IMPACTS OF MATERNAL OBESITY ON METABOLIC PROFILES IN POSTPARTUM EWES

A Thesis

by

JASON RAY MCKNIGHT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2010

Major Subject: Nutrition

Impacts of Maternal Obesity on Metabolic Profiles in Postpartum Ewes

IMPACTS OF MATERNAL OBESITY ON METABOLIC PROFILES IN POSTPARTUM EWES

A Thesis

by

JASON RAY MCKNIGHT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Chair of Committee,	Guoyao Wu
Committee Members,	Fuller W. Bazer
	M. Carey Satterfield
	Thomas E. Spencer
Intercollegiate Faculty Chair,	Stephen B. Smith

August 2010

Major Subject: Nutrition

ABSTRACT

Impacts of Maternal Obesity on Metabolic Profiles in Postpartum Ewes. (August 2010)

Jason Ray McKnight, B.S., Sam Houston State University

Chair of Advisory Committee: Dr. Guoyao Wu

This study determined the effects of gestational obesity on the long-term metabolic status of the mother and if obesity management during or after pregnancy could attenuate these effects. At 120 days prior to estrus, 8 ewes received 100% of NRC nutrient requirements (control group) and 24 ewes had free access to feed (obesity induction). Beginning on day 42 of gestation, 8 obese ewes were restricted to 65% of NRC nutrient requirements. Following parturition, controls and all but one group of obese ewes were fed 100% of NRC nutrient requirements. At postpartum days (PPD) 1 and 150, glucose tolerance tests were administered to ewes. At both PPD1 and PPD150, obesity resulted in insulin resistance, impairment of whole-body glucose utilization, increased levels of circulating leptin, and altered profiles of amino acids in plasma; however, these effects were diminished in ewes receiving obesity management during or after gestation. Additionally at PPD150, obesity increased the circulating levels of ammonia and urea in ewes, which was prevented by realimentation to 100% NRC requirements. These results indicate that weight reduction in obese dams during pregnancy or after parturition can beneficially ameliorate the adverse effects of gestational obesity on the mother.

ACKNOWLEDGEMENTS

I would first like to thank my parents for their support and guidance throughout my life. They have always been supportive of the life decisions I have made, and of the goals I have set for myself. They are always there when I need someone to confide in, and always helpful in their comments and suggestions. I am truly appreciative of all that they have done, and without them, many of my aspirations would not have been a success.

I would next like to thank my advisor, Dr. Guoyao Wu, for sharing his knowledge, guidance, and trust throughout the course of my graduate career. Without his invaluable input, completion of this research would not have been possible. Dr. Wu is an excellent role model, advisor, and scientist, and the care and compassion he shows for his research and graduate students will have a profound impact on me throughout my life. I am deeply grateful for his willingness to help me in any way needed, and for teaching me the methods and knowledge necessary to conduct quality research.

I would also like to thank all other graduate advisors, students, and technicians who helped in this work. I am very thankful for the assistance that Dr. Carey Satterfield offered throughout the course of this research, especially during the animal work and discussion of results. I would also like to thank Drs. Tom Spencer and Fuller Bazer for their help in manuscript clarification and preparation, and for their helpful suggestions. Finally, I would like to thank members and technicians of Dr. Wu's lab and Sorin Greff for all the assistance they provided throughout the course of this research.

TABLE OF CONTENTS

Page

ABSTRACT iii

ACKNOWLEDGEMENTS iv

TABLE OF CONTENTS v

LIST OF FIGURES vi

LIST OF TABLES vii

CHAPTER

I INTRODUCTION AND LITERATURE REVIEW 1

The obesity epidemic 1
Weight gain during pregnancy 3
Maternal complications of gestational obesity 6
Fetal and neonatal complications 12
Obesity management during pregnancy 14
Summary and objectives 19
Hypothesis 19

II IMPACTS OF MATERNAL OBESITY ON METABOLIC PROFILES IN POSTPARTUM EWES 21

Materials and methods 22
Results 25
Discussion 36

III SUMMARY AND DIRECTION OF FUTURE RESEARCH 41

REFERENCES 42

VITA 54

LIST OF FIGURES

Page

Figure 1 Experimental Design 23

LIST OF TABLES

Page

Table 1 Concentrations of leptin, ammonia, urea, and non-esterified fatty acids (NEFA) in plasma of ewes on PPD1 and PPD150 26

Table 2 Concentrations of insulin in plasma of ewes on PPD1 and PPD150 28

Table 3 Concentrations of glucose in plasma of ewes on PPD1 and PPD150 31

Table 4 Kinetics of glucose in plasma of ewes on PPD1 and PPD150 32

Table 5 Concentrations of amino acids in plasma of ewes on PPD1 at 0 and 120 min after i.v. administration of glucose 33

Table 6 Concentrations of amino acids in plasma of ewes on PPD150 at 0 and 120 min after i.v. administration of glucose 34

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

The obesity epidemic

The human conditions of being overweight and/or obese result from abnormal or excessive white fat accumulation in the body. The World Health Organization (WHO 2009) and the National Institutes of Health (NIH 1998) have defined overweight as a body mass index (BMI, kg/m^2) of 25 to 29.9, and obesity as a BMI of 30 or greater. Further, obesity is characterized into 3 classes by BMI: Class I (30-34.9), Class II (35-39.9), and Class III (greater than 40). Over the past few decades, obesity has become a troubling pandemic in developed nations and some developing countries (Abelson and Kennedy 2004; CDC 2009). In 2005, the WHO estimated that nearly 1.6 billion adults worldwide are overweight and at least 400 million are obese. In the United States alone, 35% of adults are obese, and approximately two-thirds of the population is overweight (Flegal et al. 2010). Similarly, in Australia, 16% of the adult population is classified as obese, while 49% are classified as overweight (Schrauwers and Dekker 2009). The obesity epidemic is not bound by societal factors, as adults and children from all groups of society are affected (CDC 2009). Associated with obesity are a number of other health problems, such as insulin resistance, type II diabetes, atherosclerosis, stroke, hypertension, and some types of cancer (including colon and breast cancers) (Pi-Sunyer 2003).

This thesis follows the style of *Amino Acids*.

As a result, obesity is claiming an increasing number of lives, and is a significant burden on the healthcare systems of the world. In the United States alone, approximately 300,000 people die annually due to obesity related diseases, and 6-8% of health care expenditures can be attributed to obesity. Indeed, the WHO (2000) has declared that obesity is now so common that it is replacing the more traditional public healthcare concerns, including under-nutrition and infectious disease, as one of the most significant contributors to poor health.

The adverse effects of obesity may be more severe for women than for men (CDC 2009). For example, obesity is associated with developing asthma in women, but not in men (Beckett et al. 2001). Further, women are more likely to be obese than men, as 34% of women are obese compared to 28% of men (Flegal et al. 2010). Also, women are twice as likely as men to experience a major weight gain over a 10-year period. Women aged 25 to 34 have the greatest risk of major weight gain as compared to either men of the same ages or older women (Williamson et al. 1990). Between 1980 and 2004, the prevalence of obesity in women has doubled from 16.5% to around 33.2% (Flegal et al. 1998; Hedley et al. 2004).

Women have two periods throughout their life in which they are extremely susceptible to high weight gains: pregnancy and menopause. This is probably due to multiple factors such as changes in hormones, physical activity, and mood. Race also seems to play a role in female obesity, as it has been determined that the problem of obesity is greatest in non-Hispanic black women (48.8%), as compared with Mexican-American (38.9%) and non-Hispanic white women (31.3%), according to the Centers for

Disease Control and Prevention (CDC 2009). Of particular interest is the vast number of women of reproductive age who are now becoming overweight (57%) or obese (30%) in the U.S. (CDC 2009), compared with the previous values of 16.5-33.2% for the reported prevalence of obesity (Abrams and Laros 1986; Naeye 1990; Taffel et al. 1993; Siega-Riz et al. 1994; Cogswell et al. 1995). This is particularly troubling, because pregnancy alone is a time of great stress on the body. Coupled with excess adipose tissue, the time during gestation could cause serious health consequences to the mother and the fetus.

Weight gain during pregnancy

Gestational weight gain is normally credited to increases in both lean and fat tissues of the mother and fetus as well as to the retention of amniotic fluid (Institute of Medicine 1990). Smooth, progressive weight gain generally represents healthy fetal growth, whereas inconsistently high weight gain is primarily indicative of excess fat deposition in the mother. Gaining too much weight during pregnancy can be attributed to unhealthy eating and inadequate physical activity. Some of the maternal characteristics associated with an increased risk in low gestational weight gain can occur in combination, such as low family income, race, young age, unmarried status, and low educational levels (Institute of Medicine 1990).

Pregnancy outcomes based on the amount of gestational weight gain are well studied, and over time, the ideal weight gain during pregnancy has been occasionally questioned and revised. In the past, gestational weight gain guidelines were restrictive due to concerns about gestational hypertensive disorders, labor and delivery

complications, and weight retention after parturition (Kiel et al. 2007; Schieve et al. 1998). More recent updates have focused on preventing low birth-weight infants, and the current guidelines provide weight gain ranges depending on pre-pregnancy BMI. These guidelines were recommended by the US National Academy of Sciences Institute of Medicine (IOM 1990) to decrease the prevalence of low birth-weight infants and prevent conditions such as macrosomia, caesarean delivery, and postpartum weight retention associated with gaining too much weight during gestation (Rode et al. 2007; Shaikh et al. 2009). These recommendations were put in place to optimize chances of delivering an infant with a birth-weight between 3 and 4 kg. Current recommendations set forth by the IOM are a weight gain of 11.2 to 15.9 kg for women with a normal BMI, 6.8 to 11.2 kg for overweight women, and at least 6.8 kg for obese women (IOM 1990). After establishing the IOM recommendations with cutoff limits for each pre-pregnancy BMI class, current research has largely focused on testing the significant association of these guidelines to important clinical outcomes (Cedergren 2007). The majority of these studies found that weight gains within the IOM's ranges were coupled to a better pregnancy outcome than weight gains outside these ranges (Abrams et al. 2000; Siega-Riz et al. 1994; Cogswell et al. 1995; Stotland et al. 2006; Hedderson et al. 2006).

One issue with the current IOM guidelines is that there is no upper limit on how much weight an obese woman should gain during pregnancy, only advising that they should gain at least 6.8 kg. Further, since obesity has now been classified into Class I, Class II, and Class III by the CDC and NIH, many researchers feel that improved weight gain guidelines should distinguish between the different levels of obesity (Kiel et al.

2007). Also called into question is the IOM's definition of a "good pregnancy outcome". These parameters are largely subjective and include birth weight, preterm delivery, Apgar scores, cesarean delivery, and labor complications. Indeed, the broad weight gain ranges resulting in good pregnancy outcomes is illustrated by results of a study from California where only 40% of women with good pregnancy outcomes had weight gains within the acceptable IOM limits. Additionally, there have been discussions about whether current IOM guidelines for weight gain are too high and contributing to the ongoing obesity epidemic (Feng and Naylor 1998). Moreover, the IOM weight gain guidelines are intended for women carrying one fetus. In women carrying multiple fetuses, weight gains appear to be greater by an amount larger than that accounted for by the weight of the additional fetus and support tissues, and so this should not be taken by healthcare providers as an instance where the pregnant woman is gaining excess weight. Finally, the weight gain guidelines set forth by the IOM were compiled from data looking largely into industrialized nations as well as mainly white populations (IOM). Therefore, weight gains for citizens in developing countries as well as non-whites may be different, as the conclusions and recommendations in the IOM report relate only to healthy women in the United States.

