>
<td>0.01</td>
</tr>
<tr>
<td>Post-weaning⁴</td>
<td>0.28ᵃ</td>
<td>0.29ᵃ</td>
<td>0.33ᵇ</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Average from wk 1 to 3 mo</strong></td>
<td>0.21ᵃ</td>
<td>0.21ᵃ</td>
<td>0.23ᵇ</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Crown rump length, cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>46.46ᵃ</td>
<td>48.14ᵇ</td>
<td>51.27ᵇ</td>
<td>1.20</td>
</tr>
<tr>
<td>3 mo</td>
<td>84.03</td>
<td>81.60</td>
<td>85.10</td>
<td>1.89</td>
</tr>
<tr>
<td>Pre-weaning³</td>
<td>60.40ᵃ</td>
<td>57.93ᵃ</td>
<td>63.83ᵇ</td>
<td>0.86</td>
</tr>
<tr>
<td>Post-weaning⁴</td>
<td>79.06</td>
<td>77.70</td>
<td>80.00</td>
<td>1.72</td>
</tr>
<tr>
<td><strong>Average from birth to 3 mo⁷</strong></td>
<td>66.14ᵃ</td>
<td>64.02ᵃ</td>
<td>68.86ᵇ</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>Heart girth, cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>38.43ᵃ</td>
<td>37.98ᵃ</td>
<td>41.72ᵇ</td>
<td>0.90</td>
</tr>
<tr>
<td>3 mo⁶</td>
<td>73.66ᵇ</td>
<td>73.03ᵃ</td>
<td>78.11ᵇ</td>
<td>1.60</td>
</tr>
<tr>
<td>Pre-weaning³</td>
<td>50.49</td>
<td>50.02</td>
<td>51.81</td>
<td>0.90</td>
</tr>
<tr>
<td>Post-weaning⁴</td>
<td>71.01</td>
<td>69.61</td>
<td>72.87</td>
<td>1.14</td>
</tr>
<tr>
<td><strong>Average from birth to 3 mo⁷</strong></td>
<td>56.81</td>
<td>56.05</td>
<td>58.06</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Body condition score⁸</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mo</td>
<td>2.91</td>
<td>2.89</td>
<td>2.95</td>
<td>0.04</td>
</tr>
<tr>
<td>Pre-weaning³</td>
<td>2.94</td>
<td>2.92</td>
<td>2.97</td>
<td>0.02</td>
</tr>
<tr>
<td>Post-weaning⁴,⁵</td>
<td>2.90ᵇ</td>
<td>2.88ᵃ</td>
<td>2.96ᵇ</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Average from wk 5 to 3 mo</strong></td>
<td>2.92ᵃ</td>
<td>2.90ᵃ</td>
<td>2.96ᵇ</td>
<td>0.02</td>
</tr>
</tbody>
</table>

ᵃ,ᵇ Means within a row with different superscripts differ \( P \leq 0.05 \).

¹ Treatments: Control = 100% NRC; Restricted = 60% NRC; Overfed = 140% NRC.

² Largest SEM across treatments for variable.

³ Average of weeks 0 to 8 of age

⁴ Average of weeks 9 to 12 of age.

⁵ Control vs. Overfed, \( P \leq 0.1 \).

⁶ Overfed vs. Restricted, \( P \leq 0.1 \).

⁷ Control vs. Restricted, \( P \leq 0.1 \).

⁸ Body condition score based on scale from (Russel, 1991).
**Figure 6.** Effect of maternal diet on average daily gain (ADG; kg/d) of lambs, calculated weekly from week 1 to 12 of age.

Average daily gain of lambs born to ewes given 1 of 3 diets from 31d of gestation until parturition (Control, 100% NRC; Restricted, 60% NRC; Overfed, 140% NRC). All lambs were fed a control diet in post-natal life. Average daily gain was analyzed in lambs monitored for 3 months (Control, n = 6; Restricted, n = 4 to 5; Overfed, n = 4 to 6/ week). Average daily gain treatment differences between overfed and control at that specific time point; * $P \leq 0.05$, # $P \leq 0.1$. Largest SEM = 0.01.
respectively; Figure 7). There were no differences among treatment groups at 3 mo of age \((P = 0.4)\). Overfed offspring \((63.83 \pm 0.86 \text{ cm})\) had greater \((P = 0.01)\) CRL during the pre-weaning period than control \((60.40 \pm 0.86 \text{ cm})\) and restricted \((57.93 \pm 0.86 \text{ cm})\) offspring, but not during post-weaning \((P = 0.5; \text{ Table 3})\).

At birth, overfed offspring had greater HG \((P \leq 0.01)\) than control and restricted offspring \((38.43 \pm 0.90, 37.98 \pm 0.90, 41.72 \pm 0.90 \text{ cm}, \text{ control, restricted, overfed, respectively; Figure 8})\). They maintained this greater \((P \leq 0.06)\) HG until 3 mo of age \((\text{ Table 3})\). There were no differences in restricted versus control lambs at birth \((P = 0.7)\) or 3 mo of age \((P = 0.7)\). There were no differences among treatment groups during pre or post weaning \((P \leq 0.3)\). When averaged across all time points, HG tended to be greater \((P = 0.1)\) in overfed vs. restricted offspring.

Body condition score was measured starting at week 5 of age until 3 mo of age \((\text{ Figure 9})\). Week 5 was the first week after weaning began and the lambs were given ad libitum creep feed and hay. Averaged across all time points, from week 5 until 3 mo of age, overfed offspring had greater \((P \leq 0.04)\) average BCS than control and restricted offspring \((2.92 \pm 0.02, 2.90 \pm 0.02, 2.96 \pm 0.02, \text{ control, restricted, overfed, respectively; Table 3})\). There were no differences in average BCS across all time points from week 5 to 3 mo of age in restricted versus control offspring \((P = 0.4)\). During post-weaning, overfed offspring tended to have greater \((P = 0.07)\) BCS than control offspring and had significantly greater \((P = 0.05)\) BCS than restricted offspring.
Figure 7. Effect of maternal nutrition on crown rump length (CRL; cm), measured from birth to 3 months of age in lambs.

