Effect of maternal undernutrition during late pregnancy on hormonal status and metabolic changes in neonatal lambs

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ABSTRACT: The study investigated the consequences of maternal undernutrition during late pregnancy on hormonal status and metabolic changes in neonatal lambs. Four ewes out of twenty-eight multiparous ewes mated at a synchronized oestrus were slaughtered at day 90 of pregnancy to collect fetal blood to serve as an initial comparison group. Twenty-four animals were divided into three groups and offered 0.18 MJ ME·kg$^{-0.75}$per day (restricted group 1, RG1), 0.33 MJ ME·kg$^{-0.75}$per day (restricted group 2, RG2), and control group (*ad libitum* CG) during late pregnancy, respectively. Immediately after parturition, blood was collected from the neonatal lambs in each group and analyzed for growth hormone (GH), insulin-like growth factor I (IGF-I), IGF-II, insulin (INS), thyroxine (T_4), triiodothyronine (T_3), glucose (GLU), nonesterified fatty acids (NEFA), and total amino acid (TAA), respectively. The results indicated that the maternal undernutrition during late gestation decreased the average lamb birth weight in both RG1 ($P < 0.01$) and RG2 ($P < 0.05$) compared to CG. During the late fetal development period, the concentrations of T4, INS, and IGF-I of neonatal lambs in CG were increased ($P < 0.05$) compared to those at day 90 of pregnancy; the secretions of T_4, INS, and IGF-I in RG1 and RG2 during restriction were suppressed. The neonatal INS concentrations in RG1 and RG2 were decreased ($P < 0.05$), but the neonatal GH concentration in RG1 was greater than that of CG ($P < 0.05$). The GLU concentrations of neonatal lambs in RG1 were lower than those of CG ($P < 0.05$). However, the neonatal NEFA ($P < 0.05$) and TAA ($P < 0.01$) concentrations in RG1 were greater than those of CG. Thus, maternal undernutrition can change the hormonal and metabolic status of neonatal lambs, which may have significant implications on postnatal growth and adult health.

Keywords: intrauterine growth restriction; physiological changes; lambs

Although there is a sufficient nutrients partitioning during gestation in order to ensure adequate placental growth and fetal development (Wallace et al., 2001), maternal undernutrition during late pregnancy can result in intrauterine growth restriction (Robinson et al., 1994), which is associated with an increased incidence of cardiovascular, glucose intolerance, insulin resistance, type 2 diabetes, and other diseases in later life (Barker, 1994). During pregnancy, maternal body should make hormonal adaptive regulations to regulate partitioning of nutrients between the maternal, placental, and fetal compartments directly or indirectly (Robinson et al., 1999; Osgerby et al., 2002). On the other hand, the fetus is also capable of making a number of physiological, neuroendocrine or metabolic adaptations in response to its suboptimal intrauterine environment(Symonds et al., 2001; Fowden and Forhead, 2004; Oliver et al., 2005). It is clear that the fetal adaptations are of critical importance in determining the health and survival of the fetus and newborn (McMillen et al., 2001; Symonds et al., 2001). Furthermore, a series of worldwide epidemiological studies has highlighted the po-

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tential importance of fetal adaptations to a poor intrauterine environment for longer term health outcomes. The specific adaptations that enable the fetus to survive a period of intrauterine deprivation would result in a reprogramming of the developmental pattern of key tissue and organ systems and pathological consequences in adult life (Barker, 1999). However, the mechanism of fetal specific adaptations to maternal undernutrition remains unclear. Thus, the objective of this study was to describe the consequences of maternal undernutrition during late pregnancy on hormonal status and metabolic changes in neonatal lambs.