Gaining too much weight during gestation has been shown in numerous studies to cause multiple problems in the both the mother and fetus/infant such as gestational diabetes, failed induction of labor, lacerations, instrumental delivery, macrosomia, postpartum weight retention, and others (DeVader et al. 2007). A dangerous trend is that a high percentage of mothers gain more weight than the IOM recommendation during

gestation. DeVader et al. (2007) found that more than 60% of the 94,696 women surveyed gained more than recommended in the IOM guidelines. Further, more than 42% of mothers gained over 16 kg. This is especially problematic since approximately one half of pregnant women beginning their pregnancy as either overweight or obese (CDC 2009), and obesity along with excess gestational weight gain can compound problems. Kiel et al. (2007) reported that 46% of obese women gained more than 11.3 kg throughout gestation. Another study found that greater than 70% of obese women gained more than the recommended amount of weight for obese pregnant women, and 21% gained greater than 16 kg, which would be considered excessive for even women of normal pre-pregnancy weight (Vesco et al. 2009). Further, Butte et al. (2004) found that overweight and obese pregnant women have a net accrual of fat mass equal to about 8.4 kg, as opposed to 5.3 kg in women with a low BMI and 4.6 kg in those with a normal BMI. Also, the increases in fat mass seems to be centralized in the intra-abdominal area (Kinoshita and Itoh 2006), which may be of metabolic significance because fat deposited in this area is more highly correlated with disease later in life than white fat in other anatomical sites. Therefore, great care needs to be taken to make sure that all women, regardless of BMI, have weight gains in pregnancy closely monitored, but also that intervention be employed in obese women to ensure a minimal gestational weight gain.

Maternal complications of gestational obesity

Excess weight increases the risk of glucose intolerance, a pre-diabetic state of dysglycemia associated with insulin resistance. Even in moderately overweight subjects, the occurrence of gestational diabetes mellitus (GDM) is 1.8 to 6.5 times greater than in

normal-weight subjects, while the incidence in obese subjects is 1.4-20 times higher (Galtier-Dereure et al. 2000). Pregnancy is already a time of insulin resistance in the mother, which may be necessary to allow an adequate nutrient supply and energy source for the developing fetus. Insulin resistance normally increases 50-60% over the course of a normal pregnancy. GDM is the result of an imbalance between pregnancy-induced insulin resistance, which is exacerbated by weight excess and inadequate compensatory hyperinsulinism. Excess adipose tissue is a source of inflammatory cytokines and other metabolically active chemical mediators, such that obesity can be characterized by a state of low-grade chronic inflammation (Retnakaran et al. 2003). Obese pregnant women have a higher concentration of C-reactive protein and higher fasting and postprandial insulin concentrations than non-obese women. All of these factors lead to an increase in GDM with increasing adipose tissue such that the risk of GDM has been shown to increase with rising maternal BMI; overweight women have relative risk of 1.7, while obese women have a 3.6 times greater risk of GDM as compared to normal weight controls (Roberts and Lain 2002). Similarly, Schrauwers and Dekker (2009) showed that overweight, obese, and morbidly obese women in gestation have a 6%, 8%, and 21.7% chance, respectively, of developing GDM as opposed to 1% for normal weight women. GDM is usually determined by an oral glucose tolerance test at week 24 of gestation, but in obese women, the condition can arise much sooner than that, and so the condition remains unrecognized until it is too late for optimal management (Galtier et al. 2004). GDM also predisposes a woman for type II diabetes mellitus later in life,

and so proper therapy should be initiated as soon as possible to prevent future health risks associated with GDM.

Excess weight also increases the prevalence of hypertension and toxemic syndromes during pregnancy (Galtier et al. 2008). In obese women, the incidence of hypertension is increased by a factor of 2.2-21. Bhattacharya et al. (2007) found a linear increase in gestational hypertension with BMI, as well as an adjusted odds ratio of 3.1 for gestational hypertension in the morbidly obese. Gestational hypertension may be the result of decreased nitric oxide (a vasodilator) synthesis, and obesity also lessens synthesis of this molecule (Poirier et al. 2006), which can lead to endothelial dysfunction and future cardiovascular disease.

Obesity, insulin resistance (such as in the form of GDM), and hypertriglyceridemia, along with endothelial dysfunction as a result of decreased nitric oxide synthesis are all important factors for the pathogenesis of preeclampsia (Ramachenderan et al. 2008). Endothelial dysfunction reduces prostacyclin secretion and enhances peroxidase production that results in vasoconstriction and platelet aggregation. Indeed, obesity has been identified as an independent and well-established risk factor for the development of preeclampsia (Roberts and Lain 2002; O'Brien et al. 2003; Eskenazi et al. 1991). Preeclampsia is a rapidly progressing condition that is manifested in maternal hypertension and proteinuria, and can result in coma or death of the mother. It is one of the major contributors to maternal morbidity and mortality worldwide, and has been associated with substantial health concerns later in life (WHO 2009). Large population studies have shown that obese women are two to three times more likely to

develop preeclampsia than women of normal body weight (O'Brien et al. 2003; Baeten et al. 2001). Another study also confirmed that increasing BMI results in an odds ratio of 7.2 for developing preeclampsia as opposed to normal BMI women (Bhattacharya et al. 2007). Even in studies in which women with GDM are excluded, obesity is still an independent risk factor for preeclampsia (Jensen et al. 2003).

Pregnancy is a hypercoagulable state, associated with increases in the plasma concentrations of coagulation factors, decreases in protein S (a plasma glycoprotein involved in anti-coagulation pathways), and inhibition of fibrinolysis, resulting in a 5-fold increase in risk for venous thrombosis (Hellgren and Blomback 1994; Greer 1994). Obesity further increases the risk of thrombosis events by promoting venous stasis, increasing blood viscosity, and promoting activation of the coagulation cascade (Ramachenderan et al. 2008). Since obesity is characterized by an excess of adipose tissue, any chemical mediators produced by this tissue is elevated, which puts anyone at risk for complications. Adipose tissue has been found to secrete numerous "adipokines", tumor necrosis factor alpha (TNFA), interleukin-6 (IL6), leptin, and others (Ikeoka et al. 2010). These peptides and non-peptide compounds, which can be involved in cardiovascular homeostasis, can slow clot degradation and result in a furthered prothrombic state. This can place obese pregnant women at an increased risk for developing deep vein thrombosis, blood clots, and other thromboembolic complications.

Also of interest is the occurrence of respiratory complications in obese pregnant women. Excess weight causes a reduction in thoracic-wall compliance and increases airway resistance (Galtier et al. 2008). Sleep-disordered breathing and snoring are fairly

common in pregnancy, and women with higher BMIs and increasing neck circumference during pregnancy report higher sleep apnea scores (Pien et al 2005).

Obesity in pregnancy can result in numerous delivery complications. It could be argued that these women have higher incidences of delivery complications due to underlying medical conditions associated with their weight. However, Callaway et al. (2006) found that associations between increasing BMI and poor delivery outcomes were still present after adjustment for the presence of other conditions such as GDM. Several studies have evaluated the effect of obesity on preterm delivery, and most have concluded that the risk of preterm birth is not increased (Ramachenderan et al. 2008). One study even found that the risk actually decreases in obese versus normal weight women (6.2% and 11.2%, respectively) (Hendler et al. 2005). However, obese women are more likely than overweight and normal weight women to progress beyond term, but the increased difficulty in determining start of gestation in these women may mean that the phenomenon is an artefactual association (Ramachenderan et al. 2008). Obese women have a higher rate of induced labor and more cesarean sections than normal weight women. Indeed, Bhattacharya et al. (2007) reported that the frequency of induced labor increased with rising BMI, such that a normal weight woman had an odds ratio of 0.8, while obese women were at 1.8. Further, both elective and emergency cesarean sections were increased in the morbidly obese group, but only emergency surgeries were significantly different from other BMI categories. Another study found that around one in two severely obese women were delivered by cesarean section and each one-unit increase in pregravid BMI increased the risk of a surgical delivery by 7% (Brost et al.

1997). Commonly reported reasons for surgical delivery included cephalopelvic disproportion, failed cervical dilatation, fetal distress, and risk of shoulder dystocia. Additionally, obesity in pregnancy is associated with more difficulties with anesthetic administration. Difficulties in inserting epidural catheters include correct positioning of the patient, midline and epidural space identification, and dislodging the catheter (Galtier et al. 2008). However, regional anesthesia seems to be the preference for obese women, as intubation of these women can be difficult, and a rapid desaturation and an increased risk of aspiration is associated with obese women during anesthesia (Saravanakumar et al. 2006). Obese women are also at greater risk for deep vein thrombosis, endometritis, postpartum hemorrhage, wound infections, urinary tract infections, and prolonged hospitalization associated with delivery complications (Hall and Neubert 2005). Indeed, Bhattacharya et al (2007) found that mean blood loss following delivery showed a linear increase with increasing BMI.

Finally, there are a host of postpartum issues that overweight and obese mothers could encounter. Excess weight gain during pregnancy and high prepregnancy weight are important predictors of long-term weight change and higher BMI later in life (Ramachenderan et al. 2008; Gore et al. 2003). Scholl et al. (1995) found that excess gestational weight gain resulted in a 12% increase in weight from prepregnancy at 6 months postpartum, and retained approximately 40% of that excess weight. This is in comparison to women who gained the recommended weight, who tended to weigh only 5% more than prepregnancy weight postpartum. This relates to obesity in pregnancy because excessive weight gainers are more likely to have been overweight or obese prior

to pregnancy. Further, women who have higher prepregnancy weights are at risk for substantial postpartum weight retention. Soltani and Fraser (2000) found that the majority of obese women were heavier 6 months postpartum than they were early in their pregnancy. Retaining this excess weight places these women at greater risks of further complications with future pregnancies, as well as higher risks for cardiovascular disease, type II diabetes, atherosclerosis, and the metabolic syndrome.

Fetal and neonatal complications

Obesity during pregnancy can also give rise to disease and complications in the offspring. Maternal obesity has been associated with markers of fetal distress, stillbirths, and neonatal deaths (Shaikh et al. 2009). Higher BMI categories have been associated with an increased odds ratio of meconium aspiration, fetal distress, and low Apgar score (Cedergren 2004). Further, the CEMACH 2006 dataset indicated that of the women who had a stillbirth or neonatal death and had a recorded BMI, 26% were overweight and 22% were obese (Confidential Enquiry into Maternal and Child Health 2008). Therefore, almost one half of the stillbirth/neonatal deaths were associated with excess maternal weight. Indeed, Huang et al. (2000) found that the factor most closely associated with unexplained fetal death was increased prepregnancy weight. Consistent with this finding is the fact that obesity was associated with a five-fold increase in the rate of stillbirths with histological placental dysfunction in the 1998-2001 Danish National Birth Cohort (Nohr et al. 2005). The mechanism of fetal compromise has not been determined and likely involves a plethora of factors, including placental insufficiency, relative fetal hypoxia, and the dysfunction of fetal organs (Sebire et al. 2001).