Crown rump length of lambs born to ewes given 1 of 3 diets from 31d of gestation until parturition (Control, 100% NRC; Restricted, 60% NRC; Overfed, 140% NRC). All lambs were fed a control diet in post-natal life. Crown rump length at the birth time point was analyzed in all 35 lambs on study. After birth, CRL was analyzed in lambs monitored for 3 months (Control, n = 6; Restricted, n = 4 to 5; Overfed, n = 4 to 6/ week). Crown rump length treatment differences between overfed and control at that specific time point; * P ≤ 0.05, # P ≤ 0.1. Largest SEM at birth = 1.20. Week 1 to 12 largest SEM = 0.85.
Heart girth of lambs born to ewes given 1 of 3 diets from 31d of gestation until parturition (Control, 100% NRC; Restricted, 60% NRC; Overfed, 140% NRC). All lambs were fed a control diet in post-natal life. Heart girth at the birth time point was analyzed in all 35 lambs on study. After birth, HG was analyzed in lambs monitored for 3 months (Control, n = 6; Restricted, n = 4 to 5; Overfed, n = 4 to 6/week). Heart girth treatment differences between overfed and control at that specific time point; * $P \leq 0.05$. Heart girth treatment differences between restricted and control at that specific time point; † $P \leq 0.05$. Largest SEM at birth = 0.90. Week 1 to 12 largest SEM = 0.91.
**Figure 9.** Effect of maternal nutrition on body condition scores (BCS) of lambs from week 5 (first week after start of weaning) until 3 months of age.

Body condition scores of lambs born to ewes given 1 of 3 diets from 31d of gestation until parturition (Control, 100% NRC; Restricted, 60% NRC; Overfed, 140% NRC). All lambs were fed a control diet in post-natal life. Body condition scores were measured from week 5 until 3 months of age (Control, n = 6; Restricted, n = 4 to 5; Overfed, n = 4 to 6/ week). Week 5 was the first week lambs were given ad libitum creep feed and hay. Body condition score treatment differences between overfed and control at that specific time point; # P ≤ 0.1. Largest SEM = 0.02.
Organ Weights

Heart weight was quantified at birth and 3 mo necropsy (Figure 10). When heart weight is expressed with BW as a covariate, at 3 mo, the restricted offspring have greater ($P = 0.04$) heart weights than the control offspring, and overfed offspring tend to have greater ($P = 0.1$) heart weights than control offspring (0.15 ± 0.01, 0.18 ± 0.01, 0.17 ± 0.01, control, restricted, overfed, respectively; Table 4). There were no differences in heart weight at birth ($P = 0.4$).

Liver weight, analyzed with BW as a covariate at birth tended to be greater in overfed offspring than control offspring ($P = 0.08$; Table 4). At 3 mo necropsy there were no differences in liver weight among treatment groups ($P = 0.6$; Figure 11). Using a grid, LEA, the cross section of the longissimus dorsi muscle, was measured at birth and 3 mo necropsy (Figure 12). When expressed with BW as a covariate at birth, restricted offspring had reduced LEA than control offspring ($P = 0.01$; Table 4). At 3 mo there was no difference when adjusted for BW among treatment groups ($P = 0.4$). Backfat thickness was measured only at 3 mo necropsy (Figure 13). When analyzed with BW as a covariate, the restricted offspring had reduced backfat versus the control offspring at 3 mo of age ($P = 0.05$; Table 4).

Metabolic Hormones

Hormone concentrations were measured at birth and once per week until 3 mo of age. There were no differences among treatment groups in GH at birth ($P = 0.3$) or averaged across time points from birth until 3 mo of age ($P = 0.6$; Figure 14). At 3 mo of age overfed offspring tended to have greater GH concentrations than control offspring ($P = 0.1$). Average GH concentrations tended to be greater ($P = 0.1$) in restricted (12.25 ± 1.78 ng/mL) versus control
Figure 10. Lamb heart weights at birth and 3 month necropsy, expressed with body weight (BW) as a covariate.

Heart weight of lambs quantified at birth (n = 6/treatment) and 3 month (Control, n = 6; Restricted, n = 4; Overfed, n = 4) necropsy. Heart weight was analyzed with BW as a covariate. Heart weight treatment differences between treatments indicated by * $P \leq 0.05$ vs. Control and # $P \leq 0.1$ vs. Control. Values are means ± SEM.
Table 4. Lamb organ weights, loin eye area, and backfat thickness measurements at birth and 3 month (mo) necropsy expressed with body weight (BW) as a covariate.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment¹</th>
<th>Control</th>
<th>Restricted</th>
<th>Overfed</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>3 mo³</td>
<td>0.15ᵃ</td>
<td>0.18ᵇ</td>
<td>0.17ᵃᵇ</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Liver weight, kg</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth³</td>
<td>0.11</td>
<td>0.11</td>
<td>0.12</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>3 mo</td>
<td>0.75</td>
<td>0.70</td>
<td>0.73</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Loin eye area, cm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>7.57ᵃ</td>
<td>6.27ᵇ</td>
<td>7.49ᵃᵇ</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>3 mo</td>
<td>23.11</td>
<td>21.01</td>
<td>20.10</td>
<td>3.00</td>
<td></td>
</tr>
<tr>
<td>Backfat thickness, cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>NDᵈ</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 mo</td>
<td>0.20ᵃ</td>
<td>0.11ᵇ</td>
<td>0.26ᵃᵇ</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ,ᵇ Means within a row with different superscripts differ (P ≤ 0.05).
¹ Treatments: Control = 100% NRC; Restricted = 60% NRC; Overfed = 140% NRC.
² Expressed with BW as a covariate.
³ Control vs. Overfed, (P ≤ 0.1).
⁴ Not Detectable.
Figure 11. Lamb liver weights from birth and 3 month necropsy expressed with body weight (BW) as a covariate.

Liver weight of lambs quantified at birth (n = 6/treatment) and 3 month (Control, n = 4; Restricted, n = 4; Overfed, n = 4) necropsy. Liver weight was analyzed with BW as a covariate. Liver weight treatment differences indicated by # P ≤ 0.1 vs. Control. Values are means ± SEM.
Figure 12. Loin eye area (LEA), cross section of the longissimus dorsi muscle, measured in lambs at birth and 3 month necropsy expressed with body weight (BW) as a covariate.

