Amino Acids (2012) 43:1593–1603
DOI 10.1007/s00726-012-1235-9

ORIGINAL ARTICLE

Arginine nutrition and fetal brown adipose tissue development in diet-induced obese sheep

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Received: 12 September 2011 / Accepted: 28 January 2012 / Published online: 12 February 2012

Abstract The global incidence of human obesity has more than doubled over the past three decades. An ovine model of obesity was developed to determine effects of maternal obesity and arginine supplementation on maternal, placental, and fetal parameters of growth, health, and well being. One-hundred-twenty days prior to embryo transfer, ewes were fed either ad libitum ($n = 10$) to induce obesity or 100% National Research Council-recommended nutrient requirements ($n = 10$) as controls. Embryos from superovulated ewes with normal body condition were transferred to the uterus of control-fed and obese ewes on day 5.5 post-estrus to generate genetically similar singleton pregnancies. Beginning on day 100 of gestation, obese ewes received intravenous administration of saline or L-arginine-HCl three times daily (81 mg arginine/kg body weight/day) to day 125, whereas control-fed ewes received saline. Fetal growth was assessed at necropsy on day 125. Maternal obesity increased (1) percentages of maternal and fetal carcass lipids and (2) concentrations of leptin, insulin, glucose, glutamate, leucine, lysine and threonine in maternal plasma while reducing (1) concentrations of progesterone, glycine and serine in maternal plasma and (2) amniotic and allantoic fluid volumes. Administration of L-arginine to obese ewes increased arginine and ornithine concentrations in maternal and fetal plasma, amniotic fluid volume, protein content in maternal carcass, and fetal brown adipose tissue (+60%), while reducing maternal lipid content and circulating leptin levels. Fetal or placental weight did not differ among treatments. Results indicate that arginine treatment beneficially reduces maternal adiposity and enhances fetal brown adipose tissue development in obese ewes.

Keywords Adipose tissue · Pregnancy · Fetus · Arginine · Sheep · Obese

Abbreviations

BAT	Brown adipose tissue
cGMP	Cyclic guanosine monophosphate
DM	Dry matter
NRC	National Research Council
WAT	White adipose tissue

Introduction

The current incidence of obesity among women of reproductive age exceeded 23% in the US (March-of-Dimes 2009). Emerging evidence indicates that maternal obesity has negative impacts on subsequent long-term health of the offspring into adulthood and possibly across generations (Satterfield et al. 2011a; Wang et al. 2012). These observations have led to the widely accepted concept of fetal programming, whereby the fetus establishes a number of set points to regulate basic physiological and metabolic processes in adulthood. These programmed events are based on signals received by the fetus in utero and drive the pattern of organ development and subsequent function (Armitage et al. 2005; Khan et al. 2005). Predisposition to a number of adult onset diseases has been linked to maternal obesity and altered expression of fetal genes (Aagaard-Tillery et al. 2008; Cox et al. 2009). Metabolic abnormalities in the

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affected offspring result in development of obesity (Muhlhausler et al. 2007a, b), hypertension (Gilbert and Nijland 2008), reduced vascular compliance, endothelial dysfunction, aortic hyperplasia (Samuelsson et al. 2008), decreased renal Na–K ATPase activity, decreased locomotor activity, insulin resistance, impaired glucose utilization, and type-II diabetes mellitus (Dunn and Bale 2009; Ford et al. 2009; McCurdy et al. 2009; Taylor et al. 2005). That maternal obesity spawns obesity in the offspring is problematic and likely contributes to the 36% increase in obese youth within the last decade (March-of-Dimes 2009; Williams et al. 2001). The cyclical nature of this epidemic presents a unique and complex challenge to stem the tide toward global obesity and the reduced quality of life associated with metabolic disorders.

To date, viable strategies to improve fetal outcome and postnatal health and well being in offspring from obese mothers have not been developed. A reduction in maternal white adipose tissue (WAT) without altering maternal intake and thus fetal nutrient availability would presumably result in a more favorable uterine environment for development. Recently, dietary supplementation with arginine to non-pregnant mammals resulted in a reduction of WAT mass in a number of species, including rats (Fu et al. 2005; Jobgen et al. 2009b; Wu et al. 2007), humans (Lucotti et al. 2006), and swine (Tan et al. 2009, 2011). Evidence from animal studies indicates that physiological levels of arginine decrease de novo synthesis of glucose and triacylglycerides, while promoting oxidation of glucose and long-chain fatty acids (Jobgen et al. 2009b; Wu et al. 2009). Arginine supplementation also increases lipolysis and inhibits lipogenesis in WAT via modulation of expression and function of key enzymes involved in antioxidative response and fat metabolism in insulin-sensitive tissues (Jobgen et al. 2009a). Nitric oxide, which is synthesized from arginine by nitric oxide synthase (Wu and Morris 1998), appears to partially mediate the effects of arginine on cell metabolism and function (Blachier et al. 2011; Eklou-Lawson et al. 2009; Gaudiot et al. 1998; Le Gouill et al. 2007). In addition to nitric oxide (Wu and Meininger 2009) and polyamines (Wu et al. 2011), physiological levels of arginine also increase the production of carbon monoxide from cells, which is known to activate guanalyl cyclase to generate cGMP and enhance oxidation of glucose and fatty acids in skeletal muscle (Li et al. 2009a). Activation of the cGMP signaling cascade reduces malonyl-coA concentrations in the cell, which inhibits fatty acid synthesis and stimulates the transport of long-chain fatty acids from cytoplasm to mitochondria for oxidation (McKnight et al. 2010). Besides its direct effects on WAT loss, arginine supplementation may reduce the incidence of the metabolic syndrome, as evidenced by decreased plasma levels of glucose, homocysteine, fatty acids, dimethylarginines, and triglycerides, as well as improved whole-body insulin sensitivity in mammals (Jobgen et al. 2009b; Kohli et al. 2004; Lucotti et al. 2006; Mendez and Balderas 2001; Wu et al. 2007).