Maternal obesity is a well-recognized risk factor for fetal macrosomia: birth weights greater than 4 kg. Certainly, multiple studies have shown strong associations between maternal obesity and macrosomia (Sebire et al. 2001; Baeten et al. 2001; Jensen et al. 2003). In agreement, Bhattacharya et al. (2007) found that macrosomia was more common in obese and morbidly obese groups with odds ratios of 1.9 and 2.1, respectively. Macrosomia can also cause its own set of complications. Fetal overgrowth brings about an increased risk of shoulder dystocia, birth injury, and neonatal death, which can further lead to delivery complications in the mother and an increased risk of perineal trauma, chorioamnionitis, or postpartum hemorrhage (Stotland et al. 2004). The mechanism by which obesity affects neonatal birth weight is unclear. However, it is thought to include obesity-related insulin resistance, in which the elevated insulin levels signal for rapid fetal growth, and genetic factors (Yogev and Catalano 2009). With the recent interest in the field of epigenetics and the developmental origins of health and disease hypothesis (Barker Hypothesis), much more knowledge will be learned as to the genetic mechanisms of how obesity affects fetal growth (reviewed in Satterfield et al. 2010).

Maternal obesity is also a risk factor for congenital abnormalities, because obesity during pregnancy can affect embryogenesis (Galtier-Dereure et al. 2000; Ramachenderan et al. 2008). Data from the National Institute of Neurological and Communicative Disorders and Stroke showed an increase of major congenital abnormalities of 35% when mothers were overweight and 37.5% when obese (Naeye 1990). The most common abnormality is neural tube defects, specifically spina bifida.

Odds ratios for neural tube defects range from 1.8 to 3 depending on the degree of maternal overweight (Galtier-Dereure et al. 2000). Due to the fact that neural tube defects are typically associated with a suboptimal folic acid intake by the mother, it has been speculated that there is potentially a decrease in the absorption of folic acid from the diet in obese women. However, this suggestion has been met with inconsistent study results (Yogev and Catalano 2009). Other birth defects have also been associated with maternal obesity, such as a doubling of the risk for omphalocele and heart defects (Watkins et al. 2003), an increase in cryptochidism in male infants, and elevated risk of fluctuating dental asymmetry indicating developmental destabilization (Berkowitz et al. 1995; Kieser et al. 1997). Related is the fact that there is an increased chance of failure to detect birth defects in obese women because of difficult interpretation of serum markers due to changes in the volume of distribution, as well as suboptimal visualization of fetal anatomy by ultrasound. Indeed, Hendler et al. (2004) found that the rate of suboptimal visualization of fetal cardiac and craniofacial structures increased by 37% and 43% in obese women, respectively. Similar studies have shown that these affected structures include the heart, umbilical cord and spine, which are all known sites of obesity related deformities (Wolfe et al. 1990).

Obesity management during pregnancy

Previous recommendations regarding weight loss during pregnancy have been unfavorable. In fact, many experts believe that losing weight during pregnancy is always inadvisable (Galtier et al. 2008), no matter the degree of obesity. However, a 2007 study regarding gestational weight gain during pregnancy showed minimal risks of

unfavorable pregnancy outcomes in greatly obese women losing 0-9 pounds (Kiel et al. 2007). Further, Artal et al. (2007) found that birth weights were more likely to be in the normal range among infants born to women who either lost weight or did not gain weight from the time of an obesity intervention to delivery, along with a lower percentage of small and large for gestational age infants. Therefore, although it may be beneficial to all that obese women consider losing weight before becoming pregnant, the means of obesity management (caloric restriction, exercise, etc.) during gestation could improve maternal health and not significantly impair growth and development of the fetus.

The current literature is sparse on reports of studies regarding forms of obesity management during pregnancy on long-term maternal and fetal outcomes. Maternal weight gain has been significantly associated with caloric intake, and therefore caloric restriction could be used to decrease weight gain during pregnancy. Severe caloric restriction (> 50% calorie restriction) has been found to increase ketonuria and ketonemia in pregnant women, and these can lead to impaired mental development in the fetus. However, a 33% reduction in caloric intake has been recommended to control weight gain while not leading to ketosis. Furthermore, it seems that caloric restriction would be less harmful in obese women, because gestational weight gain appears to benefit maternal fat stores rather than birth weight in obese women (Luke et al. 1996). Another potential strategy to reduce weight gain and/or induce weight loss during gestation is moderate aerobic exercise. Exercise has been noted to be an effective treatment option in improving outcome of pregnancy. Further, there are reports that

exercise has no effect on birth weight and that vigorous exercise may even result in a reduction in birth weight by up to 400 grams (Gavard and Artal 2008). Measures of physical activity have been observed to be reduced during pregnancy, possibly due to the belief that all forms of exercise in pregnancy are contraindicated; however, exercise has been determined to be safe in pregnancy for both maternal and fetal well-being (Shaikh et al. 2009). One factor that may have implications in fetal outcome may be the type of exercise performed. The risk of uterine contractions (and thereby adverse effects on the fetus) may be increased by lower-extremity exercise, whereas upper body exercise produces no uterine contractions (Jovanovic-Peterson and Peterson 1991). Going against long-held beliefs, it seems that neither moderate caloric restriction nor an increase in physical activity has a negative impact on fetal health, and can greatly benefit maternal well-being both during and after pregnancy.

One final obesity management method that is of possible benefit is the use of pharmaceuticals or nutraceuticals as a means to reduce weight. One enzyme that has emerged as a potential target for dissipation of adipose tissue is AMP-activated protein kinase (AMPK). AMPK is considered a master switch, regulating key proteins in metabolic pathways known to control fatty acid and glucose oxidation in the liver and skeletal muscle, as well as lipolysis in adipocytes (Winder and Hardie 1999). Since skeletal muscle and adipose tissue are the main sites of energy utilization, finding ways to modulate AMPK activity in these tissues may play a significant role in energy homeostasis and lead to depletion of fat stores. One such method is through the use of the experimental drug 5-aminoimidazole-4-carboxamide-1-β-D-riboruranoside, also

known as AICAR. AICAR is an adenosine analogue that can be absorbed into intact hepatocytes, adipocytes, and skeletal muscle, and once phosphorylated, mimics the effects of AMP on AMPK, thereby activating this regulatory protein (Song et al. 2002). AICAR has been used in numerous studies involving diet-induced obese mice, genetically obese mice, diabetic rats, and cell culture studies (Hardie et al. 2003). Overall, available evidence indicates that chronic AICAR administration causes a strong anti-lipogenic effect by increasing expression of key inhibiting factors of adipocytes differentiation such as peroxisome proliferator-activated receptor alpha and gamma (PPARα and PPARγ), and PPARγ coactivator 1-alpha (PGC-1α). Further, AICAR suppressed fatty acid uptake and promoted fatty acid oxidation in white adipose tissue (Gaidhu et al. 2009). This provides novel evidence that AICAR, through AMPK activation, can remodel white adipose tissue and other insulin-sensitive tissues to increase oxidation of energy substrates. One negative effect observed, however, is the alteration of the blood lipid profile in both normal and genetically obese mice. Song et al. (2002) found that plasma levels of non-esterified fatty acids (NEFA) were increased in rats, probably attributable to increased lipolysis in adipocytes. Regarding AICAR administration as a whole, tissue-specific and pathway-specific strategies involving AMPK must be developed to support the beneficial effects of AICAR while minimizing the adverse effects on the blood lipid profile.

Instead of using drugs, nutrients, particularly amino acids, may play a significant role in prevention and treatment of obese subjects during pregnancy (McKnight et al. 2010). As precursors for the synthesis of physiologically important substances, amino

acids have versatile roles in the maintenance of whole-body homeostasis (Wu 2009). Of particular interest, L-arginine, L-cysteine, and glycine are substrates for the generation of NO, CO, and H_2S (gaseous signaling molecules) in cells, respectively (Li et al. 2009; Tan et al. 2009b). Because obese or diabetic subjects often have vascular dysfunction (Wu and Meininger 2009), modulating the arginine-NO (a major vasodilator) pathway may be a promising therapeutic means for the management of overweight or obese women before and during pregnancy, as previously demonstrated for nonpregnant mammals (Wu et al. 2006; 2009). Notably, dietary supplementation with L-arginine reduces obesity in Zucker diabetic fatty rats (a Type-2 diabetic animal model) (Fu et al. 2005; Wu et al. 2007b), diet-induced obese rats (Jobgen et al. 2009a; Jobgen et al. 2009b), growing-finishing pigs (He et al. 2009; Tan et al. 2009a,b), and obese humans with Type-2 diabetes (Lucotti et al. 2006). Besides increasing cAMP concentrations (McKnight et al. 2010) and activating the mTOR signaling pathway (Rhoads and Wu 2009; Tan et al. 2010; Yao et al. 2008) in insulin-sensitive tissues, L-arginine acts in many of the same ways as AICAR, such as increasing the activities of AMPK and other proteins that stimulate fat and glucose metabolism (Fu et al. 2005; Nall et al. 2009; Tan et al. 2010). Additionally, Jobgen et al. (2009b) found that high fat feeding altered gene expression in white adipose tissue of adult rats, including decreases in mRNA levels for AMPK and antioxidative proteins, whereas arginine supplementation attenuated these adverse affects. Enteral or parenteral administration of L-arginine or its immediate precursor (L-citrulline) is safe and effective in increasing circulating levels of arginine in both the mother and the fetus (Lassala et al. 2009; Mateo et al. 2007; Wu et al. 2007a).

Therefore, supplementation with L-arginine or L-citrulline could result in beneficial effects on improving pregnancy outcomes in overweight or obese mammals (including women, pigs, sheep, and rats). Future research is warranted to test this novel hypothesis.

Summary and objectives

In summary, the current obesity epidemic is a major public health problem worldwide, which adversely affects an increasing number of pregnant women. Growing evidence shows that maternal obesity negatively impacts maternal health during gestation as well as fetal growth and development and postnatal metabolism and health in offspring. However, little is known about effects of obesity before and during gestation on both the short- and long-term postpartum health of mothers. This study utilized an ovine model of obesity to focus on the following objectives:

1. Evaluate the effects of maternal obesity on amino acid and glucose metabolism in ewes at parturition;
2. Assess the long-term implications of maternal obesity on amino acid and glucose metabolism in postpartum ewes;
3. Determine the effects of postpartum obesity management on amino acid and glucose metabolism in ewes; and
4. Evaluate the effects of obesity management during pregnancy and after parturition on amino acid and glucose metabolism in ewes.

Hypothesis

Our hypothesis was that gestational obesity would negatively impact amino acid and glucose metabolism in ewes, and that obesity management during pregnancy and/or after

parturition would ameliorate abnormalities of amino acid and glucose metabolism in previously obese ewes.

CHAPTER II

IMPACTS OF MATERNAL OBESITY ON METABOLIC PROFILES IN POSTPARTUM EWES

Obesity is a major health problem for both adults and children worldwide (Bray and Bellanger 2006; CDC 2009). Particularly, obesity during pregnancy can lead to major complications for both the mother and fetus, including gestational diabetes, hypertensive disorders of pregnancy, delivery complications, and congenital abnormalities (Galtier et al. 2008; Galtier-Dereure et al. 2000; Shaikh et al. 2009). This is a serious concern for healthcare specialists, because 1 in 5 women are obese at the start of pregnancy and approximately 65% of women of reproductive age are overweight or obese (CDC 2009). The best way to avoid these adverse effects of maternal obesity is to reduce body weight before pregnancy. However, this is often not practical as many women enter into pregnancy unknowingly, thereby necessitating treatment and action during gestation to minimize obesity-associated complications (Catalano 2007).