Loin eye area of lambs measured, using a grid, at birth (n = 6/treatment) and 3 month (Control, n = 6; Restricted, n = 4; Overfed, n = 4) necropsy. Loin eye area was analyzed with BW as a covariate. Loin eye area treatment differences indicated by * $P \leq 0.05$ vs. Control. Values are means ± SEM.
Figure 13. Backfat thickness of lambs measured at 3 month necropsy, expressed with body weight (BW) as a covariate.

Backfat thickness of lambs was measured at 3 month (Control, n = 6; Restricted, n = 4; Overfed, n = 4) necropsy. Backfat was analyzed with BW as a covariate. * Indicates backfat treatment difference $P \leq 0.05$ vs. Control. Values are means ± SEM.
Figure 14. Serum growth hormone (GH; ng/mL) concentrations measured from birth until 3 months of age.

Growth hormone (GH) concentrations of lambs born to ewes given 1 of 3 diets from 31d of gestation until parturition (Control, 100% NRC; Restricted, 60% NRC; Overfed, 140% NRC). All lambs were fed a control diet in post-natal life. Growth hormone at the birth time point was analyzed in all 35 lambs on study. After birth, GH was analyzed in lambs monitored for 3 months (Control, n = 6; Restricted, n = 4 to 5; Overfed, n = 4 to 6/week). Growth hormone treatment differences between overfed and control at that specific time point; # \( P \leq 0.1 \). Largest SEM at birth = 3.71. Week 1 to 12 largest SEM = 1.61.
(8.49 ± 1.78 ng/mL) and overfed (8.42 ± 1.78 ng/mL) lambs pre-weaning (wk 0 to 8), but were not different post-weaning \((P = 0.7; \text{Table 5})\).

Insulin-like growth factor-I concentrations were greater in overfed offspring \((334.67 ± 65.93 \text{ ng/mL})\) than control offspring \((149.51 ± 65.93 \text{ ng/mL}; \ P = 0.05)\) and tended to be greater in overfed versus restricted \((155.07 ± 65.93 \text{ ng/mL})\) offspring at 3 mo of age \((P = 0.08; \text{Figure 15})\). There were no differences among treatment groups at birth \((P = 0.9)\) or averaged across time points, from birth until 3 mo of age \((P = 0.2)\). During pre-weaning there was an overall treatment by time effect \((P = 0.1)\), but there were no differences among treatment groups during post-weaning \((P = 0.3; \text{Table 5})\). Concentrations of IGF-I decreased at wk 4 due to fasting in preparation for an IVGTT. After wk 4, IGF-I concentrations increased since lambs were given free access to creep feed and hay beginning at wk 4 through the remainder of the study.

Insulin-like growth factor binding protein-3 concentrations at birth were not different among treatment groups \((P = 0.6)\). At 3 mo of age, overfed offspring had greater IGFBP-3 concentrations than control offspring \((P = 0.01; \text{Table 5})\). When averaged across time points, IGFBP-3 was greater \((P = 0.04)\) in overfed \((121.99 ± 6.24 \text{ AU})\) versus restricted lambs \((103.10 ± 6.24 \text{ AU})\). Post-weaning, week 9 through 12 of age, average IGFBP-3 concentrations were greater \((P ≤ 0.05)\) in overfed \((175.27 ± 12.19 \text{ AU})\) versus control \((140.56 ± 12.19 \text{ AU})\) and restricted \((125.79 ± 12.19 \text{ AU})\) lambs (Figure 16). The only difference in IGFBP-2 among treatments was observed at 3 mo of age (Figure 17), when overfed \((18.49 ± 1.52 \text{ AU})\) lambs had greater \((P = 0.01)\) concentrations than control \((11.91 ± 1.52 \text{ AU})\) lambs (Table 5).

There were no significant differences in leptin concentrations (Table 6) at birth \((P = 0.9)\) or across time points from birth until 3 mo \((P = 0.5)\), among treatment groups (Figure 18).
Table 5. Serum concentrations of somatotropic axis hormones [growth hormone (GH), insulin-like growth factor (IGF)-1, IGF binding protein (IGFBP) -3 and -2] measured in lambs from birth until 3 months of age.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>GH, ng/mL</td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>5.47</td>
</tr>
<tr>
<td>3 mo⁵</td>
<td>2.16</td>
</tr>
<tr>
<td>Pre-weaning⁴</td>
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</tr>
<tr>
<td>Post-weaning⁵, 6, 7</td>
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</tr>
<tr>
<td>Average birth to 3 mo</td>
<td>7.35</td>
</tr>
<tr>
<td>IGF-I, ng/mL</td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>110.42</td>
</tr>
<tr>
<td>3 mo⁶</td>
<td>149.51a</td>
</tr>
<tr>
<td>Pre-weaning⁴</td>
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</tr>
<tr>
<td>Post-weaning⁵</td>
<td>187.78</td>
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<tr>
<td>Average birth to 3 mo</td>
<td>147.68</td>
</tr>
<tr>
<td>IGFBP-3, AU⁸</td>
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</tr>
<tr>
<td>Birth</td>
<td>68.15</td>
</tr>
<tr>
<td>3 mo⁷</td>
<td>120.16a</td>
</tr>
<tr>
<td>Pre-weaning⁴</td>
<td>98.16</td>
</tr>
<tr>
<td>Post-weaning⁵</td>
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<tr>
<td>Average birth to 3 mo</td>
<td>111.20ab</td>
</tr>
<tr>
<td>IGFBP-2, AU⁸</td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>Average birth to 3 mo</td>
<td>15.38</td>
</tr>
</tbody>
</table>

a, b Means within a row with different superscripts, differ (P ≤ 0.05).
¹Treatments: Control = 100% NRC; Restricted = 60% NRC; Overfed = 140% NRC.
²Largest SEM across treatments for variable.
³Control vs. Overfed, (P ≤ 0.1).
⁴Average of weeks 0 to 8 of age.
⁵Average of weeks 9 to 12 of age.
⁶Control vs. Restricted, (P ≤ 0.1).
⁷Restricted vs. Overfed, (P ≤ 0.1).
⁸Arbitrary units.
Figure 15. Serum insulin-like growth factor-I (IGF-I; ng/mL) concentrations measured from birth until 3 months of age.