In both humans and sheep, fetal brown adipose tissue begins to appear after mid-gestation (Symonds et al. 2003; Vernon 1986). Accumulation of fetal brown adipose tissue (BAT) as opposed to WAT would be beneficial for improving the metabolic profile in progeny (McKnight et al. 2010), as BAT is highly efficient at oxidizing fatty acids and glucose to produce heat (Cannon and Nedergaard 2004). In the fetal lamb, BAT development increases rapidly between days 70 and 120 of gestation and then slows to term (day 147; Alexander 1978). Although fetal WAT is affected by prolonged manipulation of maternal feed intake (Bispham et al. 2003; Gopalakrishnan et al. 2001), the effect of maternal nutrition on fetal BAT development and function is largely unknown (Satterfield and Wu 2011). Interestingly, in adult rats, dietary supplementation with arginine to reduce WAT was accompanied by an increase in BAT mass (Jobgen et al. 2009b; Wu et al. 2007).

This study tested the hypothesis that maternal obesity could alter multiple aspects of fetal growth, including organ and tissue development, and that maternal arginine administration might reduce adiposity in the mother and increase fetal BAT.

Materials and methods

All experimental and surgical procedures were approved by the Institutional Agricultural Animal Care and Use Committee of Texas A&M University.

Experimental design

One-hundred-twenty days prior to embryo transfer, recipient ewes were assigned to either ad libitum feeding resulting in obesity or fed 100% of National Research Council (NRC)-recommended nutrient requirements (NRC 1985) to maintain their normal body condition (Satterfield et al. 2011b). Ewes were group fed at this time and weighed monthly to determine changes in body weight. After 120 days on 100% NRC feeding (20 g feed/kg body weight per day) and ad libitum feeding (170% of feed intake for the 100% NRC-fed ewes on a per-animal basis), ewes with normal body condition and obese ewes were synchronized into estrus and a single embryo from a superovulated Suffolk ewe of normal body condition was transferred into the recipient's uterus on day 5.5 post-estrus (Satterfield et al. 2011b). Pregnancy was diagnosed by ultrasound on day 28 of gestation. All ewes were individually housed from day 28 to 125 of gestation. During this

period, ewes continued to receive their respective feeding regimens, body weight was analyzed every 7 days, and feed intake for 100% NRC-fed ewes (NRC 1985) was adjusted based on changes in body weight (22.6 g feed/kg body weight per day). Ewes in the 100% NRC group of this study were the same as those we used in the previously published study involving 50% NRC-fed ewes (Satterfield et al. 2011b) to reduce the number of experimental animals, and both studies were conducted simultaneously in the same facility. Obese ewes ($n = 10$) were maintained on ad libitum feeding throughout gestation (175% of feed intake for the 100% NRC-fed ewes on a per-animal basis).

On day 100, obese ewes were further assigned randomly to receive intravenous administration of either sterile saline ($n = 5$) or L-arginine-HCl ($n = 5$) three times daily (equivalent to 81 mg L-arginine/kg body weight/day; Satterfield et al. 2011b). L-arginine-HCl was dissolved in saline (pH 7.2) and filter-sterilized before use (Lassala et al. 2010). All 100% NRC-fed ewes were administered intravenously the same volume of sterile saline as the saline-obese ewe group three times daily. Administration of saline or arginine was performed at 0600, 1400, and 2200 hours daily between days 100 and 125 of gestation, which is a period of rapid growth of BAT in the ovine fetus (Alexander 1978). The rationale for providing arginine intravenously was to increase circulating concentrations of arginine in blood (Lassala et al. 2009; Wu et al. 2007) because microorganisms in the rumen of sheep extensively degrade dietary arginine (Wu 2009). Feed intake did not differ between saline- and arginine-treated obese ewes during the entire experimental period.

Blood samples from the maternal jugular vein were collected into heparinized tubes on days 113 and 125 of gestation at 1 h after administration of saline or arginine. Plasma was immediately harvested following centrifugation (2,000×*g* for 10 min at 4°C) and stored at −20°C until analyzed within 1 week (Li et al. 2009b). This procedure of plasma processing and storage did not result in a detectable loss of any amino acid. On day 125 of pregnancy, conceptus (fetal-placental unit) development was determined at necropsy.