Growing interest in the field of epigenetics, fetal programming, and the developmental origins of health and disease (DOHaD) hypothesis have led to numerous studies to define how gestational obesity affects metabolic programming and development of the neonate (Galtier-Dereure et al. 2000; Shaikh et al. 2009; Barker and Osmond 1986; Yogev and Catalano 2009; Ramachenderan et al. 2008). However, there has been limited research on maternal effects of obesity during or after parturition, while the health and well-being of the mother is also an important matter. We hypothesized

that maternal obesity would have both short- and long-term impacts on insulin sensitivity in the mother and that reducing obesity during pregnancy or after parturition can ameliorate this metabolic problem. This hypothesis was tested in the current study using the pregnant ewe (*Ovis aries*), a widely used animal model for human pregnancy (Barry and Anthony 2008).

Materials and methods

All surgical and experimental procedures were in compliance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching and approved by the Institutional Animal Care and Use Committee of Texas A&M University.

Experimental design

The experimental design is illustrated in Figure 1. At 120 days prior to estrus, multiparous Suffolk ewes were assigned randomly to either receive 100% of National Research Council (NRC) nutrient requirements (Control group, n=8) or have free access to feed (obesity induction; three groups of obese ewes; n = 8/group). After 120 days on the feeding regimens, control-fed and obese ewes were synchronized into estrus and a single blastocyst from a super-ovulated Suffolk ewe of normal body condition score was transferred into the uterus on day 6 post-estrus. Pregnancy was confirmed by ultrasound on day 28 of gestation. Ewes in the control group were maintained on 100% NRC feeding throughout gestation. Beginning on day 42 of gestation (36 days after embryo transfer), one group of obese ewes were restricted to 65% of NRC nutrient requirements (OB-NR) and the remaining two groups of obese ewes continued to have free access to feed throughout gestation. Following parturition, the control and one obese group

continued to be fed 100% NRC nutrient requirements and to have free access to feed, respectively, whereas the other obese group and OB-NR groups were realimented to 100% NRC nutrient requirements (OB-RAL and OB-NR-RAL, respectively). Two ewes in the obese group died within 1 mo after parturition, and only 6 ewes remained in this group for subsequent measurements. During pregnancy and after parturition, all ewes were individually fed and weighed weekly, whereas feed intake for control-fed and OB-NR fed ewes was adjusted based on body frame size.

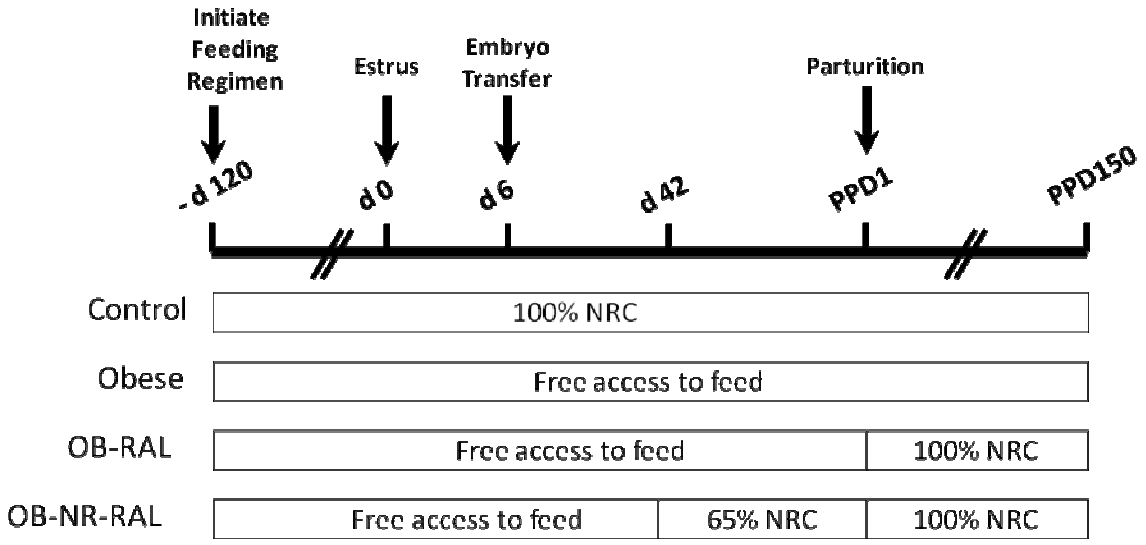

Figure 1 Experimental Design. At 120 days prior to estrus, ewes were assigned randomly to either receive 100% of NRC nutrient requirements (n = 8) or have free access to feed (obesity induction; three groups of obese ewes; n = 8/group). After 120 days on the feeding regimens, ewes received the transfer of single blastocysts from a super-ovulated ewe on day 6 post-estrus. Beginning on day 42 of gestation, one group of obese ewes were restricted to 65% of NRC nutrient requirements (OB-NR) and the remaining two groups of obese ewes continued to have free access to feed throughout gestation. Following parturition, the control and one obese group continued to be fed 100% NRC nutrient requirements and to have free access to feed, respectively, whereas the other obese group and OB-NR groups were realimented to 100% NRC nutrient requirements (OB-RAL and OB-NR-RAL, respectively).

Glucose Tolerance Test (GTT)

On postpartum day 1 (PPD1) and postpartum day 150 (PPD150), following a 12 h fast, a glucose tolerance test was administered to ewes by i.v. infusion of a 50% glucose solution (0.25 mg/kg body weight) (Ford et al. 2007). Blood samples were obtained from the jugular vein in 3 mL EDTA-K_2 tubes (BD Vacutainer) at 0, 5, 10, 15, 30, 60, 120, and 180 min after the bolus injection of glucose solution. Blood samples were immediately centrifuged at 3,500 x g for 5 min to obtain plasma which was aliquoted into 1.5 mL tubes and stored at -20° C until analyzed.

Biochemical analyses of plasma from ewes

Concentrations of leptin in plasma were determined as described by Delavaud et al. (2000). Concentrations of insulin in plasma were measured by EIA (Catalog number 80-INSOV-E01, ALPCO Diagnostics) according to manufacturer's recommendations. Non-esterified fatty acids (NEFA) were determined using a commercial colorimetric assay (Wako Chemicals, Richmond, VA). Amino acids, glucose, ammonia, and urea in plasma were analyzed using HPLC and enzymatic methods (Wu et al. 1997; Kohli et al. 2004; Wu 1995; Wu et al. 1995).

Statistical analysis

Values are least square means and pooled SE. Data were analyzed by ANOVA using the General Linear Model procedures of the Statistical Analysis System (SAS Institute, Inc.). Leptin was analyzed by 1-way ANOVA, while all others were analyzed by 2-way ANOVA with diet and time being main effects. Values of $P \leq 0.05$ were taken to indicate statistical significance.

Results

Body weights of ewes on PPD1 and PPD150

On PPD1, body weights of control, obese, and OB-NR ewes were 80.0, 116, and 86.4 kg (pooled SEM = 3.75 kg), respectively. Obese ewes were 45% and 34% heavier ($P < 0.01$) than control and OB-NR ewes, respectively. On PPD150, body weights of control, obese, OB-RAL, and OB-NR-RAL ewes were 70, 117, 98, and 80 kg (pooled SEM = 4.1 kg), respectively ($P < 0.0001$). Body weights of ewes differed ($P < 0.05$) among the obese, OB-RAL, and OB-NR-RAL groups, whereas no difference was detected between the control and OB-NR-RAL groups.

Concentrations of leptin in plasma

On PPD1, concentrations of leptin in plasma were greater in both OB-NR ($P < 0.05$) and obese ($P < 0.01$) ewes than in control-fed ewes (Table 1). On PPD150, plasma leptin levels in OB-RAL and obese ewes were elevated ($P < 0.05$ and $P < 0.01$, respectively) in comparison to the control group, but values for OB-NR-RAL and control ewes did not differ (Table 1). Additionally, obese ewes had a 79% increase ($P < 0.05$) in concentrations of leptin in plasma over values in OB-RAL ewes.

Table 1 Concentrations of leptin, ammonia, urea, and non-esterified fatty acids (NEFA) in plasma of ewes on PPD1 and PPD150[1]

Group	*n*	Leptin	Ammonia	Urea	NEFA
		µg/L	µmol/L	mmol/L	mmol/L
PPD1					
Control	8	4.2^{c}	126	5.0	0.36^{c}
Obese	16	19.4^{a}	156	6.6	0.63^{a}
OB-NR	8	8.9^{b}	141	5.6	0.49^{b}
SEM		1.5	7.2	0.35	0.03
P-Value		<0.01	0.25	0.21	<0.05
PPD150					
Control	8	4.7^{c}	142^{a}	4.3^{b}	0.20^{c}
Obese	6	26.2^{a}	79^{b}	5.6^{a}	0.57^{a}
OB-RAL	8	14.6^{b}	109^{ab}	4.2^{b}	0.38^{b}
OB-NR-RAL	8	8.6^{bc}	104^{b}	4.6^{b}	0.23^{c}
SEM		1.4	6.0	0.13	0.02
P-Value		<0.01	<0.01	<0.01	<0.01

[1]Values are means with pooled SEM. Means within a column without a common letter differ, $P < 0.05$.

Concentrations of insulin in plasma

On PPD1, concentrations of insulin in plasma differed ($P < 0.01$) due to time and treatment after i.v. administration of glucose solution (Table 2). Baseline concentrations of insulin in plasma did not differ among treatment groups. Peak concentrations of insulin occurred at 10 min for control-fed and obese ewes and at 30 min for OB-NR

ewes. Circulating levels of insulin returned to baseline values by 30 min for control-fed ewes and by 120 min for obese and OB-NR ewes.

On PPD150, there were effects of both treatment and time on concentrations of insulin inn plasma ($P < 0.01$), but their interaction was not significant (Table 2). Baseline concentrations of insulin in plasma did not differ among treatment groups. In both control-fed and OB-RAL ewes, concentrations of insulin in plasma peaked at 10 min. However, circulating levels of insulin returned to baseline by 60 min for control-fed ewes and 120 min for OB-RAL ewes. In contrast, concentrations of insulin in plasma of obese and OB-NR-RAL ewes peaked at 30 min and returned to basal levels by 60 and 120 min, respectively. OB-RAL ewes had higher ($P < 0.01$) concentrations of insulin in plasma at 10 min compared with all other groups and at 30 min, compared with control-fed and OB-NR-RAL ewes. Concentrations of insulin in plasma of obese ewes remained higher ($P < 0.01$) than all other groups at 120 min post administration of glucose solution.