MATERIAL AND METHODS

Animals

All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals in China (The State Science and Technology Commission of China, 1988). The experimental design and detailed procedures were outlined previously (Gao et al., 2007, 2008, 2009). Briefly, twenty-eight multiparous ewes with body weight of about 43.80 ± 0.60 kg were mated at a synchronized oestrus after treatment with intravaginal progestagen pessaries (each contains 0.3 g progesterone in inert silicone elastomer) and an injection of PMSG (pregnant mare serum gonadotropin) for twelve days. Pregnancies were confirmed by ultrasound scanning (Medison-SA-600, Shanghai, P.R.China) at approximately day 40 of gestation. Based on the fact that the fetus is considered to have achieved 80–85% of its final birth weight during the last two months of pregnancy (Robinson et al., 1999; Symonds et al., 2001), the maternal undernutrition was carried out from day 90 of gestation until parturition, and three maternal treatments were designed during late pregnancy: restricted group 1 (RG1: 0.18 MJ ME·kg$^{-0.75}$ per day), restricted group 2 (RG2: 0.33 MJ ME·kg$^{-0.75}$ per day), and control group (CG: *ad libitum*). Out of 28 ewes at day 90 of pregnancy, four ewes were slaughtered to collect fetal umbilical plasma to serve as an initial comparison group. The 24 animals were allocated to three different groups. All ewes were housed in individual pens, fed chopped natural hay (Table 1), and the animals had free access to water and mineral mixture block. The daily intake of hay offered in RG1 and RG2 was calculated by the ewe body weight, nutrition value of hay, and restricted energy level, and the amount was constant throughout the restricted period (Table 2). Restricted ewes were fed at 8:30 and 16:00 each day. *Ad libitum* fed ewes were fed at 8:30, 11:00, and 16:00 daily (the feed refusals were approximately 10% of the total amount offered). The feed refusals were collected daily and recorded before feeding at 8:30 and sampled for chemical analysis. During restriction, one ewe in each group miscarried and one ewe in RG1 died.

Sampling and assays

Immediately after birth, lamb birth weight was recorded, and five neonatal lambs (at birth) in each group were selected randomly to collect the jugular blood samples (10 ml) before suckle. The blood samples were put into heparinized tubes and centrifuged within 2 h of collection for the analysis of growth hormone (GH), insulin-like growth factor I (IGF-I), IGF-II, insulin (INS), thyroxine (T_4), triiodothyronine (T_3), glucose (GLU), nonesterified fatty acids (NEFA), and total amino acid (TAA). The plasma concentrations of INS, GH, IGF-I, and IGF-II were determined using radioimmunoassay (RIA) kits (Kangyuan Ruide Biological Technology, Beijing, P.R. China). Intra and inter assay coefficients of variation were 4.8 and 9.3%, 8.4 and 13.7%, 9.8 and 14.9%, 4.7 and 8.9%, 3.9 and 8.5%, 8.9 and 12.7% for INS, IGF-I and IGF-II, T_3, T_4, GH, respectively. The plasma concentrations of NEFA (NJJCBIO, Nanjing, P.R. China), TAA (NJJCBIO, Nanjing, P.R. China),

Table 1. Chemical composition and nutritive value of grass fed and residua during restriction period (late pregnancy)

	ME (MJ/kg)	DM (%)	CP (%)	EE (%)	NDF (%)	ADF (%)	Ash (%)	Ca (%)	P (%)
Fed grass	8.79	85.89	8.49	2.59	76.20	49.43	5.23	0.56	0.24
Residual grass	–	82.60	6.45	1.83	81.75	56.03	5.79	0.56	0.24

ME = metabolisable energy, DM = dry matter, CP = crude protein, EE = ether extract, NDF = neutraldetergent fiber, ADF = acid detergent fiber

Table 2. Levels of nutrition during restriction period (late pregnancy) in different groups

Levels of nutrition	CG	RG2	RG1
Mean daily grass intake (g/day)	1332.52	719.33	383.99
Mean daily crude protein intake (g/day)	113.13	61.07	32.60
Daily metabolizable energy intake (MJME·kg$^{-0.75}$/day)	0.61	0.33	0.18

CG = control group (*ad libitum*), RG1 = restricted group 1, RG2 = restricted group 2

and GLU (Zhong Sheng Beikong BIO-Technology, Beijing, P.R. China) were analyzed with the aid of commercial kits by Hitachi Clinical Analyzer 7600-020 (Hitachi, Tokyo, Japan).