Insulin-like growth factor-I (IGF-I) concentrations of lambs born to ewes given 1 of 3 diets from 31d of gestation until parturition (Control, 100% NRC; Restricted, 60% NRC; Overfed, 140% NRC). All lambs were fed a control diet in post-natal life. At the birth time point, IGF-I was analyzed in all 35 lambs on study. After birth, IGF-I was analyzed in lambs monitored for 3 months (Control, n = 6; Restricted, n = 4 to 5; Overfed, n = 4 to 6/ week). Insulin-like growth factor treatment differences between overfed and control at that specific time point: *P ≤ 0.05, # P ≤ 0.1. Largest SEM at birth = 22.10. Week 1 to 12 largest SEM = 25.33.
Figure 16. Serum insulin-like growth factor binding protein-3 (IGFBP-3) concentrations measured in arbitrary units (AU) from birth until 3 months of age.

Insulin-like growth factor binding protein-3 (IGFBP-3) concentrations of lambs born to ewes given 1 of 3 diets from 31d of gestation until parturition (Control, 100% NRC; Restricted, 60% NRC; Overfed, 140% NRC). All lambs were fed a control diet in post-natal life. At the birth time point, IGFBP-3 was analyzed in all 35 lambs on study. After birth, IGFBP-3 was analyzed in lambs monitored for 3 months (Control, n = 6; Restricted, n = 4 to 5; Overfed, n = 4 to 6/week). Insulin-like growth factor binding protein-3 treatment differences between overfed and control at that specific time point; *$P \leq 0.05$, # $P \leq 0.1$. Insulin-like growth factor binding protein-3 treatment differences between restricted and control at that specific time point; □ $P \leq 0.05$. Largest SEM at birth = 5.13. Week 1 to 12 largest SEM = 6.24.
Figure 17. Serum concentrations of insulin-like growth factor binding protein-2 (IGFBP-2) measured in arbitrary units (AU) from birth until 3 months of age in lambs.

Insulin-like growth factor binding protein-3 concentrations of lambs born to ewes given 1 of 3 diets from 31d of gestation until parturition (Control, 100% NRC; Restricted, 60% NRC; Overfed, 140% NRC). All lambs were fed a control diet in post-natal life. At the birth time point, IGFBP-3 was analyzed in all 35 lambs on study. After birth, IGFBP-3 was analyzed in lambs monitored for 3 months (Control, n = 6; Restricted, n = 4 to 5; Overfed, n = 4 to 6/ week). Insulin-like growth factor binding protein-2 treatment differences between overfed and control at that specific time point; *P ≤ 0.05, # P ≤ 0.1. Insulin-like growth factor binding protein-2 treatment differences between restricted and control at that specific time point; □ P ≤ 0.05, ‡ P ≤ 0.1. Largest SEM at birth = 1.06. Week 1 to 12 largest SEM = 0.58.
Figure 18. Plasma leptin concentrations measured in ng/mL of lambs from birth until 3 months of age.

Plasma leptin concentrations of lambs born to ewes given 1 of 3 diets from 31d of gestation until parturition (Control, 100% NRC; Restricted, 60% NRC; Overfed, 140% NRC). All lambs were fed a control diet in post-natal life. At the birth time point, leptin was analyzed in all 35 lambs on study. After birth, leptin was analyzed in lambs monitored for 3 months (Control, n = 6; Restricted, n = 4 to 5; Overfed, n = 4 to 6/ week). Leptin treatment differences between overfed and control at that specific time point; *$P \leq 0.05$. Leptin treatment differences between overfed and restricted at that specific time point; # $P \leq 0.05$. Largest SEM at birth = 0.20. Week 1 to 12 largest SEM = 0.09.
Table 6. Plasma leptin and serum insulin measured in ng/mL, and serum total cholesterol (TC) and triglyceride (TG) concentrations measured in mg/dL from lamb birth until 3 months (mo) of age.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Restricted</th>
<th>Overfed</th>
<th>SEM²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Leptin, ng/mL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>1.22</td>
<td>1.17</td>
<td>1.32</td>
<td>0.20</td>
</tr>
<tr>
<td>3 mo</td>
<td>1.03ᵃᵇ</td>
<td>0.78ᵃ</td>
<td>1.24ᵇ</td>
<td>0.13</td>
</tr>
<tr>
<td>Pre-weaning ³</td>
<td>1.15</td>
<td>1.30</td>
<td>1.29</td>
<td>0.11</td>
</tr>
<tr>
<td>Post-weaning ⁴</td>
<td>1.01</td>
<td>0.98</td>
<td>1.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Average birth to 3 mo</td>
<td>1.11</td>
<td>1.21</td>
<td>1.23</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Insulin, ng/mL</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Birth</td>
<td>1.17ᵃ</td>
<td>1.95ᵇ</td>
<td>1.06ᵃ</td>
<td>0.36</td>
</tr>
<tr>
<td>3 mo</td>
<td>0.41</td>
<td>0.50</td>
<td>0.66</td>
<td>0.24</td>
</tr>
<tr>
<td>Pre-weaning ³, ⁵</td>
<td>0.34ᵃ</td>
<td>0.49ᵇ</td>
<td>0.47ᵇ</td>
<td>0.05</td>
</tr>
<tr>
<td>Post-weaning ⁴, ⁵</td>
<td>0.33</td>
<td>0.50</td>
<td>0.61</td>
<td>0.14</td>
</tr>
<tr>
<td>Average birth to 3 mo</td>
<td>0.33ᵃ</td>
<td>0.49ᵇ</td>
<td>0.49ᵇ</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>TC, mg/dL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>35.13</td>
<td>38.42</td>
<td>30.28</td>
<td>4.39</td>
</tr>
<tr>
<td>3 mo</td>
<td>30.18</td>
<td>37.70</td>
<td>28.70</td>
<td>9.78</td>
</tr>
<tr>
<td><strong>TG, mg/dL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>22.52</td>
<td>15.52</td>
<td>23.96</td>
<td>5.22</td>
</tr>
<tr>
<td>3 mo</td>
<td>15.31</td>
<td>16.86</td>
<td>15.38</td>
<td>2.73</td>
</tr>
</tbody>
</table>

ᵃ,ᵇ Means within a row, with different superscripts, differ (P ≤ 0.05).