Table 2 Concentrations of insulin in plasma of ewes on PPD1 and PPD150[1]

Group	*n*	Time after i.v. administration of glucose (min)					
		0	5	10	30	60	120
		ng/mL					
PPD1							
Control	8	0.8	1.8^{a}	2.2^{a}	1.6^{a}	1.9^{a}	1.1^{a}
Obese	16	0.6	1.6^{a}	2.4^{a}	1.8^{a}	1.8^{a}	0.7^{ab}
OB-NR	8	0.4	1.1^{b}	1.0^{b}	1.1^{b}	1.2^{b}	0.6^{b}
SEM		0.02	0.02	0.03	0.03	0.02	0.02
P-Value		0.48	<0.05	<0.01	<0.05	<0.05	<0.05
PPD150							
Control	8	0.3^{c}	1.2^{b}	1.8^{b}	1.7^{b}	0.7^{c}	0.3^{b}
Obese	6	1.0^{a}	2.1^{b}	2.1^{b}	2.1^{b}	2.1^{a}	1.7^{a}
OB-RAL	8	0.7^{ab}	2.3^{a}	3.1^{a}	3.0^{a}	1.7^{ab}	0.6^{b}
OB-NR-RAL	8	0.5^{bc}	1.6^{ab}	1.7^{b}	2.1^{b}	1.2^{bc}	0.3^{b}
SEM		0.01	0.02	0.02	0.01	0.02	0.01
P-Value		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[1]Values are means with pooled SEM. Means within a column without a common letter differ, $P < 0.05$.

Concentrations of ammonia, urea, and non-esterified fatty acids in plasma

On PPD1 and PPD150, there were no effects of time for either levels of ammonia or urea in plasma; therefore, data were pooled across time for analysis by ANOVA. On PPD1, concentrations of ammonia and urea in plasma were not different among the three groups of ewes (Control, Obese, and OB-NR), but circulating levels of NEFA were affected ($P < 0.05$) by dietary treatment (Table 1). Obese ewes had higher ($P < 0.05$)

levels of NEFA in plasma than control-fed ewes, and OB-NR ewes had intermediate levels of circulating NEFA.

On PPD150, concentrations of ammonia and urea in plasma were affected by dietary treatment (Table 1). Obese and OB-NR-RAL ewes had lower ($P < 0.05$) levels of ammonia in plasma than control-fed ewes, but values for OB-RAL ewes did not differ from other treatments. Obese ewes had higher ($P < 0.05$) concentrations of urea in plasma than control, OB-NR-RAL, and OB-RAL ewes. An effect of treatment was detected ($P < 0.01$) for concentrations of NEFA in plasma (Table 1). In particular, obese ewes had higher ($P < 0.01$) concentrations of NEFA in plasma than OB-RAL, OB-NR-RAL, and control-fed ewes. Further, OB-RAL ewes had higher ($P < 0.05$) levels of NEFA than OB-NR-RAL and control-fed ewes. Concentrations of NEFA in plasma did not differ between OB-NR-RAL and control-fed ewes.

Concentrations of glucose in plasma

Whole-body utilization of glucose, indicated by GTT results, was affected by dietary treatments on both PPD1 and PPD150 (Table 3). On PPD1, basal concentrations of glucose in plasma did not differ among the three treatment groups. In response to the intravenous bolus of glucose, concentrations of glucose in plasma increased to peak values at 5 min post-administration for control-fed and OB-NR ewes and at 10 min for obese ewes. Between 10 and 30 min post- administration, both OB-NR and obese ewes had higher ($P < 0.05$) concentrations of glucose in plasma than control-fed ewes, with the exception of obese ewes at 15 min. Circulating levels of glucose returned to baseline values by 60 min for control-fed ewes, but not until 120 min for both OB-NR and obese

ewes. The area under the curve (AUC) and half-life ($T_{1/2}$) were greater ($P < 0.05$), but clearance rate (CL) was lower ($P < 0.05$) for obese compared to control-fed ewes (Table 4). Maximum concentrations (C_{max}) of glucose in plasma did not differ among treatment groups (Table 4).

On PPD150, basal levels of glucose in plasma did not differ among the four groups of ewes, but peak concentrations of glucose were at 5 min post-administration of glucose for all treatment groups (Table 3). Concentrations of glucose were higher ($P < 0.05$) in obese, OB-RAL and OB-NR-RAL ewes than in control-fed ewes. At 10 to 15 min post administration, obese, OB-RAL, and OB-NR-RAL ewes had higher ($P < 0.05$) concentrations of glucose than control-fed ewes. At 30 min, OB-NR-RAL ewes did not differ from control-fed ewes in concentrations of glucose in plasma, and at 60 min, neither realimented group differed from control-fed ewes. At 180 min, obese ewes still had higher ($P < 0.001$) concentrations of glucose in plasma than the control group. Concentrations of glucose returned to baseline concentrations by 60 min in control-fed ewes, 120 min in OB-NR-RAL and OB-RAL ewes, and 180 min in obese ewes (Table 3).

Glucose kinetics were affected by dietary treatments (Table 4). Area under the curve (AUC) for glucose did not differ among control-fed, OB-NR-RAL, and OB-RAL ewes, but obese ewes had greater ($P < 0.05$) values than all other treatment groups. Further, the half-life ($T_{1/2}$) of glucose in plasma was longer ($P < 0.05$) in obese ewes than in all other treatment groups. In contrast, maximum concentrations (C_{max}) were greater ($P < 0.001$) in obese ewes than in control-fed and OB-NR-RAL ewes. Finally,

clearance rate (CL) of glucose in plasma was lower ($P < 0.05$) in obese compared with control-fed ewes.

Table 3 Concentrations of glucose in plasma of ewes on PPD1 and PPD150[1]

Group	*n*	Time after i.v. administration of glucose (min) (min)							
		0	5	10	15	30	60	120	180
		mmol/L							
PPD1									
Control	8	3.8	12.7	9.8^{b}	9.7^{b}	7.6^{b}	5.9	3.9	3.0
Obese	16	3.5	13.2	13.7^{a}	10.9^{b}	9.9^{a}	7.0	4.7	3.5
OB-NR	8	3.7	13.2	12.3^{a}	13.4^{a}	9.9^{a}	7.6	4.4	3.4
SEM		0.03	0.03	0.03	0.04	0.03	0.04	0.03	0.03
P-Value		0.82	0.67	<0.01	<0.01	<0.01	0.16	0.53	0.69
PPD150									
Control	8	2.6	10.3^{b}	9.1^{b}	8.2^{b}	5.9^{c}	3.8^{b}	2.5^{b}	2.5^{b}
Obese	6	3.6	12.6^{a}	12.1^{a}	11.3^{a}	9.9^{a}	9.3^{a}	6.1^{a}	4.6^{a}
OB-RAL	8	3.1	12.4^{a}	11.1^{a}	10.3^{a}	7.7^{b}	5.2^{b}	3.0^{b}	2.6^{b}
OB-NR-RAL	8	2.8	11.9^{a}	10.9^{a}	9.7^{a}	7.2^{bc}	5.0^{b}	3.7^{b}	2.7^{b}
SEM		0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
P-Value		0.24	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

[1]Values are means with pooled SEM. Means within a column without a common letter differ, $P < 0.05$.

Table 4 Kinetics of glucose in plasma of ewes on PPD1 and PPD150[1]

Group	*n*	AUC	CL	C_{max}	$T_{1/2}$
		(min•mmol)/L	mL/(min•kg)	mmol/L	min
PPD1					
Control	8	1548[b]	0.93[a]	9.3	111[b]
Obese	16	2021[a]	0.74[b]	9.5	148[a]
OB-NR	8	1479[b]	0.95[a]	9.9	100[b]
SEM		91	0.04	0.33	6
P-Value		<0.05	<0.05	0.70	<0.05
PPD150					
Control	8	1280[b]	1.63[a]	6.6[c]	104[b]
Obese	6	2312[a]	0.78[c]	9.5[a]	167[a]
OB-RAL	8	1235[b]	1.17[b]	8.3[ab]	103[b]
OB-NR-RAL	8	1327[b]	1.04[bc]	7.8[b]	116[b]
SEM		127	0.05	0.21	6
P-Value		<0.05	<0.01	<0.01	<0.05

[1]Values are means with pooled SEM. Means within a column without a common letter differ, $P < 0.05$. Abbreviations: AUC- area under the glucose concentration curve; CL- clearance rate; C_{max}- maximum concentration; $T_{1/2}$- half-life.

Table 5 Concentrations of amino acids in plasma of ewes on PPD1 at 0 and 120 min after i.v. administration of glucose[1]

	0 min			120 min				*P*-Value		
	Control	Obese	OB-NR	Control	Obese	OB-NR	SEM	Diet	Time	Diet x Time
					µmol/L					
Ala	202	192	202	179	206	179	11	0.93	0.63	0.73
Arg	188	176	163	178	142	127	10	0.30	0.19	0.85
Asn	26	34	37	22	29	33	2	0.10	0.30	0.99
Asp	8^{a}	5^{ab}	5^{ab}	6^{ab}	4^{b}	4^{b}	0.5	<0.05	0.18	0.98
β-Ala	20	19	27	26	22	24	2	0.49	0.50	0.55
Cit	255^{a}	156^{c}	218^{ab}	245^{ab}	141^{c}	219^{ab}	11	<0.01	0.73	0.96
Cys	82^{b}	169^{a}	97^{b}	72^{b}	145^{a}	91^{b}	8	<0.01	0.42	0.89
Gln	207^{b}	270^{a}	302^{a}	218^{b}	227^{b}	295^{a}	8	<0.01	0.44	0.36
Glu	75	94	107	85	102	116	6	0.13	0.47	0.99
Gly	403^{c}	473^{b}	807^{a}	512^{b}	465^{b}	886^{a}	30	<0.01	0.36	0.71
His	42^{b}	62^{a}	77^{a}	43^{b}	62^{a}	71^{a}	4	<0.05	0.86	0.92
Ile	60^{b}	95^{a}	64^{b}	50^{b}	61^{b}	62^{b}	4	<0.05	<0.05	0.19
Leu	77^{b}	159^{a}	84^{b}	74^{b}	103^{b}	93^{b}	6	<0.01	0.15	0.06
Lys	94	124	92	106	112	91	7	0.14	0.64	0.47
Met	19^{b}	24^{b}	20^{b}	46^{a}	27^{b}	23^{b}	2	<0.05	<0.01	<0.01
Orn	99^{a}	85^{a}	60^{b}	88^{a}	64^{b}	51^{b}	6	<0.05	0.23	0.90
Phe	49^{ab}	61^{a}	53^{ab}	43^{b}	45^{ab}	47^{ab}	3	0.24	<0.05	0.73
Pro	87^{c}	116^{bc}	96^{c}	141^{ab}	167^{a}	173^{a}	7	0.22	<0.01	0.72
Ser	82^{a}	52^{b}	97^{a}	50^{b}	47^{b}	100^{a}	5	<0.01	0.29	0.38
Tau	46	61	68	52	75	52	5	0.12	0.64	0.66
Thr	70	87	74	74	102	96	5	0.09	0.34	0.54
Trp	32^{a}	34^{a}	23^{b}	10^{c}	24^{b}	17^{bc}	2	0.22	<0.01	0.29
Tyr	66^{ab}	68^{a}	62^{ab}	47^{b}	53^{ab}	52^{ab}	3	0.80	<0.05	0.80
Val	151^{b}	210^{a}	107^{c}	100^{c}	128^{bc}	110^{c}	9	<0.05	<0.05	0.18
Total	2357	2662	2681	2449	2425	2491	68	0.52	0.42	0.56

[1]Values are means with pooled SEM. Means within a row without a common letter differ, $P < 0.05$.