Statistical analysis

The CRL, thoracic girth, umbilical girth, GH, IGF-I, IGF-II, INS, T_4, T_3, GLU, NEFA, and TAA were analyzed using the MIXED procedures of SAS (Software Analysis System, Version 8.01, 2001). Fixed effects in the model included treatment, term, and treatment × term interaction. Differences among treatments were analyzed using Tukey's multiple range tests. Data were presented as standard error of the means. Significance was declared at $P < 0.05$ and $P < 0.01$.

RESULTS

Lamb birth weight and measurements

Effects of maternal undernutrition during late pregnancy on fetal weight and other measurements are shown in Table 3. Lamb birth weights in RG1 ($P < 0.01$) and RG2 ($P < 0.05$) were lower than those of CG. From day 90 of gestation to birth, the fetal crown-rump length (CRL), thoracic girth, and umbilical girth for each group were increased significantly ($P < 0.01$). There were significant differences ($P < 0.01$) in neonatal thoracic girth among RG1, RG2, and CG. CRL in RG1 was significantly reduced compared to CG ($P < 0.01$), but there was no significant difference in umbilical girth of the neonatal lambs between CG and nutrient-restricted groups ($P > 0.05$).

Hormonal status

Effects of maternal undernutrition during late pregnancy on fetal and neonatal hormonal status are shown in Figure 1. During the late fetal development period, the plasma concentrations of T_4, INS, and IGF-I of neonatal lambs in CG were increased ($P < 0.05$) compared to those at day 90 of pregnancy; the secretions of T_4, INS, and IGF-I in RG1 and RG2 during restriction were suppressed. The neonatal INS concentrations in RG1 and RG2 were lower than in CG ($P < 0.05$), but the neonatal GH concentration in RG1 was greater than that of CG ($P < 0.05$).

Table 3. Effects of maternal undernutrition on the change of lamb birth weight and measurements in all groups during late pregnancy

Item	Day 90 of gestation	Neonatal lambs (at birth)		
		CG	RG1	RG2
Body weight (kg)	0.48 ± 0.02	3.82 ± 0.24^{a*}	2.55 ± 0.15^{c*}	3.11 ± 0.19^{bc*}
Crown-rump length (cm)	26.25 ± 1.26	51.33 ± 2.31^{a*}	45.75 ± 1.71^{c*}	48.25 ± 2.22^{ac*}
Thoracic girth (cm)	16.95 ± 1.10	38.00 ± 0.00^{a*}	34.25 ± 1.50^{c*}	35.00 ± 1.15^{c*}
Umbilical girth (cm)	18.75 ± 0.50	35.00 ± 1.73^{a*}	32.00 ± 2.94^{a*}	32.25 ± 2.87^{a*}

CG = control group, RG1 = restricted group 1, RG2 = restricted group 2

$^{a–c}$means within a row for neonatal lambs followed by the same letters are different at $P > 0.05$, adjacent letters are different at $P < 0.05$ and by interval superscripts are different at $P < 0.01$

*significant differences are described for the same group between day 90 of gestation and term in the text and indicated by $P < 0.05$