Tissue collection and handling following necropsy

At the time of necropsy, ewes were stunned using a captive bolt gun followed immediately by exsanguination. Immediately upon exsanguination, the uterus was exposed and venous blood samples were collected from the uterine vein into a heparinized tube. Plasma was harvested following centrifugation (2,000×*g* for 10 min at 4°C) and then stored at −20°C until analyzed. Amniotic and allantoic fluids were obtained and volumes recorded by puncturing the amniochorion and chorioallantois, respectively (Kwon et al. 2003). Following collection of the fetal fluids and umbilical artery and umbilical vein blood samples, the fetus was removed, weighed, measured, and dissected. For determination of BAT mass, only peri-renal BAT was utilized as it represents >80% of the BAT depot in the fetal lamb (Clarke et al. 1997). Uncoupling protein-1 was detected in the ovine fetal BAT (Satterfield and Wu 2011). Samples from fetal organs were preserved in either 4% paraformaldehyde or snap frozen in liquid nitrogen and stored at −80°C for analyses. Following dissection of the fetus, the fetal carcass was further divided into soft tissue and bone by scalpel dissection. Fetal bone was weighed while fetal soft tissues were frozen at −20°C for chemical analyses. The placenta was further dissected to isolate all placentomes for the assessment of placentome number, gross morphology, and weight. Placentomes were fixed in either 4% paraformaldehyde or snap frozen in liquid nitrogen. Simultaneously, the maternal organs were dissected and weighed. Upon removal of maternal organs, the pelt and head were removed and the carcass was split along the spine. One half of the remaining maternal carcass was broken down by knife to collect all soft tissues and bone for subsequent carcass composition analyses. Maternal bone was weighed immediately while maternal soft tissue was frozen at −20°C for subsequent analyses.

Analyses of carcass minerals, dry matter, and total lipids

For analyses of maternal carcass composition, soft tissues were thawed and subsequently homogenized using a Seydelmann Cutter K64 (Strasser; Stuttgart, Germany). A subsample of the homogenized soft tissue was further homogenized in a food processor, aliquoted and stored at −20°C. Fetal soft tissues were homogenized in a food processor, aliquoted and stored at −20°C. Total lipids (∼1 g) were extracted from soft tissue homogenates (∼1 g sample) using a mixture of chloroform and methanol (2:1, vol/vol) according to the procedure of Folch et al. (1957). Total nitrogen in soft tissue homogenates (∼1 g sample) was analyzed using LECO Model FP-528 Analyzer (St. Joseph, MI, USA) (Li et al. 2011a). Crude protein was calculated as total nitrogen multiplied by 6.25 on the basis of the assumption that protein generally contains 16% nitrogen (Wu 2010). Ash was determined to indicate the amount of minerals by placing tissue in a 550°C furnace for 12 h (Wu et al. 1999).

Analyses of amino acids in maternal and fetal plasma

Fetal and maternal plasma (0.5 ml) obtained on day 125 of gestation were deproteinized with an equal volume of 1.5 M $HClO_4$, followed by addition of 0.25 ml of 2 M K_2CO_3 (Wu et al. 1996). Amino acids in the neutralized

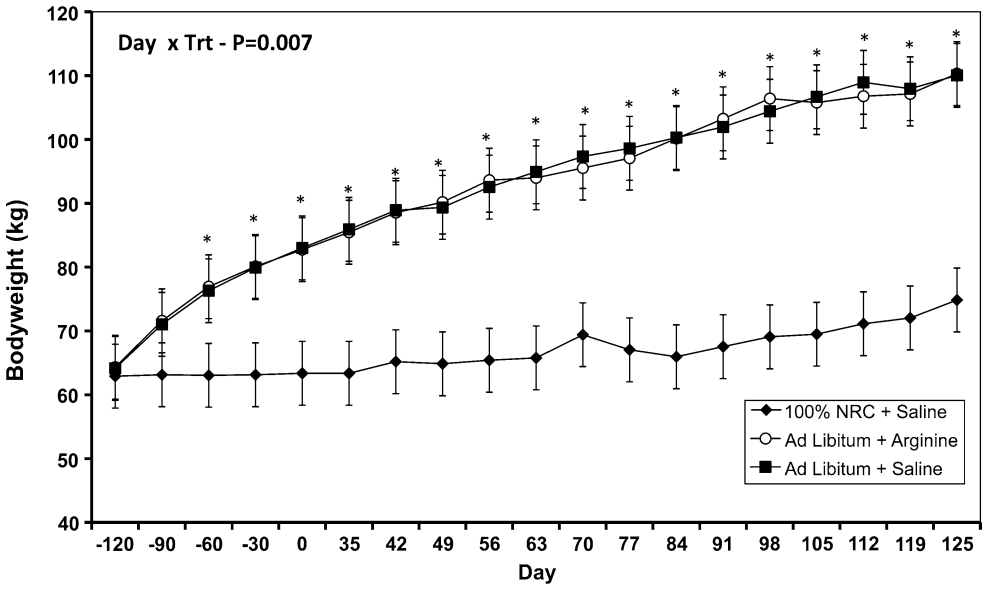

Fig. 1 Effects of obesity and arginine administration on maternal weight change in gestating ewes. Maternal weight in obese ewes was greater ($P < 0.01$) than that of 100% NRC-fed ewes beginning at day 60 after the initiation of ad libitum feeding and remained elevated to necropsy on day 125 of gestation. There was no difference ($P > 0.10$) in maternal weight between arginine- and saline-treated obese ewes at any time point

extract were determined by fluorometric HPLC methods involving precolumn derivatization with *o*-phthaldialdehyde, as described previously (Wu et al. 1997). The integration of chromatographic peaks was performed using Millenium-32 Software (Waters, Milford, MA).