Table 6 Concentrations of amino acids in plasma of ewes on PPD150 at 0 and 120 min after i.v. administration of glucose[1]

	0 min				120 min					*P*-Value		
	Control	Obese	OB-RAL	OB-NR-RAL	Control	Obese	OB-RAL	OB-NR-RAL	SEM	Diet	Time	Diet x Time
					µmol/L							
Ala	107^{c}	248^{a}	164^{b}	153^{b}	93^{c}	191^{b}	110^{c}	88^{c}	5	<0.01	<0.01	0.28
Arg	169	227	176	213	199	165	160	169	7	0.56	0.13	0.16
Asn	26	27	28	35	30	28	23	29	1	0.14	0.36	0.23
Asp	3	4	4	4	4	5	4	6	0.3	0.16	0.06	0.46
β-Ala	48^{a}	30^{bc}	36^{ab}	45^{a}	20^{cd}	16^{d}	11^{d}	21^{cd}	3	0.45	<0.01	0.86
Cit	200	208	224	213	179	195	176	202	8	0.87	0.15	0.82
Cys	88^{bc}	135^{a}	104^{b}	92^{b}	76^{c}	103^{b}	72^{c}	63^{c}	3	<0.01	<0.01	0.72
Gln	207^{c}	208^{c}	220^{c}	270^{ab}	288^{ab}	294^{a}	239^{b}	311^{a}	7	<0.05	<0.01	0.34
Glu	42^{c}	74^{a}	70^{ab}	68^{ab}	40^{c}	58^{abc}	46^{c}	52^{bc}	0.4	<0.01	<0.01	0.40
Gly	311^{d}	378^{cd}	470^{ab}	480^{ab}	427^{abc}	341^{cd}	486^{ab}	506^{a}	19	<0.05	0.43	0.56
His	110^{a}	71^{a}	100^{a}	107^{a}	47^{b}	33^{b}	37^{b}	47^{b}	5	0.26	<0.01	0.80
Ile	61^{ab}	71^{a}	69^{a}	57^{ab}	54^{b}	65^{ab}	48^{b}	46^{b}	2	<0.05	<0.01	0.26
Leu	86^{ab}	110^{a}	87^{ab}	91^{ab}	62^{bc}	84^{b}	46^{c}	56^{c}	3	<0.01	<0.01	0.68
Lys	78^{ab}	82^{ab}	87^{ab}	96^{a}	74^{ab}	62^{bc}	44^{c}	57^{c}	3	0.59	<0.01	0.15
Met	17^{a}	19^{a}	18^{a}	19^{a}	15^{ab}	16^{ab}	12^{b}	12^{b}	0.6	0.35	<0.01	0.28
Orn	109^{ab}	117^{a}	108^{ab}	93^{ab}	84^{bc}	62^{c}	59^{c}	65^{c}	4	0.41	<0.01	0.46
Phe	45^{c}	66^{a}	57^{ab}	52^{bc}	30de	42^{cd}	28^{e}	28^{e}	1	<0.01	<0.01	0.38
Pro	134^{a}	71^{c}	141^{a}	131^{a}	112^{ab}	139^{a}	121^{ab}	96^{bc}	4	0.13	0.77	<0.01
Ser	48^{c}	51^{c}	74^{b}	80^{a}	43^{c}	45^{c}	46^{c}	69^{b}	3	<0.01	0.06	0.60
Tau	34^{bc}	64^{a}	41^{bc}	44^{b}	31^{cd}	41^{bc}	23^{d}	30^{cd}	2	<0.05	<0.01	0.46
Thr	125^{ab}	116^{bc}	142^{ab}	161^{a}	90^{c}	87^{c}	98^{c}	83^{c}	9	0.81	<0.05	0.76
Trp	25^{c}	41^{a}	34^{ab}	33^{b}	35^{ab}	40^{a}	36^{ab}	30^{bc}	1	<0.01	0.48	0.21
Tyr	64^{ab}	69^{a}	69^{a}	72^{a}	56^{b}	75^{a}	59^{b}	61^{b}	1	<0.01	<0.05	0.08
Val	140^{ab}	169^{a}	116^{bc}	158^{a}	102^{c}	126^{b}	87^{c}	88^{c}	5	<0.05	<0.01	0.45
Total	2228^{abc}	2474^{ab}	2542^{ab}	2617^{a}	2174^{bc}	2302^{abc}	2039^{c}	2199^{bc}	49	0.43	<0.01	0.35

[1]Values are means with pooled SEM. Means within a row without a common letter differ, $P < 0.05$.

Concentrations of amino acids in plasma

Concentrations of total amino acids in plasma were unaffected by diet or time on PPD1 (Table 5), but there were effects of treatment on specific amino acids. Concentrations of histidine increased ($P < 0.05$) in both obese and OB-NR compared with the control-fed ewes, while aspartate and citrulline decreased ($P < 0.05$ and $P < 0.01$, respectively). Concentrations of serine, glutamine, and glycine were greater ($P < 0.01$) in OB-NR ewes, while ornithine levels were lower ($P < 0.05$). Circulating levels of all branched-chain amino acids (BCAA; isoleucine, leucine and valine) and cysteine were also higher ($P < 0.05$) in obese ewes compared to ewes in the other treatment groups. Time after administration of the glucose tolerance test also had an effect on some amino acids, as concentrations of isoleucine, phenylalanine, tryptophan, tyrosine, and valine (control-fed and obese ewes) decreased over time ($P < 0.05$). Only proline increased ($P < 0.05$) after administration of the bolus of glucose. Concentrations of all other amino acids remained unchanged by either diet or time. Of note, methionine levels did not differ among treatments or across time, except for control-fed ewes in which values were higher at 120 min ($P < 0.01$).

On PPD150, concentrations of most amino acids in plasma were affected ($P < 0.05$) by either diet or time (Table 6). Concentrations of alanine, glutamate, tyrosine, and tryptophan in plasma were higher in obese ewes with or without weight loss compared with control-fed ewes ($P<0.01$). Concentrations of glycine in plasma were greater in both realimented groups (OB-NR-RAL and OB-RAL) ($P<0.05$), but not obese ewes. Circulating levels of glutamine ($P < 0.05$) and serine ($P < 0.01$) were increased in OB-

NR-RAL ewes compared to control ewes. Concentrations of taurine, all BCAA, cysteine, and phenylalanine were higher ($P < 0.05$) in plasma of obese than control-fed ewes. Concentrations of glutamine increased ($P < 0.05$) over time, while alanine, β-alanine, cysteine, glutamine, leucine, phenylalanine, histidine, isoleucine, lysine, methionine, ornithine, taurine, threonine, tyrosine, valine, and total amino acids in plasma decreased ($P < 0.05$). Proline concentrations were initially lower in obese ewes, but by 120 min after the glucose tolerance test, these ewes had greater levels of proline while all other groups exhibited a decrease ($P < 0.05$, trt x time). Concentrations of arginine, asparagine, aspartate, and citrulline did not change ($P > 0.05$) in response to diet or time.

Discussion

Obesity during gestation is a common and growing problem around the world (CDC 2009). Although the literature is vast on fetal implications of maternal obesity during pregnancy (Yogev and Catalano 2009; Ford et al. 2007), very little is known about either short-term or long-term metabolic impacts of maternal obesity on the mother. The present study involved an obese ovine ewe model to elucidate some of the consequences of this growing problem in obese gestating women. Obesity was evident in ewes before pregnancy to mimic obese women who unknowingly become pregnant. Our results indicate that gestational obesity impaired insulin sensitivity on both PPD1 and PPD150, which resulted in a vast amount of downstream adverse effects, including reduced utilization of glucose (Tables 3 and 4) as well as altered metabolism of fatty acids (Table 1) and amino acids (Tables 5 and 6).

Obesity can impair the oxidation of fatty acids and glucose (Jobgen et al. 2009a). Such an effect, coupled with increased intake of energy substrates (including lipids and carbohydrates) from the diet, causes high levels of NEFA (Table 1) in plasma and particularly glucose in response to its administration (Table 3). Long-chain unsaturated fatty acids and glucose are known to result in insulin resistance in skeletal muscle and other tissues of obese subjects, as reported for type-2 diabetic patients (Marliss et al. 2006). An important finding of this work is that reducing body weight (mainly white fat) during pregnancy or after parturition improved the metabolic profiles of the ewes. Specifically, ewes assigned to obesity management treatments exhibited improvements in concentrations of leptin, NEFA, insulin, and urea in plasma, as well as glucose metabolism, in comparison to the obese ewes on both PPD1 and PPD150 (Tables 1 and 2). Because the ewes were followed for 5 months, the long-term effects of both maternal obesity and the management of this condition could be evaluated. To the best of our knowledge, this is the first report of effective and safe intervention methods for ameliorating the metabolic syndrome in obese mothers.

Obesity is known to affect protein metabolism (Jobgen et al. 2009b; Marliss et al. 2006) and gene expression (Jobgen et al. 2009c) in non-pregnant adult animals. However, little is known about effects of maternal obesity on this biochemical event in the mothers either immediately or in a longer term following parturition. As an initial step to address this question, we determined concentrations of amino acids in ewes on PPD1 and PPD150. As in non-pregnant rats, maternal obesity altered the amino acid profile in plasma on both days (Tables 5 and 6). Of particular note, circulating levels of

all BCAA were greater in obese than control-fed ewes. This suggests impaired mitochondrial function in skeletal muscle, the major site for initiation of BCAA degradation in mammals (Wu 2009). In contrast, concentrations of serine and citrulline were reduced in the plasma of obese ewes on PPD1 (Table 5), possibly due to reduced synthesis from glycine and glutamine, respectively. In support of this view, concentrations of glycine and glutamine were higher in obese than control-fed ewes on PPD1 (Table 5). Altered metabolism of serine and impaired synthesis of citrulline likely results in adverse metabolic effects because of the following two reasons. First, serine is a major component of the one-carbon unit metabolism that is essential to cell growth and differentiation (Wu 2009). Second, citrulline is the immediate substrate for intracellular synthesis of arginine, which is the precursor of nitric oxide (a major vasodilator, a key angiogenic factor, and a gaseous signaling molecule) (Wu et al. 2009).

Concentrations of urea in plasma can be a good indicator of altered metabolism of protein and amino acids in animals, including sheep (Satterfield et al. 2010). In keeping with this notion, circulating levels of urea were elevated in obese ewes, but reduced in obese ewes that lost weight either during pregnancy or after parturition (Table 1). Due to insulin resistance, protein synthesis is reduced, but protein degradation is increased in skeletal muscle of obese subjects, resulting in increased amounts of amino acids for oxidation and urea formation (Jobgen et al. 2009a). This metabolic problem is diminished in obese mothers when their whole-body insulin action is enhanced through weight management, as indicated by reduced concentrations of urea in plasma (Table 1).