¹ Treatments: Control = 100% NRC; Restricted = 60% NRC; Overfed = 140% NRC.
² Largest SEM across treatments for variable.
³ Average of weeks 0 to 8 of age.
⁴ Average of weeks 9 to 12 of age.
⁵ Control vs. Overfed, (P < 0.01).
At 3 mo of age, overfed offspring (1.24 ± 0.13 ng/mL) had greater \((P = 0.03)\) leptin concentrations than restricted offspring (0.78 ± 0.13 ng/mL).

Averaged across all time points, from birth until 3 mo of age, both overfed (0.49 ± 0.05 ng/mL) and restricted (0.49 ± 0.05 ng/mL) offspring had increased \((P \leq 0.05)\) serum insulin concentrations when compared with the control (0.33 ± 0.05 ng/mL) offspring (Figure 19). At birth, restricted offspring had greater \((P = 0.1)\) insulin concentrations than control and overfed offspring. There were no differences in insulin concentrations at 3 mo \((P = 0.7)\) of age (Table 6). During pre-weaning control offspring had reduced \((P \leq 0.1)\) insulin concentrations compared with overfed and restricted offspring. During post-weaning, overfed offspring tended to have greater \((P = 0.1)\) insulin concentrations than control offspring.

Total cholesterol was measured from lambs at birth and 3 mo of age. There were no differences among treatment groups at birth \((P = 0.4)\) or at 3 mo of age \((P = 0.8)\) due to the variation among lambs within a treatment (Table 6). Total cholesterol concentrations decreased with age (Figure 20). Triglyceride concentration was measured from lambs at birth and 3 mo of age (Table 6). Triglyceride concentrations decreased with age, but there were no significant differences among treatment groups at either time point, birth \((P = 0.5)\) or 3 mo of age \((P = 0.9;\) Figure 21).

Intravenous Glucose Tolerance Test

Plasma glucose and insulin concentrations (Table 7) were measured from an IVGTT performed on lambs at 3 mo of age. There were no differences \((P = 0.7)\) in glucose concentrations (Figure 22) over the 2 h 30 min challenge among treatments, but insulin concentration (Figure 23) in overfed offspring tended to be greater than control
Figure 19. Serum insulin concentrations measured in ng/mL of lambs from birth until 3 months of age.

Serum insulin concentrations of lambs born to ewes given 1 of 3 diets from 31d of gestation until parturition (Control, 100% NRC; Restricted, 60% NRC; Overfed, 140% NRC). All lambs were fed a control diet in post-natal life. At the birth time point, insulin was analyzed in all 35 lambs on study. After birth, insulin was analyzed in lambs monitored for 3 months (Control, n = 6; Restricted, n = 4 to 5; Overfed, n = 4 to 6/week). Insulin treatment differences between overfed and control at that specific time point; *$P \leq 0.05$, # $P \leq 0.1$. Insulin treatment differences between restricted and control at that specific time point; □ $P \leq 0.1$. Largest SEM at birth = 0.36. Week 1 to 12 largest SEM = 0.05.
Figure 20. Total cholesterol (TC; mg/dL) concentrations measured from lambs at birth and 3 months of age.

Total cholesterol was measured from birth and 3 month serum samples collected from lambs. There was no difference observed among treatment groups at birth or 3 month time points. Values are means ± SEM.
Figure 21. Triglyceride (TG; mg/dL) concentrations measured from lambs at birth and 3 months of age.

Triglyceride concentrations were measured in mg/dL, from serum samples collected from lambs at birth and 3 months of age. There was no difference among treatment groups at either time point. Values are means ± SEM.
Table 7. Plasma glucose and insulin concentrations from the intravenous glucose tolerance test (IVGTT) performed on lambs at 3 months of age.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment (^1)</th>
<th>Control</th>
<th>Restricted</th>
<th>Overfed</th>
<th>SEM (^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average of all time points, mg/dL</td>
<td>137.08</td>
<td>128.88</td>
<td>127.48</td>
<td>10.19</td>
<td></td>
</tr>
<tr>
<td>Baseline, mg/dL</td>
<td>91.08</td>
<td>97.16</td>
<td>88.42</td>
<td>8.46</td>
<td></td>
</tr>
<tr>
<td>Peak concentration, mg/dL (^3)</td>
<td>219.44</td>
<td>211.28</td>
<td>180.54</td>
<td>16.75</td>
<td></td>
</tr>
<tr>
<td>AUC, AU (^4)</td>
<td>18,201.90</td>
<td>15,717.20</td>
<td>16,916.30</td>
<td>1467.69</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average of all time points, ng/mL (^3)</td>
<td>0.84</td>
<td>1.17</td>
<td>1.41</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>Baseline, ng/mL (^3)</td>
<td>0.30(^a)</td>
<td>0.61(^b)</td>
<td>0.42(^{ab})</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Peak concentration, ng/mL (^5)</td>
<td>1.75(^a)</td>
<td>2.46(^{ab})</td>
<td>3.66(^b)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>AUC, AU (^4)</td>
<td>106.31</td>
<td>149.06</td>
<td>200.50</td>
<td>47.37</td>
<td></td>
</tr>
<tr>
<td>1(^st) phase response, ng/mL</td>
<td>1.69</td>
<td>2.20</td>
<td>1.75</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Insulin:glucose ratio, ng/mL, mg/dL</td>
<td>0.005(^a)</td>
<td>0.009(^b)</td>
<td>0.01(^c)</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a, b, c}\) Means within a row with different superscripts, differ \((P \leq 0.05)\).

\(^1\) Treatments: Control = 100% NRC; Restricted = 60% NRC; Overfed = 140% NRC.

\(^2\) Largest SEM across treatments for variable. \(^3\) Control vs. Overfed, \((P \leq 0.1)\). \(^4\) Expressed in arbitrary units (AU).

\(^5\) Overfed vs. Restricted, \((P \leq 0.1)\).
**Figure 22.** Plasma glucose concentrations and glucose area under the curve (AUC) from intravenous glucose tolerance test (IVGTT) performed on lambs at 3 months of age.

