Neonatal hormone changes and growth in lambs born to dams receiving differing nutritional intakes and selenium supplementation during gestation

Leticia E Camacho, Allison M Meyer, Tammi L Neville, Carolyn J Hammer, Dale A Redmer, Lawrence P Reynolds, Joel S Caton and Kimberly A Vonnahme

Department of Animal Sciences, Center for Nutrition and Pregnancy, North Dakota State University, Fargo, North Dakota 58108, USA

Correspondence should be addressed to K A Vonnahme; Email: kim.vonnahme@ndsu.edu

A M Meyer is now at Department of Animal Science, University of Wyoming, Laramie, Wyoming

Abstract

To investigate the effects of maternal selenium (Se) supplementation and nutritional intake during gestation on hormone changes, percentage body weight (BW) change, and organ mass in neonatal lambs, ewes were allocated to differing Se levels (adequate Se (ASe, 11.5 µg/kg BW) or high Se (HSe, 77.0 µg/kg BW)) initiated at breeding and nutritional intake (60% (RES), 100% (CON), or 140% (HIGH) of NRC requirements) initiated at day 40 of gestation. At parturition, all lambs were removed from dams, fed common diets, and BW and blood samples were collected until day 19. There was a Se × nutritional intake × day interaction for percentage BW change from birth. Lambs born to ASe-HIGH ewes tended to have decreased BW change compared with ASe-CON and ASe-RES groups on day 7. Lambs from HSe-HIGH ewes tended to have increased BW change compared with HSe-RES and HSe-CON groups from days 7 to 19. At birth, there was a Se × sex of offspring interaction, in which male lambs from HSe ewes had decreased cortisol concentrations compared with all other lambs. By 24 h, lambs from RES ewes had decreased cortisol compared with those from HIGH ewes, with lambs from CON ewes being intermediate. Lambs from RES- and CON-fed ewes had greater thyroxine than HIGH ewes at 24 h. Organ masses on day 19 were mainly impacted by maternal nutritional intake and sex of the offspring. Birth weight alone did not predict growth performance during neonatal life. Moreover, despite a similar postnatal diet, maternal nutritional plane and Se status did impact neonatal endocrine profiles. Exact mechanisms of how neonatal endocrine status can influence later growth and development need to be determined.

Reproduction (2012) **144** 23–35

Introduction

Maternal nutrition during pregnancy plays an important role in supporting adequate fetal and placental growth and development. Intrauterine growth restriction (IUGR) is associated with altered fetal organ development and subsequent performance of offspring (Wu *et al.* 2006, Neville *et al.* 2010, Reynolds *et al.* 2010, Vonnahme *et al.* 2010). Our laboratory has reported that maternal nutrient restriction during mid-to-late gestation in ewes decreased fetal body weight (BW; Reed *et al.* 2007) as well as birth weight (Neville *et al.* 2010). However, ewes given supranutritional selenium (Se) levels had greater fetal BW compared with ewes receiving adequate Se (ASe) levels (Reed *et al.* 2007).

IUGR is associated with accelerated growth and increased adiposity in the early neonatal period; although the mechanisms are unknown, they could include altered secretion or metabolism of thyroid

hormones (DeBlasio *et al.* 2006). DeBlasio *et al.* (2006) suggested that placental restriction and small size at birth may increase activation of thyroxine (T_4) to triiodothyronine (T_3) and sensitivity of soft tissue to thyroid hormones, which may contribute to the accelerated neonatal growth following IUGR. Moreover, T_4 is positively correlated with size at birth (DeBlasio *et al.* 2006). Additionally, Mellor & Pearson (1977) demonstrated a positive correlation between lamb BW and concentrations of T_4 at birth, which could be reflected by the effects of T_4 on fetal growth.

Glucocorticoids have major effects on the differentiation, morphology, and function of numerous tissues including the lungs, liver, kidneys, muscle, fat, and gastrointestinal tract (Challis *et al.* 2000, Fowden & Forhead 2004). Glucocorticoids may also be essential for survival postnatally, as they induce maturation of several organs, including fetal lungs, brain, and gastrointestinal tract (Challis *et al.* 2000, Fowden & Forhead 2004).

However, the combined effects of dietary Se and nutritional intake on neonatal cortisol, T_3 , and T_4 are not well defined. We hypothesized that supplementation with Se during gestation would offset the negative effects of limited fetal nutrient availability and therefore enhance growth and development of the offspring during neonatal life. Therefore, our objectives were to determine the effects of maternal dietary Se supplementation and plane of nutrition during gestation on neonatal growth, organ mass, and serum concentrations of cortisol, T_3 , and T_4 .