Obesity along with excess gestational weight gain can negatively impact maternal health (Institute of Medicine 1990). Kiel et al. (2007) reported that 46% of obese women gained more than 11.3 kg throughout gestation. Another study found that greater than 70% of obese women gained more than the recommended amount of weight for obese pregnant women, and 21% gained greater than 16 kg, which would be considered excessive even for women of normal pre-pregnancy weight (Vesco et al. 2009). Optimal weight loss for obese women during pregnancy remains to be established. In this regard, results of this animal study may have important implications for the management of obese pregnant women. Sheep have similar metabolic profiles to humans, and so the findings obtained in this study should be easily translatable to conditions in humans (Barry and Anthony 2008; Ford et al. 2007). Furthermore, the addition of obesity management treatments either during pregnancy or after parturition can give insight into how much the maternal condition can be improved when weight loss is induced, a critical subject area not studied in previous research involving maternal obesity. Reducing food intake of obese ewes to 65% of NRC nutrient requirements resulted in a desirable weight loss (primarily white fat). Furthermore, our results indicate that initiation of weight loss even after parturition was highly beneficial for improving the metabolic profile in obese mothers (Tables 1-6). These results can be helpful in recommending weight loss for obese pregnant women.

In summary, maternal obesity during gestation led to higher levels of leptin and NEFA in plasma, impaired glucose utilization, and an altered amino acid profile. Importantly, obesity management during gestation ameliorated these negative effects.

Further, continued obesity for long-term periods after parturition can exacerbate the problem of high leptin and NEFA levels, which can further impair actions of insulin, as well as the metabolism of amino acids, lipids, and glucose in mothers. We conclude that obesity management beginning immediately after parturition greatly improved their metabolic conditions. These new findings greatly enhance the base of knowledge on effects of maternal obesity on the mother and outcomes of dietary interventions for successful management of obesity in women during gestation and during the postpartum period.

CHAPTER III

SUMMARY AND DIRECTION OF FUTURE RESEARCH

To our knowledge, this study is the first report of safe and effective intervention methods for ameliorating the metabolic syndrome in obese mothers. Although weight loss during pregnancy has generally been considered unfavorable, the discovery by Kiel et al. (2007) that a weight loss of up to 9 pounds can be achieved in obese gestating women with minimal risk of an unfavorable pregnancy outcome may lead the way to new recommendations regarding gestational weight gain/loss in the obese woman. Results of this study could have important implications for the management of obesity in the pregnant woman. Because sheep have similar metabolic profiles to humans, our data should be easily translatable to clinical medicine.

The results of this novel study provides a new database for (1) desiging future research regarding the effects of maternal obesity on the metabolic syndrome in the mother; (2) understanding how excess white fat affects maternal health in the short- and long-term periods postpartum; and (3) develop means to minimize/ameliorate these negative consequences of this rapidly growing problem. Therefore, the present work has important implications for improving the health and well-being of pregnant women.

REFERENCES

Abelson P, Kennedy D (2004) The obesity epidemic. Science 304:1413

Abrams BF, Laros RK Jr (1986) Prepregnancy weight, weight gain, and birth weight. Am J Obstet Gynecol 154:503-509

Abrams B, Altman SL, Pickett KE (2000) Pregnancy weight gain: still controversial. Am J Clin Nutr 71:1233S-1241

Artal R, Catanzaro RB, Gavard JA et al (2007) A lifestyle intervention of weight-gain restriction: diet and exercise in obese women with gestational diabetes mellitus. Appl Physiol Nutr Metab 32:596-601

Baeten JM, Bukusi EA, Lambe M (2001) Pregnancy complications and outcomes among overweight and obese nulliparous women. Am J Public Health 91:436-440

Barker DJP, Osmond C (1986) Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet 327:1077-1081

Barry JS, Anthony RV (2008) The pregnant sheep as a model for human pregnancy. Theriogenology 69:55-70

Beckett WS, Jacobs DR Jr, Yu X et al (2001) Asthma is associated with weight gain in females but not males, independent of physical activity. Am J Respir Critical Care Med 164:2045-2050

Berkowitz GS, Lapinski RH, Godbold JH et al (1995) Maternal and neonatal risk factors for cryptorchidism. Epidemiology 6:127-131

Bhattacharya S, Campbell DM, Liston WA et al (2007) Effect of Body Mass Index on pregnancy outcomes in nulliparous women delivering singleton babies. BMC Public Health 7:168-175

Bray GA, Bellanger T (2006) Epidemiology, trends, and morbidities of obesity and the metabolic syndrome. Endocrine 29:109-117

Brost BC, Goldenberg RL, Mercer BM et al (1997) The preterm prediction study: association of cesarean delivery with increases in maternal weight and body mass index. Am J Obstet Gynecol 177:333-337

Butte NF, Wong WW, Treuth MS et al (2004) Energy requirements during pregnancy based on total energy expenditure and energy deposition. Am J Clin Nutr 79:1078-1087

Callaway LK, Prins JB, Chang AM et al (2006) The prevalence and impact of overweight and obesity in an Australian obstetric population. MJA 184:56-59

Catalano PM (2007) Management of obesity in pregnancy. Obstet Gynecol 109:419-433

CDC (2009) Obesity and overweight for professionals: data and statistics. http://www.cdc.gov/obesity/data/index.html. Accessed Nov 20, 2009

Cedergren MI (2004) Maternal morbid obesity and the risk of adverse pregnancy outcome. Obstet Gynecol 103:219-224

Cedergren MI (2007) Optimal gestational weight gain for Body Mass Index categories. Obstet Gynecol 110:759-764

Cogswell ME, Serdula MK, Hungerford DW et al (1995) Gestational weight gain among average-weight and overweight women- what is excessive? Am J Obstet Gynecol 172:705-712

Confidential Enquiry into Maternal and Child Health. In: Acolet D, editor. Perinatal mortality 2006: England, Wales, and Northern Ireland. London: CEMACH; 2008

Delavaud C, Bocquier F, Chilliard Y et al (2000) Plasma leptin determination in ruminants: effects of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J Endocrinol 165:519-526

DeVader SR, Neeley HL, Myles TD et al (2007) Evaluation of gestational weight guidelines for women with normal prepregnancy Body Mass Index. Obstet Gynecol 110:745-751

Eskenazi B, Fenster L, Sidney S (1991) A multivariate analysis of risk factors for preeclampsia. JAMA 266:237-241

Feng D, Naylor C (1998) Eating for two: are guidelines for weight gain during pregnancy too liberal? Lancet 351:1054-1055

Flegal KM, Carroll MD, Kuczmarski RJ et al (1998) Overweight and obesity in the United States: prevalence and trends, 1960-1994. Int J Obes Relat Metab Disord 22:39-47

Flegal KM, Carroll MD, Ogden CL et al (2010) Prevalence and trends in obesity among US adults, 1999-2008. JAMA 303:235-241

Ford SP, Hess BW, Schwope MM et al (2007) Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. J Anim Sci 85:1285-1294

Fu WJ, Haynes TE, Kohli R et al (2005) Dietary L-arginine supplementation reduces fat mass in Zucker diabetic fatty rats. J Nutr 135:714-721

Gaidhu MP, Fediuc S, Anthony NM et al (2009) Prolonged AICAR-induced AMP-kinase activation promotes energy dissipation in white adipocytes: novel mechanisms integrating HSL and ATGL. J Lipid Res 50:704-715

Galtier F, Farret A, Bringer J (2004) Grossesse et Obesite. In: Basdevant A, Guy-Grand B, editors. Medecine de l'Obesite. Paris: Flammarion Medecine Sciences p. 347-350

Galtier F, Raingeard I, Renard E et al (2008) Optimizing the outcome of pregnancy in obese women: from pregestational to long-term management. Diabetes & Metabolism 34:19-25

Galtier-Dereure F, Boegner C, Bringer J (2000) Obesity and pregnancy: complications and cost. Am J Clin Nutr 71:1242S-1248

Gavard JA, Artal R (2008) Effect of exercise on pregnancy outcome. Clin Obstet Gynecol 51:467-480

Gore SA, Brown DM, West DS (2003) The role of postpartum weight retention in obesity among women: a review of the evidence. Ann Behav Med 26:149-159

Greer IA (1994) Haemostasis and thrombosis in pregnancy. In: Bloom AL, Forbes CD, Thomas DP, et al, editors. Haemostasis and Thrombosis in Pregnancy. Edinburgh (UK): Churchill Livingstone; p. 987-1015

Hall LF, Neubert A (2005) Obesity and pregnancy. Obstet Gynecol Survey 60:8-9

Hardie DG, Scott JW, Pan DA et al (2003) Management of cellular energy by the AMP-activated protein kinase system. FEBS Lett 546:113–120

He QH, Kong XF, Wu G, et al (2009) Metabolomic analysis of the response of growing pigs to dietary L-arginine supplementation. Amino Acids 37: 199-208

Hedderson MM, Weiss NS, Sacks DA et al (2006) Pregnancy weight gain and risk of neonatal complications: macrosomia, hypoglycemia, and hyperbilirubinemia. Obstet Gynecol 108:1153-1161

Hedley AA, Ogden CL, Johnson CL et al (2004) Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. JAMA 291:2847-2850

Hellgren M, Blomback M (1981) Studies on blood coagulation and fibrinolysis in pregnancy, during delivery and in the puerperium. I. Normal condition. Gynecol Obstet Invest 12:141-154

Hendler I, Blackwell SC, Bujold E et al (2004) The impact of maternal obesity on midtrimester sonographic visualization of fetal cardiac and craniospinal structures. Int J Obes 28:1607-1611

Hendler I, Goldenberg RL, Mercer BM et al (2005) The preterm prediction study: association between maternal body mass index and spontaneous and indicated preterm birth. Am J Obstet Gynecol 192:882-886

Huang DY, Usher RH, Kramer MS et al (2000) Determinants of unexplained antepartum fetal deaths. Obstet Gynecol 95:215-221

Ikeoka D, Mader JK, Pieber TR (2010) Adipose tissue, inflammation and cardiovascular disease. Rev Assoc Med Bras 56:116-121

Institute of Medicine (1990) Nutritional status and weight gain. In: Nutrition during pregnancy. Washington, DC: National Academies Press p. 27-233

Jensen DM, Damm P, Sorensen B et al (2003) Pregnancy outcome and prepregnancy body mass index in 2459 glucose-tolerant Danish women. Am J Obstet Gynecol 189:239-244

Jobgen WJ, Meininger CJ, Jobgen SC et al (2009a) Dietary L-arginine supplementation reduces white-fat gain and enhances skeletal muscle and brown fat masses in diet-induced obese rats. J Nutr 139: 230-237

Jobgen W, Fu WJ, Gao H et al (2009b) High fat feeding and dietary L-arginine supplementation differentially regulate gene expression in rat white adipose tissue. Amino Acids 37:187-198

Jobgen W, Fu WJ, Gao H et al (2009c) High fat feeding and dietary L-arginine supplementation differentially regulate gene expression in rat white adipose tissue. Amino Acids 37:187-198

Jovanovic-Peterson L, Peterson C (1991) Is exercise safe or useful for gestational diabetic women? Diabetes 40:S179-181

Kiel DW, Dodson EA, Artal R et al (2007) Gestational weight gain and pregnancy outcomes in obese women. Obstet Gynecol 110:752-758

Kieser JA, Groeneveld HT, Da Silva PC (1997) Dental asymmetry, maternal obesity, and smoking. Am J Phys Anthropol 102:133-139

Kinoshita T, Itoh M (2006) Longitudinal variance of fat mass deposition during pregnancy evaluated by ultrasonography: the ratio of visceral fat to subcutaneous fat in the abdomen. Gynecol Obstet Invest 61:115-118