Plasma glucose concentrations were analyzed from samples collected during the intravenous glucose tolerance test (IVGTT) from 30 minutes before glucose infusion to 120 minutes after glucose infusion. The glucose tolerance test was performed at 3 months of age on 14 lambs [Control (CON), n = 6; Restricted (RES), n = 4; Overfed (OVER), n = 4]. Baseline glucose concentrations were calculated by the average of -30, -15, and 0 time points. Glucose peak concentrations were measured during the tolerance test. Inset shows AUC expressed in arbitrary units (AU). Glucose treatment differences between overfed and control at that specific time point; # $P \leq 0.1$. Glucose treatment differences between restricted and control at that specific time point; *$P \leq 0.05$. Largest SEM = 10.19. AUC largest SEM = 1467.69.
Plasma insulin concentrations were analyzed from samples collected during the intravenous glucose tolerance test (IVGTT) from 30 minutes before glucose infusion to 120 minutes after glucose infusion. The glucose tolerance test was performed at 3 months of age on 14 lambs [Control (CON), n = 6; Restricted (RES), n = 4; Overfed (OVER), n = 4]. Baseline insulin concentrations were calculated by the average of -30, -15, and 0 time points. Insulin peak concentrations were analyzed at 10 minutes relative to glucose infusion and AUC were measured during the tolerance test. Inset shows AUC expressed in arbitrary units (AU). First phase insulin response was calculated by the sum of concentrations at 2 and 5 min minus the average baseline concentration. Insulin treatment differences between overfed and control at that specific time point; * \( P \leq 0.05 \). Largest SEM = 0.27. AUC largest SEM = 47.37.
offspring ($P = 0.1$). There were also no significance differences in glucose baseline or AUC among treatments ($P > 0.1$). Overfed (180.54 ± 16.75 mg/dL) offspring tended to have reduced ($P = 0.1$) glucose peak concentration than control (219.44 ± 16.75 mg/dL) offspring. Insulin baseline concentrations were greater in restricted offspring than control ($P = 0.02$) and overfed ($P = 0.1$) offspring. There was no difference in AUC or first phase insulin response among treatment groups ($P = 0.4$). Ten minutes after glucose infusion, insulin peak concentration was reached and was greater in overfed offspring than control ($P = 0.003$) and restricted ($P = 0.07$) offspring (Figure 24). Both overfed and restricted offspring exhibited a greater insulin:glucose ratio than the control offspring ($P \leq 0.05$; Figure 24).
Figure 24. Plasma insulin:glucose ratio from the intravenous glucose tolerance test (IVGTT) performed on lambs at 3 months of age.

The insulin to glucose ratio was significantly greater in overfed and restricted offspring than control offspring. Ten minutes after glucose infusion overfed lambs had a greater insulin:glucose ratio than the control offspring and maintained this greater ratio until 30 minutes after infusion. Insulin:glucose treatment differences between overfed and control at that specific time point; * $P \leq 0.05$. Largest SEM = 0.001.
DISCUSSION

Maternal nutrition has been implicated in developmental programming associated with pre- and post-natal changes affecting health and performance of offspring (Barker, 2004; Wu et al., 2006). Fetal growth restriction and maternal under-nutrition result in negative impacts on growth efficiency and body composition (Greenwood et al., 2000; Wu et al., 2006; Larson et al., 2009; Neville et al., 2010). This current study focused on determining the effects poor maternal nutrition, during gestation, has on post-natal growth of offspring. Specifically studying the changes in concentrations of hormones involved in growth and differences in organ size and body composition. These changes could potentially be linked to altered fetal programming as a result of poor maternal nutrition. Adverse consequences, such as these, can become more evident with increasing age as compensatory adaptations in other tissues and organ systems fail (McMillen and Robinson, 2005).

Reed et al. (2007) and Swanson et al. (2008) demonstrated that maternal nutrient-restriction during the last two-thirds of gestation in sheep reduced birth weights. However, Gardner et al. (2005) and Ford et al. (2007) have reported that maternal restriction significantly altered composition of growth and metabolism in the absence of birth weight differences of offspring born to ewes nutrient-restricted in either early gestation, early to mid gestation, or late gestation, which support the data from the current study where there were no differences in birth weight observed in lambs born to nutrient-restricted ewes from d 31 of gestation until parturition. However, developmental programming can occur independently of birth weight (Barker, 2004). Thus, long-term insults of poor maternal nutrition can occur even when BW is not different at birth.
In the current study, we observed heavier birth weight and BW throughout the 3 mo in lambs born to ewes overfed from 31 d of gestation until parturition. Similar to our data, Meyer et al. (2010) reported at 20 d of age, BW was greater in lambs born to overfed ewes versus control-fed ewes. However, these data are not consistent with several studies using an obese or over-nourished sheep model, who reported reduced birth weight of lambs born to ewes overfed during mid to late or late gestation (Muhlhausler et al., 2006; Swanson et al., 2008) or no differences in birth weight when ewes were overfed from 60 d before conception (Long et al., 2010). These differences between these studies and the current study are possibly attributed to different timing of changes in nutrition. All three of these studies began overfeeding ewes either before conception or later (d 50 or d 90) in gestation than the current study.

A number of studies have reported a U-shaped relationship between birth weight and adult fat mass, with a greater prevalence of obesity occurring at lighter and heavier birth weights (McMillen and Robinson, 2005). Since we observed a greater increase in BW after weaning in lambs born to overfed ewes, this could suggest that if these lambs had been monitored for a longer period of time, development of obesity would have been likely, especially given that maternal obesity at conception is associated with an increased risk of obesity in the offspring (Shankar et al., 2008; Long et al., 2010).

Low and high planes of maternal nutrition can impact growth of fetal organs (Reed et al., 2007; Carlson et al., 2009; Caton et al., 2009). Since the heart is fairly mature at birth, any environmental stimulus that alters the timing of the transition from hyperplastic to hypertrophic growth of cardiomyocytes may have long lasting consequences for heart growth and function. This includes effects in post-natal life of offspring exposed to poor maternal nutrition (Li et al., 2003). Studies have reported changes in cardiac morphometry and function in fetuses of
nutrient-restricted ewes (Vonnahme et al., 2003) and overfed (Dong et al., 2008; Fan et al., 2011; Wang et al., 2011) ewes and rats. Vonnahme et al. (2003) observed cardiac ventricular hypertrophy in fetuses of nutrient restricted ewes. These data support our findings that lambs born to restricted and overfed ewes exhibited increased heart weight compared with lambs born to control-fed ewes. This could suggest cardiovascular dysfunction and hypertrophy were programmed in-utero.