Glucose and hormone analyses in maternal plasma

Maternal plasma obtained on day 113 of gestation was analyzed for glucose and hormones (Satterfield et al. 2011b). Briefly, glucose was determined using a spectrophotometric method involving hexokinase and glucose-6-phosphate dehydrogenase as previously described (Fu et al. 2005). Leptin was determined by a specific radioimmunoassay as previously described (Delavaud et al. 2000). Progesterone was determined according to manufacturer's specifications using an antiserum highly specific for progesterone (DSL-3900 ACTIVE Progesterone Coated-Tube Radioimmunoassay Kit, Diagnostic Systems Laboratories, Webster, TX), as previously described (Satterfield et al. 2006). Insulin was assayed using an ovine-specific enzyme-linked immunoassay (80-INSOV-E01, ALPCO Diagnostics, Salem, NH) according to manufacturer's instructions. Assay results were calculated using the AssayZap Version 3.1 program (Biosoft, Ferguson, CA).

Statistical analyses

A total of four ewes were removed from the study (all from the 100%-NRC fed group), due to death (2 ewes), inappropriately timed fetal collection (1 ewe), and an unexpected twin pregnancy (1 ewe). Statistical analyses of maternal weight changes were determined by two-way ANOVA for repeated measures using day and treatment as independent variables and the presence of an interaction between day and treatment (Wei et al. 2011). All other maternal and fetal measures were subjected to least-squares analysis of variance using the general linear models procedures of the statistical analysis system (SAS Institute, Cary, NC) with preplanned pair-wise comparisons. Fetal organ weights presented are relative weights after adjustment for fetal weight using fetal weight as a covariate in the statistical model. Data are presented as the least-squares mean (LSM) with standard error of the mean (SEM). Differences in mean were considered to be statistically significant when a P value was ≤ 0.05 while a P value of <0.1 was considered to indicate a tendency toward significance.

Results

Maternal bodyweight changes

A day by treatment interaction ($P < 0.01$) was detected for maternal bodyweight (Fig. 1). Compared with the 100% NRC group, maternal bodyweight was greater ($P < 0.01$) for ad libitum fed ewes as early as 60 days after initiation of ad libitum feeding and remained greater to day 125 of gestation. Administration of arginine to obese ewes between days 100 and 125 of gestation did not affect ($P > 0.10$) maternal body weight.

Maternal endocrine status

Maternal dietary treatment altered the endocrine profile of a number of circulating glucose and hormones in the plasma of ewes on day 113 of gestation (Table 1). Maternal glucose levels were greater ($P < 0.01$) in obese ewes than

Table 1 Effects of maternal obesity and arginine administration on maternal circulating levels of hormones

Variable	100% NRC-fed ewes	Obese ewes	Obese ewes + Arginine	SEM	*P* value
Glucose (mM)	2.89[b]	3.78[a]	3.82[a]	0.16	<0.01
Insulin (ng/mL)	0.60[b]	2.7[a]	2.5[a]	0.5	<0.05
Leptin (ng/mL)	3.4[c]	24.8[a]	22.1[b]	0.8	<0.001
Progesterone (ng/mL)	9.7[a]	6.6[b]	7.5[b]	0.6	<0.05

Data are mean with pooled standard error of the mean (SEM). Values within a row sharing different superscripts differ ($P < 0.05$)

Table 2 Effects of maternal obesity and arginine administration on maternal organ weights in gestating ewes

Variable	100% NRC-fed ewes	Obese ewes	Obese ewes + arginine	SEM	*P* value
Heart (g)	306[b]	345[a,b]	374[a]	18	0.05
Left ventricle (g)	129[b]	158[a]	171[a]	12	<0.05
Right ventricle (g)	85	100	100	7	>0.10
Septum (g)	44	43	43	4	>0.10
Kidney (g)	138[b]	178[a]	179[a]	10	<0.01
Liver (g)	737[b]	1,529[a]	1,137[a]	219	<0.05
Lung (g)	685	813	811	49	>0.10
Adrenal gland (g)	6.3	9.2	9.0	1.3	>0.10
Spleen (g)	109[b]	132[a]	145[a]	8	<0.05
Pancreas (g)	79	62	54	12	>0.10
Small intestine (g)	1,354	1,538	1,075	207	>0.10
Large intestine (g)	384[b]	638[a]	344[b]	104	<0.05
Stomach (kg)	2.8	2.6	2.6	0.4	>0.10
Mammary gland (kg)	1.2	1.2	1.3	0.2	>0.10

Data are mean with SEM. Values within a row sharing different superscripts differ ($P < 0.05$)

in 100% NRC-fed ewes. Arginine administration to obese ewes did not affect ($P > 0.10$) glucose concentrations in maternal plasma. Maternal insulin levels were 4.5-fold higher ($P < 0.05$) in plasma of ad libitum fed ewes than in 100% NRC-fed ewes. Arginine treatment did not affect ($P > 0.10$) concentrations of insulin in maternal plasma of ewes. Circulating levels of leptin in maternal plasma were 7.3-fold higher ($P < 0.05$) in saline-treated obese ewes than in 100% NRC-fed ewes. Within obese ewes, maternal arginine administration decreased ($P < 0.05$) concentrations of leptin in plasma as compared to saline-infused obese dams. Obese ewes had lower ($P < 0.05$) concentrations of progesterone than 100% NRC-fed ewes. Administration of arginine had no effect ($P > 0.10$) on concentrations of progesterone in maternal plasma of obese ewes.

Maternal organ, tissue, and placental measures

Maternal nutritional regimen affected ($P < 0.05$) weights of a number of maternal organs (Table 2) and maternal body composition (Table 3). Weights of the maternal kidney, liver, and spleen, as well as weight of the maternal left ventricle, were greater ($P < 0.05$) in obese ewes than in 100% NRC-fed ewes. Maternal heart weight increased ($P = 0.05$) in obese ewes as compared to 100% NRC-fed ewes. Weight of the large intestine was heavier ($P < 0.05$) in saline-treated obese ewes than in arginine-treated obese ewes and in 100% NRC-fed ewes.