Kohli R, Meininger CJ, Haynes TE et al (2004) Dietary l-arginine supplementation enhances endothelial nitric oxide synthesis in streptozotocin-induced diabetic rats. J Nutr 134:600-608

Lassala A, Bazer FW, Cudd TA et al (2009) Intravenous administration of L-citrulline to pregnant ewes is more effective than L-arginine for increasing arginine availability in the fetus. J Nutr 139:660-665

Li XL, Bazer FW, Gao H et al (2009) Amino acids and gaseous signaling. Amino Acids 37: 65-78

Lucotti P, Setola E, Monti LD et al (2006) Beneficial effects of a long-term oral L-arginine added to a hypocaloric diet and exercise training program in obese, insulin-resistant type 2 diabetic patients. Am J Physiol Endocrinol Metab 291:E906-912

Luke B, Hediger ML, Scholl TO (1996) Point of diminishing returns: when does gestational weight gain cease benefiting birth weight and begin adding to maternal obesity? J Matern Fetal Med 5:168-173

Marliss EB, Chevalier S, Gougeon R et al (2006) Elevations of plasma methylarginines in obesity and ageing are related to insulin sensitivity and rates of protein turnover. Diabetologia 49:351-359

Mateo RD, Wu G, Bazer FW et al (2007) Dietary L-arginine supplementation enhances the reproductive performance of gilts. J Nutr 137:652-656

McKnight JR, Satterfield MC, Jobgen WS et al (2010) Beneficial effects of L-arginine on reducing obesity: potential mechanisms and important implications for human health. Amino Acids. DOI: 10.1007/s00726-010-0598-z

Naeye RL (1990) Maternal body weight and pregnancy outcome. Am J Clin Nutr 52:273-279

Nall JL, Wu G, Kim KH et al (2009) Dietary supplementation of L-arginine and conjugated linoleic acid reduces retroperitoneal fat mass and increases lean body mass in rats. J Nutr 139:1279-1285

National Institute of Health (NIH 1998) Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults. NIH Publication No. 98-4083, Bethesda, MD

Nohr EA, Bech BH, Davies MJ et al (2005) Prepregnancy obesity and fetal death: a study within the Danish National Birth Cohort. Obstet Gynecol 106:250-259

O'Brien TE, Ray JG, Chan WS (2003) Maternal body mass index and the risk of preeclampsia: a systematic overview. Epidemiology 14:368-374

Pien GW, Fife D, Pack AI et al (2005) Changes in symptoms of sleep-disordered breathing during pregnancy. Sleep 28:1299-1305

Pi-Sunyer X (2003) A clinical view of the obesity problem. Science 299:859-860

Poirier P, Giles T, Bray G et al (2006) Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. Circulation 113:898-918

Ramachenderan J, Bradford J, McLean M (2008) Maternal obesity and pregnancy complications: A review. Aust N Z J Obstet Gynaecol 48:228-235

Retnakaran R, Hanley AJ, Raif N et al (2003) C-reactive protein and gestational diabetes: The central role of maternal obesity. J Clin Endocrinol Metab 88:3507-3512

Rhoads JM, Wu G (2009) Glutamine, arginine, and leucine signaling in the intestine. Amino Acids 37: 111-122

Roberts JM, Lain KY (2002) Recent insights into the pathogenesis of preeclampsia. Placenta 23:359-372

Rode L, Hegaard HK, Kjaergaard H et al (2007) Association between maternal weight gain and birth weight. Obstet Gynecol 109:1309-1315

Saravanakumar K, Rao SG, Cooper GM (2006) Obesity and obstetric anesthesia. Anaesthesia 61:36-48

Satterfield MC, Bazer FW, Spencer TE et al (2010) Sildenafil citrate treatment enhances amino acid availability in the conceptus and fetal growth in an ovine model of intrauterine growth restriction. J Nutr 140:251-258

Schieve LA, Cogswell ME, Scanlon KS (1998) An empiric evaluation of the Institute of Medicine's pregnancy weight gain guidelines by race. Obstet Gynecol 91:878-884

Scholl TO, Hediger ML, Schall JI et al (1995) Gestational weight gain, pregnancy outcome, and postpartum weight retention. Obstet Gynecol 86:423-427

Schrauwers C, Dekker G (2009) Maternal and perinatal outcome in obese pregnant patients. J Maternal-Fetal Neonatal Med 3, 218-226

Sebire NJ, Jolly M, Harris JP et al (2001) Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London. Int J Obes Relat Metabol Disord 25:1175-1182

Shaikh H, Robinson S, Teoh TG (2009) Management of maternal obesity prior to and during pregnancy. Sem Fetal Neonatal Med 15:77-82

Siega-Riz AM, Adair LS, Hobel CJ (1994) Institute of Medicine maternal weight gain recommendations and pregnancy outcome in a predominantly Hispanic population. Obstet Gynecol 84:565-573

Soltani H, Fraser RB (2000) A longitudinal study of maternal anthropometric changes in normal weight, overweight and obese women during pregnancy and postpartum. Br J Nutr 84:95-101

Song XM, Fiedler M, Galuska D et al (2002) 5-Aminoimidazole-4-carboxamide ribonucleoside treatment improves glucose homeostasis in insulin-resistant diabetic (ob/ob) mice. Diabetologia 45:56-65

Stotland NE, Caughey AB, Breed EM et al (2004) Risk factors and obstetric complications associated with macrosomia. Int J Gynecol Obstet 87:220-226

Stotland NE, Cheng YW, Hopkins LM et al (2006) Gestational weight gain and adverse neonatal outcome among term infants. Obstet Gynecol 108:635-643

Taffel SM, Keppel KG, Jones GK (1993) Medical advice on maternal weight gain and actual weight gain. Results from the 1988 National Maternal and Infant Health Survey. Ann N Y Acad Sci 678:293-305

Tan BE, Yin YL, Liu ZQ et al (2009a) Dietary L-arginine supplementation increases muscle gain and reduces body fat mass in growing-finishing pigs. Amino Acids 37: 169-175

Tan BE, Li XG, Kong XF et al (2009b) Dietary L-arginine supplementation enhances the immune status in early-weaned piglets. Amino Acids 37: 323-331

Tan BE, Yin YL, Kong XF et al (2010) L-Arginine stimulates proliferation and prevents endotoxin-induced death of intestinal cells. Amino Acids 38:1227-1235

Vesco KK, Dietz PM, Rizzo J et al (2009) Excessive gestational weight gain and postpartum weight retention among obese women. Obstet Gynecol 114:1069-1075

Watkins ML, Rasmussen SA, Honein MA et al (2003) Maternal obesity and risk for birth defects. Pediatrics 111:1152-1158

WHO (2009) World Health Organization. World Health Statistics – 2009. www.who.int. Accessed March 2, 2010

Williamson DF, Kahn HS, Remington PL et al (1990) The 10-year incidence of overweight and major weight gain in US adults. Arch Intern Med 150:665-672

Winder WW, Hardie DG (1999) AMP-activated protein kinase, a metabolic master switch: possible roles in Type 2 diabetes. Am J Physiol Endocrinol Metab 277:E1-10

Wolfe HM, Sokol RJ, Martier SM et al (1990) Maternal obesity: a potential source of error in sonographic prenatal diagnosis. Obstet Gynecol 76:339-342

World Health Organization (2000) Obesity: preventing and managing the global epidemic. Geneva (Switzerland): World Health Organization. WHO technical report series 894

Wu G (1995) Urea synthesis in enterocytes of developing pigs. Biochem J 312:717-723

Wu G (2009) Amino acids: metabolism, functions, and nutrition. Amino Acids 37: 1-17

Wu G, Meininger CJ (2009) Nitric oxide and vascular insulin resistance. BioFactors 35:21-27

Wu G, Knabe DA, Yan W et al (1995) Glutamine and glucose metabolism in enterocytes of the neonatal pig. Am J Physiol Regulatory Integrative Comp Physiol 268:334-342

Wu G, Davis PK, Flynn NE et al (1997) Endogenous synthesis of arginine plays an important role in maintaining arginine homeostasis in postweaning growing pigs. J Nutr 127:2342-2349

Wu G, Bazer FW, Wallace JM et al (2006) Intrauterine growth retardation: implications for the animal sciences. J Anim Sci 84:2316-2337

Wu G, Collins JK, Perkins-Veazie P et al (2007a) Dietary supplementation with watermelon pomace juice enhances arginine availability and ameliorates the metabolic syndrome in Zucker diabetic fatty rats. J Nutr 137:2680-2685

Wu G, Bazer FW, Cudd TA et al (2007b) Pharmacokinetics and safety of arginine supplementation in animals. J Nutr 137:1673S-1680S

Wu G, Bazer FW, Davis TA et al (2009) Arginine metabolism and nutrition in growth, health and disease. Amino Acids 37: 153-168

Yao K, Yin YL, Chu WY et al (2008) Dietary arginine supplementation increases mTOR signaling activity in skeletal muscle of neonatal pigs. J Nutr 138:867-872

Yogev Y & Catalano PM (2009) Pregnancy and obesity. Obstet Gynecol Clin N Am 36:285-300

VITA

Jason Ray McKnight
219 McKnight Lane, Corrigan, TX 75939
jrm001@tamu.edu

Education

May 2008 B.S. in Animal Science Pre-veterinary Medicine, Sam Houston State University, Huntsville, TX

August 2010 M.S. in Nutrition, Texas A&M University, College Station, TX

Work Experience

August 2008-January 2010 Graduate Teaching Assistant, Dept. of Biology, Texas A&M University

August 2008- August 2010 Graduate Research Assistant, Texas A&M University

Award

First place prize, Nutrition Graduate Student research competition, TAMU, April 2010

Publications

McKnight JR, Satterfield MC, Jobgen WS, Smith SB, Spencer TE, Meininger CJ, McNeal CJ, Wu G (2010) Beneficial effects of L-arginine on reducing obesity: Potential mechanisms and important implications for human health. *Amino Acids* DOI: 10.1007/s00726-010-0598-z.

Li XL, Bazer FW, Gao H, Jobgen W, Johnson GA, Li P, **McKnight JR**, Satterfield MC, Spencer TE, Wu G (2009) Amino acids and gaseous signaling. *Amino Acids* 37: 65-78.

Wu G, Li XL, **McKnight JR**, Satterfield MC (2010) Amino acids: utilization, gene expression and cell signaling. In: Encyclopedia of Animal Science (W.G. Pond, D. Ullrey and C. Kirk-Baer, eds.), Taylor & Francis Group, New York, NY. (In press)

McNeal C, Wu G, Vasquez S, Wilson DP, Satterfield MC, **McKnight JR**, Malbari H (2010) The role of arginine for treating obese youth. In: Global Perspectives on Childhood Obesity (D. Bagchi, ed.), Elsevier, New York, NY. (In press)

Satterfield MC, **McKnight JR**, Li XL, Wu G (2010) Nutrition, epigenetics, and vascular function. In: Nutrition, Epigenetic Mechanisms, and Human Disease (N. Maulik, ed.), CRC Press, New York (In press)

Oral presentation at Scientific Meeting

McKnight JR, Satterfield MC, Bazer FW, Spencer TE, Wu G (2010) Pre-partum nutrition extremes impair post-partum maternal glucose utilization in sheep. Experimental Biology April 24-28, 2010 (Anaheim, CA)