Lambs born to overfed ewes tended to have increased liver weight at birth compared with lambs born to control ewes, when expressed with BW as a covariate. This may suggest an increase in liver metabolic function due to nutrient repartitioning to allow for proper development based on findings reported in Vonnahme et al. (2003). It could also suggest an increase in hepatic triglyceride concentrations, which are associated with maternal over-nutrition and lead to fatty liver disease as observed in rats and nonhuman primates (Buckley et al., 2005; McCurdy et al., 2009). Vonnahme et al. (2003) reported that fetuses from nutrient-restricted ewes (fed 50% NRC) at 78 d of gestation had increased liver weight. In the current study we observed an increase in liver weight in lambs born to overfed ewes. Therefore, both maternal over-nutrition and nutrient-restriction can lead to altered fetal organ growth, although offspring of restricted mothers did not differ in liver weight in the current study.

Loin eye area, a predictor of muscularity, was reduced in lambs born to nutrient-restricted ewes at birth when compared with lambs born to control-fed ewes, which could indicate that offspring from nutrient-restricted mothers at birth exhibited reduced muscularity. Decreased muscularity is a normal result of maternal under-nutrition due to the decrease in available energy (Wu et al., 2006). These lambs are also expected to have increased adiposity (Greenwood and Thompson, 2007; Ford et al., 2007). However, the lambs born to restricted ewes on our study
did not exhibit increased adiposity. Backfat thickness, a predictor of adiposity, was reduced in lambs born to restricted ewes at 3 mo of age compared with those born to control and overfed ewes. The lambs exposed to under-nutrition in fetal life could have still been recovering from this restriction at 3 mo of age since this is still an early time point in post-natal life. This could potentially explain why we observed a reduction in backfat thickness of these lambs. However, at 3 mo of age we did observe an up regulation of factors involved in adipogenesis (Bush et al., 2013), suggesting that if we had monitored these lambs for a longer period of time they would have exhibited an increase in adiposity. Reduced nutrient intake at various time points before mating and during gestation can result in offspring with increased adipose depot weights at term or later in life (Gardner et al., 2005; Ford et al., 2007). This supports our hypothesis that these lambs would have exhibited increased adiposity if studied until a later time point.

Measures of the somatotropic axis are known to be predictive of nutritional status, energy balance, and growth rate (Freake et al., 2001; Rausch et al., 2002). Many of the studies analyzing the relationship between hormones of the somatotropic axis and poor maternal nutrition focused on changes in these hormones during fetal development. Few studies have been done determining if these changes persist in post-natal life and most of these studies used a model of maternal under-nutrition (Greenwood et al., 2000; Wu et al., 2006; Caton et al., 2007; Ford et al., 2007; Larson et al., 2009; Neville et al., 2010). We are now beginning to determine that maternal over-nutrition has similar negative consequences associated with the somatotropic axis of offspring in post-natal life. Thus, our objective was to design an experiment consisting of both a maternal under-nutrition model and a maternal over-nutrition model and study changes associated with the somatotropic axis of the offspring.
Intrauterine growth retardation is normally observed in animals with reduced birth weight and body composition. In the current study, lambs born to nutrient restricted ewes did not differ in BW from lambs born to control ewes at any time point during the study. However, this does not necessarily mean that these lambs did not exhibit IUGR. The reduced LEA at birth and reduced backfat at 3 months of age suggests that body composition of these lambs was altered. Therefore, these lambs could have exhibited IUGR.

Circulating concentrations of somatotropic hormones in the mother and the fetus are under strong nutritional regulation from mid to late gestation (Oliver et al., 1999). In fetal sheep, fetal plasma IGF-I, IGF-II, IGFBP-3, insulin, and glucose are decreased during severe maternal nutrient-restriction, while IGFBP-2 concentrations are increased (Gallaher et al., 1994). Growth hormone in fetal sheep is increased in response to under-nutrition and exhibits a pattern of response similar to that seen during post-natal life (Bauer et al., 1995). Growth hormone concentrations in normal growing animals are known to decrease with age (Govoni et al., 2003, 2004) and in nutrient-restricted animals concentrations are known to increase (Rausch et al., 2002). Growth hormone concentrations in lambs born to nutrient-restricted ewes in the current study tended to be greater than control lambs before bottle weaning at week 8 of age, demonstrating that the somatotropic axis of these lambs in post-natal life was affected by their exposure to maternal nutrient-restriction in-utero.

Most IGF-I in circulation is bound in a complex with IGFBP-3 (Breier, 1999), so it was expected that IGFBP-3 concentrations would be parallel to IGF-I concentrations and both are known to increase with age and growth rate (Govoni et al., 2003, 2004). Both IGF-I and IGFBP-3 concentrations decreased at week 4 of age, which was indicative of fasting in preparation for a glucose tolerance test (Katz et al., 2002). Concentrations of IGFBP-3 are known to increase with
growth rate, similar to IGF-I, which was demonstrated in this study with lambs born to overfed ewes exhibiting increased concentrations of IGFBP-3 and IGF-I that parallel increases in BW. We observed an increase in IGFBP-2 concentrations at 3 mo of age in lambs born to overfed ewes, which was not expected since IGFBP-2 concentrations are normally opposite that of IGFBP-3 concentrations (Govoni et al., 2003, 2004). This was the only time point IGFBP-2 was different among treatments and this may be because IGFBP-2 concentrations were variable and are known to be greater at birth and decrease with age (Govoni et al., 2003, 2004).