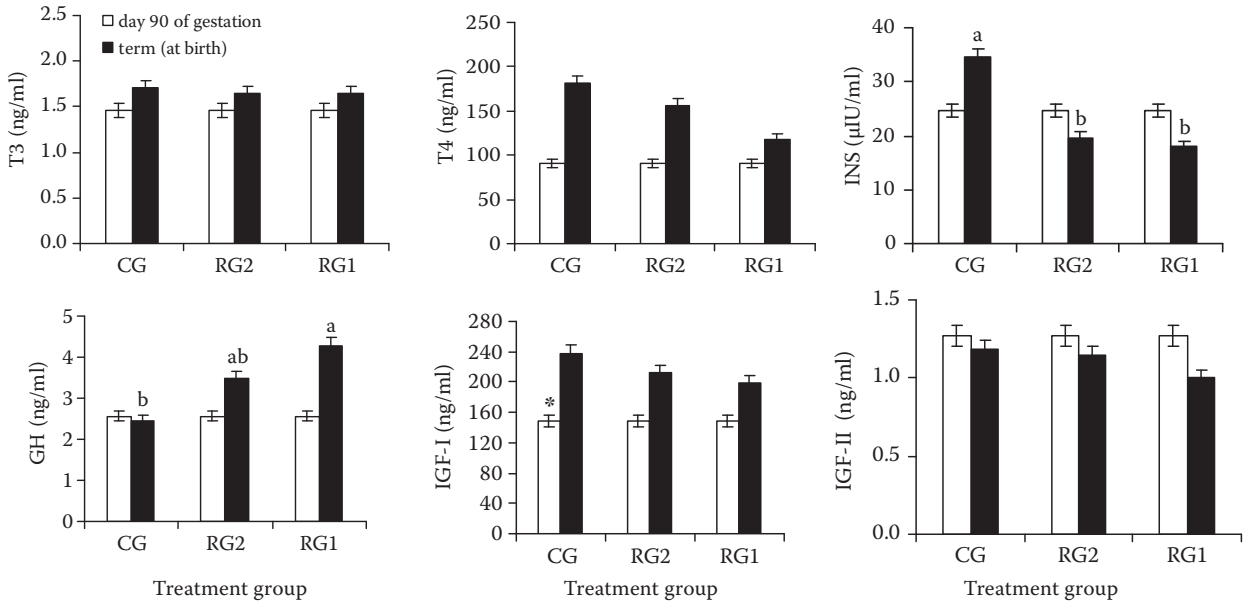

Figure 1. Effect of maternal undernutrition on the concentrations of T3, T4, INS, GH, IGF-I, and IGF-II in neonatal lambs

CG = control group, RG1 = restricted group 1, RG2 = restricted group 2

[a–c]means for neonatal lambs followed by adjacent letters are different at $P < 0.05$ and by interval superscripts are different at $P < 0.01$

*significant differences are described for the same group between at day 90 of gestation and term in the text and indicated by $P < 0.05$

Metabolite status

Effects of maternal undernutrition during late pregnancy on fetal and neonatal metabolite status are shown in Figure 2. The GLU concentrations of neonatal lambs in RG1 and RG2 were lower than those of CG, and the difference was significant between RG1 and CG ($P < 0.05$). However, the neonatal NEFA ($P < 0.05$) and TAA ($P < 0.01$) concentrations in RG1 were greater than those of CG.

DISCUSSION

The present investigations documented that although the fetal body weight, CRL, thoracic girth, and umbilical girth for each group were increased significantly during late pregnancy, the lamb birth weight and thoracic girth in restricted groups were reduced significantly with the decreasing of maternal nutrition level, which is in agreement with the results of Louey et al. (2000) and Bloomfield

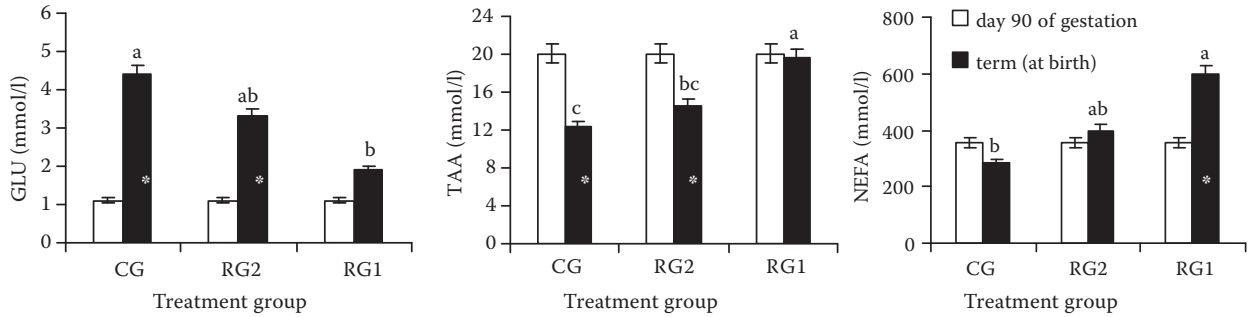

Figure 2. Effect of maternal undernutrition on the concentrations of GLU, TAA, and NEFA in neonatal lambs