Results

The impacts of maternal diet on ewe growth throughout gestation, as well as gestation length, lamb BW at birth, size, average daily gain (ADG), and offspring adiposity has been previously reported by our laboratory (Meyer *et al.* 2010).

Percentage weight change

Our laboratory has previously published (Meyer *et al.* 2010) that in ASe ewes, lambs from RES ewes were lighter than CON and HIGH; however, within the high Se (HSe) ewes, nutritional plane did not impact birth weight. Moreover, lambs from HSe-RES ewes were heavier than lambs from ASe-RES ewes (Meyer *et al.* 2010). Although male lambs were heavier ($P=0.02$) at birth compared with female lambs (4.73 ± 0.14 vs 4.31 ± 0.10 kg), there was no effect of sex of offspring, or its interaction with maternal Se supplementation, maternal nutritional intake, or day ($P \geq 0.35$) on percentage weight change from birth to day 19. However, there was a Se $\times$ nutritional intake $\times$ day interaction ($P=0.0002$; Fig. 1). Percentage weight change from birth to days 1 and 3 was similar within each ewe dietary treatment ($P \geq 0.18$). Thereafter, the percentage weight change from birth in all lambs increased until day 19 ($P \leq 0.02$), with the exception of

Figure 1 A maternal Se supplementation $\times$ maternal nutritional intake $\times$ day of age interaction (A) from birth to 19 days of age. Maternal Se supplementation treatments were 11.5 μ g Se/kg of body weight (adequate Se; ASe) or 77.0 μ g Se/kg of body weight (high Se (HSe)) throughout gestation. Maternal nutritional intake treatments were RES (60% of control), CON (control; 100% requirements for gestating ewe lambs), and HIGH (140% of control) fed from day 40 to term. Lambs were raised similarly after birth. Mean separations within a comparison are shown: (B) impacts of maternal nutritional plane within the ASe treatment; (C) impacts of maternal nutritional plane within the HSe treatment; (D) impacts of maternal Se status within the RES treatment; (E) impacts of Se status within the CON treatment; and (F) impacts of Se status within the HIGH treatment. *Within a comparison, percentage change in lamb weight differed.

ASe-HIGH, in which percentage weight change was similar ($P>0.16$) from birth to day 7 and then increased ($P<0.0001$) at days 14 and 19. Lambs born to ASe-HIGH ewes had decreased ($P=0.03$; Fig. 1B) percentage weight change on day 7 compared with ASe-CON and ASe-RES groups, which did not differ ($P=0.98$). In contrast, lambs from HSe-HIGH ewes had increased ($P=0.01$; Fig. 1C) percentage weight change by day 7 compared with HSe-RES, which did not differ ($P=0.35$) from HSe-CON. Moreover, lambs from ASe-HIGH ewes had a decrease ($P=0.001$) in percentage weight change on day 7 compared with those from HSe-HIGH ewes (Fig. 1F). Percentage weight change by day 7 was similar between lambs from ASe-RES and HSe-CON ewes ($P=0.16$; Fig. 1D) and ASe-CON and HSe-CON ewes ($P=0.62$; Fig. 1E).

Although lambs from ASe ewes were not different among nutritional intake groups ($P\geq 0.13$) by day 14, lambs from HSe-CON and HSe-HIGH ewes had an increase ($P\leq 0.02$) in percentage weight change compared with those from HSe-RES ewes (Fig. 1C). Furthermore, by day 14, lambs born to ASe-RES ewes had an increase ($P=0.003$) in percentage weight change compared with those from HSe-RES (Fig. 1D), whereas lambs from ASe-CON were similar ($P=0.72$) to those from HSe-CON (Fig. 1E), and lambs from ASe-HIGH ewes had a decrease ($P=0.01$) in percentage weight change compared with those from HSe-HIGH (Fig. 1F).

By day 19, lambs from ASe-HIGH ewes tended to have a reduced ($P=0.06$) percentage weight change compared with those from ASe-RES, with those from ASe-CON ewes being intermediate (Fig. 1B). Moreover, as maternal nutritional intake increased in lambs from HSe ewes, the percentage weight change in the lambs also increased ($P\leq 0.01$; Fig. 1C). Furthermore, by day 19, lambs born to ASe-RES ewes had an increase ($P=0.0004$) in percentage weight change compared with those from HSe-RES ewes (Fig. 1D), whereas lambs from the ASe-CON group were similar ($P=0.74$) to those from the HSe-CON group (Fig. 1E), and lambs from ASe-HIGH ewes had a decrease ($P=0.0005$) in percentage weight change compared with HSe-HIGH (Fig. 1F).