Indicators of maternal body composition, such as the twelfth-rib backfat thickness, body-wall thickness, total internal fat, weight of gastrocnemius muscle, weight of the whole hindlimb, weight of the carcass soft tissue, and percentage of total lipids within soft tissue were greater ($P < 0.05$) in obese ewes than in 100% NRC-fed ewes. Backfat thickness, body-wall thickness, total internal fat, weight of the whole hindlimb, and carcass soft tissue weight were not different ($P > 0.10$) between arginine and saline-infused obese ewes. However, within obese ewes, arginine treatment reduced ($P < 0.05$) the percentage of total lipids within carcass soft tissue by 25%. Carcass bone weight was greater ($P < 0.05$) in obese ewes treated with arginine than in 100% NRC-fed ewes. Dry matter (DM) content of maternal soft tissue was lower ($P < 0.01$) in 100% NRC-fed ewes as compared to arginine- or saline-treated obese ewes. Within obese ewes, DM content was lower ($P < 0.01$) in arginine-treated than saline-treated dams. The percentage of ash (minerals) in maternal soft tissue was reduced ($P < 0.01$) in obese ewes as compared to 100% NRC-fed ewes. Within obese ewes, protein content of maternal soft tissue was higher ($P < 0.01$) in arginine-treated compared with saline-treated ewes, but maternal ash content did not differ ($P > 0.10$) between these two groups of animals. Protein content of maternal soft tissue was higher ($P < 0.01$) in 100% NRC-fed ewes than in obese ewes receiving intravenous administration of arginine or saline.

Analyses of the placenta indicated that placentome number and average placentome weight did not differ ($P > 0.10$) between treatment groups (Table 4). In contrast, volume of amniotic fluid was reduced ($P < 0.01$) in saline-treated obese ewes as compared to arginine-treated obese ewes and 100% NRC-fed ewes. Among the three groups of animals, ewes in the 100% NRC group had the highest ($P < 0.01$) volume of amniotic fluid. Volume of allantoic fluid was reduced ($P = 0.07$) in obese ewes irrespective of treatment, compared with 100% NRC-fed

Table 3 Effects of maternal obesity and arginine administration on maternal carcass composition in gestating ewes

Variable	100% NRC-fed ewes	Obese ewes	Obese ewes + Arginine	SEM	*P* value
Gastrocnemius muscle (g)	560[b]	950[a]	883[a]	83	<0.05
Whole hindlimb (kg)	2.6[b]	3.8[a]	3.7[a]	0.2	<0.001
Last Rib backfat (cm)	0.4[b]	2.5[a]	2.6[a]	0.4	<0.001
Body-wall thickness (cm)	1.40[b]	5.03[a]	5.03[a]	0.3	<0.001
Internal white fat (g)	1,192[b]	8,500[a]	8,847[a]	677	<0.001
Carcass soft tissue (kg)	12[b]	27[a]	24[a]	1.4	<0.001
Total lipids in CST (kg)	3.9[c]	17.0[a]	12.7[b]	0.7	<0.001
Carcass bone (kg)	3.9[b]	4.3[a,b]	4.8[a]	0.2	<0.05
Soft tissue DM content (%)	40[c]	62[a]	58[b]	1.3	<0.001
Soft tissue lipid content (%)	32[c]	63[a]	53[b]	4	<0.001
Soft tissue protein content (%)	56[a]	40[c]	47[b]	2	<0.001
Soft tissue ash content (%)	0.90[a]	0.51[b]	0.51[b]	0.03	<0.001

Data are mean with SEM. Values within a row sharing different superscripts differ ($P < 0.05$)

CST carcass soft tissue

ewes. Allantoic fluid volume did not differ ($P > 0.10$) between arginine- and saline-treated obese ewes.

Fetal weight, organ weights, and carcass composition

Fetal weight did not differ ($P > 0.10$) between treatments on day 125 of gestation (Table 4). Weight of the fetal adrenal gland was reduced ($P = 0.05$) in fetuses from saline-treated obese ewes versus 100% NRC-fed ewes. There was no difference ($P > 0.10$) in weight of the fetal adrenal gland between saline- and arginine-treated obese ewes. Weight of BAT did not differ ($P > 0.10$) between 100% NRC-fed ewes and saline-treated obese ewes. However, maternal administration of arginine to obese ewes increased ($P < 0.01$) fetal peri-renal BAT mass by 60% in comparison with saline-treated ewes.

Composition of the fetal carcass was altered by maternal dietary intake (Table 5). Dry matter content of the fetal soft tissue was increased ($P < 0.05$) in fetuses from arginine-treated obese ewes as compared to 100% NRC-fed ewes. Fetal soft tissue lipid content was increased ($P < 0.01$) but protein content was decreased ($P < 0.01$) in both saline- and arginine-treated obese ewes compared to 100% NRC-fed ewes (Table 6).