Of interest is the comparison of these hormonal concentrations between pre-weaning and post-weaning, since weaning occurred at the same time that the rumen was fully developing (Parish et al., 2009). The difference in GH concentrations among treatments was observed pre-weaning, but not post-weaning. However, the prominent differences in IGF-I and IGFBP-3 were observed in lambs born to overfed ewes post-weaning. These changes in hormone concentrations may demonstrate differences in intake, digestion and nutrient utilization due to adaptation from pre-ruminant to functional ruminant (Seal and Reynolds, 1993). Muhlhausler et al. (2006) and Samuelsson et al. (2008) have demonstrated that pre-natal overfeeding in mice and sheep leads to altered appetite regulation in post-natal offspring. Altered response of the central appetite regulatory system can signal to increase feed intake and adiposity after birth (Muhlhusler et al., 2006). Long et al. (2010) demonstrated, that feed intake was increased by 10% in lambs born to obese ewes compared with offspring of control ewes. These data show that the appetitive regulation system of lambs exposed to over-nutrition in pre-natal life is altered, causing an increase in appetite. This could explain the differences in hormone concentrations between pre-and post-weaning of lambs born to overfed ewes in the current study.
Most of the changes in these hormones were exhibited by lambs born to overfed ewes, not lambs born to restricted ewes, similar to BW differences observed in the current study. This may suggest that concentrations of IGF-I may be related more to size at birth and current weight rather than maternal nutritional group (Oliver et al., 2002). This suggests that the factors that program post-natal phenotype are more closely related to size at birth than to maternal nutrition in late gestation (Oliver et al. 2002). This is in agreement with the current study since overfed lambs at 3 mo of age had greater BW and greater IGF-I and IGFBP-3 concentrations than control lambs. However, the increase in BW and IGF-I concentrations after weaning in lambs born to overfed ewes could be due to an increase in feed intake; however we did not measure intake of these lambs. We expected overfed offspring to have greater IGF-I and IGFBP-3 concentrations, but another experiment would need to be done to determine if these changes are due to BW or maternal nutrition.

Hyperinsulinemia can be caused by compensation for insulin resistance, which is one of the many conditions associated with metabolic syndrome. Hyperinsulinemia has been reported in lambs and rats exposed to poor maternal nutrition (Ford et al., 2007, 2009; Shankar et al., 2008; Samuelsson et al., 2008). In the current study, lambs born to both overfed and restricted ewes had greater insulin concentrations than control born lambs throughout their life time, suggesting insulin-glucose homeostasis was altered during fetal life. Increased insulin concentrations may also indicate impaired insulin signaling pathways leading to insulin resistance, which has been shown as another long-term effect of poor maternal nutrition (Thorn et al., 2011). In normal growing lambs, insulin secretion, sensitivity and action are known to decrease with age (Gatford et al., 2004), but we did not observe this decrease in lambs from the current study, which also indicates an impairment in homeostasis. This could suggest compensatory changes to insulin
secretion in the current study to maintain glucose tolerance. Maturation of the ovine pancreas does not occur until two mo of age (Titlbach et al., 1985); therefore, poor maternal nutrition could contribute to pancreatic dysfunction. This could be a result of fetal programming, and thus alter insulin regulatory and signaling mechanisms (Armitage et al., 2004; Ford et al., 2009).

We performed an IVGTT on all lambs at 1 and 3 mo of age to assess glucose tolerance and insulin sensitivity and we observed that lambs born to overfed ewes had greater insulin response to glucose than lambs born to control-fed ewes. Lambs born to overfed ewes exhibited reduced glucose peak and increased insulin peak concentrations, but no significant difference in glucose or insulin AUC. Insulin AUC was increased in lambs born to overfed ewes, but was not significant due to variation among animals, agreeing with the increase in insulin AUC in lambs born to nutrient-restricted ewes at 63 d of age observed in Ford et al. (2007).

A study performed by Gatford et al. (2004) established post-natal ontogeny of glucose homeostasis and insulin action in lambs through IVGTT at different ages. This study determined glucose homeostasis of lambs from pre-weaning to adulthood by assessing insulin secretion, insulin sensitivity, and glucose tolerance. They concluded that glucose homeostasis, insulin secretion, insulin sensitivity, and insulin action decrease with maturation and age from before weaning to early adulthood in sheep. Insulin secretion in young lambs was predictive of insulin secretion in young adults, suggesting that events in early life may permanently determine the capacity for glucose-stimulated insulin secretion (Gatford et al., 2004). Findings in the current study, along with Ford et al. (2007), could suggest that both maternal over-nutrition and under-nutrition result in lambs with deficits in insulin regulation of glucose, more so than what would have been observed with increasing age. This could lead to problems such as insulin resistance and obesity.
We did not observe differences in leptin concentrations among treatment groups throughout the study. Leptin decreases feed intake and increases energy expenditure. Therefore we expected to observe an increase in leptin concentration associated with the increase in adiposity, known to result from exposure to poor maternal nutrition (Long et al., 2010). However, we did not observe an increase in adiposity in any treatment group, nor did we observe a difference in leptin concentrations due to maternal treatment. At 3 mo of age, lambs born to restricted ewes tended to have reduced leptin concentrations compared with lambs born to overfed ewes. This same time point is when we observed a reduction in backfat of lambs born to restricted ewes, suggesting that the reduced leptin concentrations are in response to reduced adiposity which allows for increased intake and increased accumulation of energy stores (Friedman, 2002). This is in agreement with Blache et al. (2000) who reported that plasma leptin concentrations were highly correlated with backfat thickness in sheep. Tokuda and Yano (2001), Tokuda (2003), and Altmann (2005) reported similar findings.

The current study demonstrates that growth, development, and metabolism are altered in offspring exposed to poor maternal nutrition. Most changes in body composition and the somatotropic axis observed were in lambs exposed to maternal over-nutrition, specifically larger body size, greater heart weight, increased concentrations of IGF-I, IGFBP-3, and insulin. These changes, along with the greater insulin response to glucose observed in lambs born to overfed ewes, demonstrates that both the somatotropic axis and insulin-glucose homeostasis were altered during fetal life and could be a result of fetal programming. The data we reported are similar to changes observed in humans who develop metabolic syndrome later in life, which is characterized by conditions such as cardiovascular disease, insulin resistance, and obesity. These findings suggest that this study, if carried out until lambs reached adult life, could have
resulted in lambs exhibiting deficits that parallel with metabolic syndrome in humans. We now know how poor maternal nutrition alters hormones involved in metabolic function, but we still need to investigate possible metabolic control mechanisms that lead to these changes. Understanding this will give us more insight into how the development of diseases in adult life are associated with changes in these hormones in early post-natal life, as a consequence of fetal programming.
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