CG = control group, RG1 = restricted group 1, RG2 = restricted group 2

[a–c]means for neonatal lambs followed by adjacent letters are different at $P < 0.05$ and by interval superscripts are different at $P < 0.01$

*significant differences are described for the same group between day 90 of gestation and term in the text and indicated by $P < 0.05$

et al. (2003). The fetal growth trajectories in RG1 and RG2 were changed by maternal undernutrition during late pregnancy. During nutrition restriction, adaptive regulations in the maternal endocrine system should ensure uterine nutritional partitioning to maximize maintaining fetal growth (McMillen et al., 2001; Osgerby et al., 2002). Nevertheless, when the nutrition density is lower than a certain level and maternal mobilized reserves could not satisfy the requirements of the body, the protective buffer system of maternal body would be challenged (Gao et al., 2007). As a consequence of exposure to maternal undernutrition challenges, the fetal development and endocrine organ function could be reset (Symonds et al., 2001).

In this study, the plasma concentrations of T_3 and IGF-II in each group were at similar levels, but the secretions of T_4, INS, and IGF-I in RG1 and RG2 were suppressed by maternal undernutrition during late pregnancy. The neonatal INS concentrations in RG1 and RG2 were decreased with the supply of nutrition restricted, but the neonatal GH concentration in RG1 was greater than that of CG, which is consistent with the findings of Bauer et al. (1995), Brameld et al. (2000), and Yuen et al. (2002). As one of the important regulating systems of animals, the endocrinology of metabolic homeostasis in sheep fetuses is well adapted to respond to a range of maternal undernutrition (Symonds et al., 2001). Once fetal substrate partitioning is modified by maternal altered metabolic status, the fetal endocrine system should make adaptive regulations according to the degree of restriction instinctively to mobilize more body reserves for maintaining the basal survival requirement. In the present study, the GLU concentrations of neonatal lambs in RG1 and RG2 were lower, but the neonatal NEFA and TAA concentrations in RG1 were greater than those of CG. Because the energy demands of fetal growth are supplied from 30–40% by glucose, from 55% by amino acid, and from 5–10% by acetic acid during late pregnancy for sheep and cow (Bell, 1993; Fowden and Hill, 2001), the glucose deficiency in RG1 and RG2 might restrict fetal growth partly. Fetal insulin acted as a signal of nutrient plane is positively related to the fetal glucose levels and body weight at birth (Fowden, 1995). The INS deficiency in RG1 and RG2 caused by maternal undernutrition would enhance glucose concentration and improve lipolysis. In addition, the suppression of IGF-I secretion and the increasing of GH concentrations in RG1 and RG2 may improve the lipolytic effect and inhibit the anabolism of protein, which resulted in the greater NEFA and TAA concentrations in RG1 and RG2. During feed restriction, there is a shift in the energy balance due to reduced levels of blood glucose, and at this point, in order to keep normal life activity, the utilization of fatty acids becomes the major source of energy (Brockman and Laarveld, 1986). Fetal glucose and amino acid are spared by mobilizing more adipose tissues, which would more effectively satisfy the fetal nutrition requirements. The mechanisms in fetus are consistent with those of in the maternal system (Gao et al., 2007).

As an adaptation, the alternations of fetal metabolic status regulated by endocrine system are important for maintaining the relative growth and the survival of animals in adversity (Oliver et al., 2005). However, hormones in the fetal circulation play important roles in regulating fetal growth and the development of individual fetal tissues (Fowden and Forhead, 2004). As fetal growth-promoting hormones (Fowden and Forhead, 2004), the deficiency of T_4, INS, and IGF-I in RG1 and RG2 groups would retard the fetal growth and lead to lower lamb birth weight.

In summary, maternal undernutrition during late pregnancy suppressed the secretions of T_4, INS, IGF-I in restricted groups and changed the metabolic status of neonatal lambs. The adaptive regulation of fetal endocrine system had a negative influence on fetal development and resulted in compromised growth, which may have significant implications on postnatal growth and health.

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