Concentrations of amino acids in maternal and fetal plasma

A number of amino acids in maternal plasma were altered by maternal dietary intake or arginine administration (Table 7). Specifically, intravenous administration of arginine to obese ewes increased ($P < 0.01$) concentrations of arginine and ornithine in maternal plasma as compared to saline-treated obese ewes and 100% NRC-fed ewes. Concentrations of β-alanine were reduced ($P < 0.05$) and concentrations of glutamate were increased ($P < 0.05$) in saline-treated obese ewes as compared to 100% NRC-fed ewes. Concentrations of glycine and serine were reduced ($P < 0.05$) in both saline and arginine-treated obese ewes than in 100% NRC-fed ewes. In contrast, concentrations of leucine and threonine were greater ($P < 0.05$) while concentrations of alanine tended to be greater ($P = 0.08$) in saline and arginine-treated obese ewes versus 100% NRC-fed ewes. Concentrations of lysine were elevated

Table 4 Effects of maternal obesity and arginine administration on placental parameters in gestating ewes

Variable	100% NRC-fed ewes	Obese ewes	Obese ewes + arginine	SEM	*P* value
Amniotic fluid (ml)	908[a]	348[c]	606[b]	112	<0.01
Allantoic fluid (ml)	587[a]	220[b]	198[b]	144	0.07
Placentome number (n)	73	64	63	5	>0.10
Placentome weight (g)	7.5	7.7	8.8	0.7	>0.10

Data are mean with pooled SEM. Values within a row sharing different superscripts differ ($P < 0.05$)

Table 5 Effects of maternal obesity and arginine administration on fetal organ weights in gestating ewes

Variable	100% NRC-fed ewes	Obese ewes	Obese ewes + Arginine	SEM	*P* value
Fetal weight (kg)	4.0	3.8	3.9	0.2	>0.10
Fetal adrenal gland (g)	0.21[a]	0.13[b]	0.16[a,b]	0.02	0.05
Fetal brown adipose tissue (g)	7.2[b]	7.1[b]	11.3[a]	0.6	<0.001

Data are mean with SEM. Values within a row sharing different superscripts differ ($P < 0.05$)

Table 6 Effects of maternal obesity and arginine administration on fetal carcass composition in gestating ewes

Variable	100% NRC-fed ewes	Obese ewes	Obese ewes + Arginine	SEM	*P* value
Soft tissue DM content (%)	19.9[b]	20.8[a,b]	21.5[a]	0.4	<0.05
Soft tissue lipid content (%)	4.1[b]	6.1[a]	5.5[a]	0.5	<0.01
Soft tissue protein content (%)	63.5[a]	60.9[b]	59.4[b]	0.8	<0.001
Total lipids in fetus (g)	160[b]	236[a]	217[a]	20	<0.01

Data are mean with SEM. Values within a row sharing different superscripts differ ($P < 0.05$)

Table 7 Effects of maternal obesity and arginine administration on concentrations of amino acids in maternal plasma of gestating ewes

Amino acids	100% NRC-fed ewes	Obese ewes	Obese ewes + arginine	SEM	*P* value
ALA	179[b]	238[a]	259[a]	22	0.08
ARG	193[b]	178[b]	602[a]	26	<0.001
ASN	30	26	25	3	>0.10
ASP	6	7	8	1	>0.10
β-ALA	21[a]	13[b]	21[a]	2	<0.05
CIT	259	162	261	43	>0.10
GLN	242	254	264	20	>0.10
GLU	68[b]	116[a]	93[a,b]	12	<0.05
GLY	768[a]	487[b]	501[b]	70	<0.05
HIS	45	43	45	3	>0.10
ILE	75	94	105	13	>0.10
LEU	86[b]	139[a]	139[a]	17	<0.05
LYS	86[c]	109[b]	149[a]	14	<0.05
MET	27	29	26	3	>0.10
ORN	65[b]	68[b]	324[a]	7	<0.001
PHE	86	102	95	5	>0.10
SER	75[a]	42[b]	47[b]	7	<0.05
TAU	109	137	143	21	>0.10
THR	61[b]	104[a]	130[a]	14	<0.01
TRP	39	32	37	3	>0.10
TYR	78	54	80	15	>0.10
VAL	125[b]	167[a,b]	183[a]	17	0.07

Data (nmol/ml) are mean with SEM. Values within a row sharing different superscripts differ ($P < 0.05$)

Table 8 Effects of maternal obesity and arginine administration on amino acid concentrations in fetal umbilical vein plasma of gestating ewes

Amino acid	100% NRC-fed ewes	Obese ewes	Obese ewes + arginine	SEM	*P* value
ALA	362[b]	460[a]	464[a]	38	>0.10
ARG	197[b]	207[b]	271[a]	18	<0.05
ASN	60	78	61	9	>0.10
ASP	16	14	16	1	>0.10
β-ALA	172	126	108	20	>0.10
CIT	215	191	180	20	>0.10
GLN	453	661	543	74	>0.10
GLU	181[a]	91[b]	113[b]	26	0.07
GLY	607	511	435	85	>0.10
HIS	56	74	58	7	>0.10
ILE	91	104	104	10	>0.10
LEU	158	213	198	24	>0.10
LYS	123	169	129	22	>0.10
MET	35	45	35	5	>0.10
ORN	110[b]	90[b]	167[a]	12	<0.001
PHE	124	148	129	9	>0.10
SER	758	780	784	91	>0.10
TAU	264	214	146	44	>0.10
THR	275[b]	427[a]	456[a]	46	<0.05
TRP	59	63	60	6	>0.10
TYR	86	98	88	9	>0.10
VAL	248[b]	318[a]	338[a]	26	0.06

Data (nmol/ml) are mean with SEM. Values within a row sharing different superscripts differ ($P < 0.05$)

($P < 0.05$) in saline-treated obese ewes versus 100% NRC-fed ewes and were further elevated by arginine administration. Concentrations of valine tended to be higher ($P = 0.07$) in arginine-treated obese ewes as compared to 100% NRC-fed ewes.

Concentrations of glutamate tended to decrease ($P = 0.07$) in fetal umbilical vein plasma of obese versus 100% NRC-fed ewes (Table 8). Concentrations of arginine and ornithine were increased ($P < 0.05$) in fetal umbilical vein plasma of arginine-treated obese ewes as compared to saline-treated obese ewes and 100% NRC-fed ewes. In contrast, concentrations of threonine and alanine were increased ($P < 0.05$) and valine tended to increase ($P = 0.061$) in fetal umbilical vein plasma of saline-treated obese ewes compared to 100% NRC-fed ewes.

Discussion

A number of studies from our laboratory and others have indicated that arginine administration to non-pregnant obese animals (Fu et al. 2005; Jobgen et al. 2009b) and humans (Lucotti et al. 2006) reduces WAT mass. In the present study, we evaluated effects of maternal arginine supplementation on maternal, fetal, and placental

parameters of growth, health, and well being in obese ewes. Results of the present study indicate that a 25-day period of arginine administration during late gestation reduces maternal soft tissue lipid content and this reduction coincided with a reduction in circulating levels of leptin. Maternal obesity did not affect fetal weight on day 125 of gestation yet resulted in a nearly 50% increase in fetal soft tissue lipid content. Arginine treatment had no effect on lipid deposition within the fetal carcass soft tissue. However, fetal peri-renal BAT in obese ewes was markedly increased by 60% in response to maternal arginine administration, as we reported for underfed ewes (Satterfield et al. 2011b). Although the present study does not elucidate the molecular mechanisms responsible for the effect of arginine on enhancing fetal BAT growth in obese ewes, our findings provide a much-needed database for future mechanistic investigation.

In the US, 23.7% of reproductive aged women (18–44 years) are classified as obese (body mass index $\geq$30) (March-of-Dimes 2009). The aim of the present study was to develop an ovine model of maternal obesity that mimicked obesity in pregnant women. To that end, ewes were fed ad libitum to induce obesity prior to conception so that the embryo was exposed to the effects of an obese environment from the time of embryo transfer. Arginine was administered based on our findings that dietary supplementation with arginine decreased WAT mass and improved the metabolic profile in adult obese rats (Fu et al. 2005; Wu and Meininger 2009) and growing swine (Geng et al. 2011; Tan et al. 2009, 2011). The observation that arginine supplementation to pregnant obese sheep reduces maternal soft tissue lipid content and circulating levels of leptin in maternal plasma is in keeping with the anti-lipogenic properties of arginine observed in other mammalian species (Tan et al. 2011; Wu et al. 2009). Whether a reduction in circulating leptin and potentially other adipose-derived cytokines during pregnancy has a beneficial effect on the fetus warrants further investigation. Clearly, earlier and more protracted maternal administration of arginine would likely promote a greater loss of maternal WAT and a more favorable uterine milieu to support fetal growth and development. Given the current state of knowledge about pregnancy, an increase in fetal BAT is not expected to impact fetal metabolism (Symonds et al. 2003). This is because the placenta produces inhibitors of BAT activation, including adenosine (Hayashi et al. 1964) and prostaglandin E2 (Thorburn 1991), which have strong anti-lipolytic actions (Jobgen et al. 2006). Furthermore, BAT activation requires significant oxygenation (Cannon and Nedergaard 2004), which would contradict the metabolic adaptation of having a physiologically hypoxic fetus. Thus, in contrast to postnatal animals (Jobgen et al. 2006; Petrovic et al. 2010), intra-fetal lipolysis in WAT is likely suppressed during gestation despite an increase in fetal BAT.

Previous results from studies involving overnourished ewes indicated that fetal weights between days 65 and 75 of gestation were greater for obese than adequately fed ewes of normal body condition (Osgerby et al. 2003; Zhu et al. 2008). However, there was no difference in birth weights of lambs born to normal versus overfed mature ewes (Wallace et al. 2005; Zhu et al. 2009). Consistent with these observations, maternal obesity increased fetal carcass lipid content by nearly 50% but had no effect on fetal weight on day 125 of gestation. Similarly, Ford et al. (2009) reported that term lambs born to obese ewes had a higher percentage of body fat than offspring from control-fed ewes. In addition, maternal obesity increased the expression of key adipogenic genes in peri-renal and subcutaneous fat depots of late prenatal and early postnatal lambs (Muhlhausler et al. 2007a, b). Furthermore, maternal obesity in sheep resulted in increased concentrations of fetal β-cell insulin content and the percentage of insulin-positive cells per unit area on gestational day 75, providing a potential mechanism for development of the metabolic syndrome in adulthood (Ford et al. 2009). Given that arginine treatment increased peri-renal BAT, a presumably beneficial fat, although carcass lipid content in fetuses from saline and arginine-treated obese ewes did not differ, the ratio of WAT to BAT in the carcass tissue was lowered by arginine treatment.

To our knowledge, this is the first report of the effect of maternal obesity on concentrations of amino acids in the ovine conceptus. Maternal obesity results in a decrease in concentrations of glycine and serine in maternal plasma. Interestingly, obesity also reduces levels of glycine in plasma of non-pregnant adult humans (Felig et al. 1969). The conversion of serine to glycine is catalyzed by the enzyme serine hydroxymethyltransferase that is responsible for the production of one-carbon units to facilitate the conversion of homocysteine to methionine (Appaji Rao et al. 2003), which can then act as a methyl donor to alter DNA methylation (Stover and Schirch 1990). In addition to its role in one-carbon unit metabolism, glycine suppresses production of inflammatory cytokines, such as tumor necrosis factor-α, interleukin 6, interleukin-1β, and interferon-γ (Alarcon-Aguilar et al. 2008; Garcia-Macedo et al. 2008; Takahashi et al. 2008). Thus, a reduction in glycine in obese individuals may contribute to the development of a pro-inflammatory phenotype and the development of the metabolic syndrome and cardiovascular disease.

Maternal obesity resulted in increased concentrations of alanine in both maternal and fetal plasma of obese ewes. Alanine is the major substrate for gluconeogenesis (Wu 2009) and thus elevated levels of alanine in plasma may result from increased synthesis or reduced oxidation of this

amino acid in mothers and fetuses. Interestingly, in humans, excretion of high levels of alanine in urine is associated with high blood pressure (Holmes et al. 2008). Concentrations of threonine and leucine (amino acids that cannot be synthesized by animal cells) were also increased in both maternal and fetal plasma of obese ewes. The increased concentration of threonine in maternal plasma may result from a reduced rate of its catabolism by the mitochondrial threonine dehydrogenase pathway, which may also contribute to reduced synthesis of glycine. Likewise, leucine degradation also involves mitochondrial branched-chain amino acid transaminase and branched-chain α-ketoacid dehydrogenase complex (Li et al. 2011b). Thus, we suggest that mitochondrial metabolism and function are impaired in maternal obesity.

The observation that maternal arginine treatment increased fetal peri-renal BAT has potentially significant implications to both animal agriculture and medicine. In neonates of most species, including humans and sheep, the production of heat to maintain core body temperature is mediated mainly by non-shivering thermogenesis (Satterfield and Wu 2011). During this process, a large amount of heat is generated via the mitochondrial uncoupling protein-1 that uncouples ATP synthesis from substrate oxidation (Asakura 2004). Power (1989) indicated that BAT can generate 150–300 times more heat per gram of tissue than non-BAT tissues. Furthermore, metabolism of BAT for non-shivering thermogenesis is responsible for approximately 50% of the heat generated in newborn lambs although BAT constitutes only 2% of birth weight (Symonds and Lomax 1992). Therefore, a 60% increase in fetal BAT mass has the potential to have significant metabolic effects on postnatal lambs.

In addition to its well-documented role of maintaining core body temperature in neonates, an ability to increase BAT mass during fetal growth may also provide a novel mechanism to combat the development of obesity in adulthood (McKnight et al. 2010). Indeed, fetuses born from obese mothers are at greater risk for the development of obesity and the metabolic syndrome (Armitage et al. 2005; Dunn and Bale 2009; Khan et al. 2005; Samuelsson et al. 2008; Taylor et al. 2005). The importance of BAT as a significant mediator of whole-body metabolism has recently gained acceptance, with the emergence of data conclusively showing active BAT in adult humans (Cypess et al. 2009; Pfannenberg et al. 2010; Saito et al. 2009; Virtanen et al. 2009; Yoneshiro et al. 2011). In an adult human, BAT makes up between 0.05 and 0.1% of the total body weight (Enerback 2010), corresponding to 35–70 g of BAT in a 70-kg man. In addition, the activity of BAT is reduced in overweight and obese individuals, while BAT activity was positively correlated with resting metabolic rate (Cypess et al. 2009; Pfannenberg et al. 2010). These results are supported by the work of Saito and coworkers who showed an inverse correlation between BAT activity and BMI, total amounts of fat, or visceral fat mass (Saito et al. 2009). Thus, increasing the amount of BAT in postnatal mammals is expected to play a key role in reducing whole-body WAT. Consistent with this view, Stock and Rothwell (1983) estimated that as little as 80–100 g of BAT generating heat at half of its maximal capacity could account for approximately 20% of daily energy expenditure in a 70-kg man.

In summary, the present study involving an ovine model of diet-induced maternal obesity revealed that the presence of obesity from the outset of pregnancy resulted in: (1) altered plasma profile of amino acids (particularly leucine, glycine and serine) in both maternal and fetal plasma; and (2) altered maternal and fetal body composition characterized by an increase in lipid accumulation and a decrease in soft tissue protein content. These results advance understanding of the effects of maternal obesity on fetal growth and development and the potential long-term consequences of nutrient excess in utero. In addition, novel results of this study establish that maternal arginine supplementation reduces maternal adiposity and increases fetal BAT growth in obese mothers. Future research is needed to determine whether the increased development of BAT in the fetus by maternal arginine administration is sustained to adulthood; and if so, whether the increase in BAT during development can protect the offspring from the development of obesity even if predisposed to this condition by having an obese mother.

Acknowledgments The authors thank Kendrick LeBlanc for assistance with animal husbandry, Xilong Li, Peng Li, Reza Rezai, and Jason McKnight for technical assistance, and the members of the Laboratory for Uterine Biology and Pregnancy for assistance with tissue collection. This work was supported, in part, by National Institutes of Health 1R21 HD049449 and National Research Initiative Competitive Grants from the Animal Growth & Nutrient Utilization Program (2009-35206-05211) of the USDA National Institute of Food and Agriculture.

Conflict of interest The authors declare that they have no conflict of interest.

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