Neonatal hormone profiles

No maternal Se $\times$ maternal nutritional intake $\times$ offspring sex interactions were observed ($P\geq 0.23$) for cortisol, T_4 , or T_3 at birth or 24 h after birth. Moreover, there were no main effects ($P\geq 0.09$) of maternal Se, maternal nutritional intake, or sex of offspring on T_4 concentrations at birth, which averaged 115.1 ± 3.6 ng/ml. There were, however, main effects of maternal Se supplementation on cortisol concentrations ($P=0.01$; Fig. 2A), maternal nutritional intake on T_3 concentrations ($P=0.02$; Fig. 2B), and sex of offspring on T_3 concentrations ($P=0.02$; Fig. 2C) at birth. Offspring from HSe ewes had decreased cortisol at birth ($P=0.01$) compared with

Figure 2 Circulating concentrations of cortisol (A) and T_3 (B and C) in blood collected immediately at birth in lambs born from ewes fed: 11.5 μ g Se/kg of body weight (adequate Se (ASe)) or 77.0 μ g Se/kg of body weight (high Se (HSe)) throughout gestation (A); maternal nutritional intake treatments were RES (60% of control), CON (control; 100% requirements for gestating ewe lambs), and HIGH (140% of control) fed from day 40 to term (B). Lamb sex influenced T_3 concentrations (C). ^{ab}LSMean $\pm$ S.E.M. within a panel differs; $P<0.05$.

lambs born from ASe ewes (Fig. 2A). Ewes in the RES intake group gave birth to lambs with lower ($P \leq 0.05$) T_3 concentrations compared with lambs from the CON- and HIGH-fed ewes, which did not differ ($P = 0.12$; Fig. 2B). Male lambs had increased ($P = 0.02$) T_3 concentrations at birth compared with female lambs.

By 24 h after birth, maternal Se supplementation, maternal nutritional intake, and sex of the offspring did not ($P \geq 0.19$) impact T_3 concentrations, which averaged 3.99 ± 0.13 ng/ml. There was a main effect ($P \leq 0.04$) of maternal nutritional intake on T_4 and cortisol, with lambs from RES-fed ewes having increased ($P = 0.04$) circulating T_4 but decreased ($P = 0.01$) cortisol concentrations compared with lambs from HIGH-fed ewes (Fig. 3A and B). Moreover, T_4 concentrations were also higher ($P = 0.01$) in lambs from CON-fed ewes than lambs from HIGH-fed ewes (Fig. 3A). Male lambs had less ($P = 0.01$) circulating cortisol concentrations than female offspring at 24 h after birth (Fig. 3C).

Concentrations of circulating T_3 , T_4 , and cortisol from days 3 to 19 were not affected ($P \geq 0.11$) by maternal Se supplementation, maternal nutritional intake, offspring sex, or their interactions. There were offspring sex $\times$ day interactions for T_3 ($P = 0.02$; Fig. 4A) and T_4 concentrations ($P = 0.005$; Fig. 4B) and a tendency for an offspring sex $\times$ day interaction for cortisol ($P = 0.08$; Fig. 4C) concentration. Concentrations of T_3 were higher ($P = 0.02$) on day 3 and decreased more from days 3 to 7 in male lambs compared with female lambs (Fig. 4A). Thereafter, concentrations of T_3 were similar ($P > 0.23$) through day 19 in both sexes. Concentrations of T_4 were similar ($P = 0.61$) on day 3 between male and female lambs, but male lambs had a tendency ($P = 0.09$) for a greater decline of T_4 concentration by day 7 compared with female lambs (Fig. 4B). Thereafter, concentrations of T_4 were similar ($P > 0.17$) between male and female lambs. Cortisol concentrations were increased ($P = 0.03$) in female compared with male lambs on day 3. By day 7, cortisol concentrations were similar ($P = 0.76$) and remained similar ($P > 0.34$) through the end of the study (Fig. 4C).

Organ mass data

We have previously reported (Meyer *et al.* 2010) that lamb BW on day 19 was less ($P \leq 0.01$) in lambs from RES compared with lambs from CON and HIGH ewes (Table 1). Moreover, male lambs remained heavier ($P = 0.03$) than female lambs at day 19 (Table 2). There were no main effects of Se ($P \geq 0.17$, data not shown) for any organ mass data collected; further, there were no maternal Se supplementation $\times$ maternal nutritional intake $\times$ offspring sex interactions ($P > 0.15$). Empty BW (EBW) was reduced ($P < 0.01$) in lambs from RES ewes compared with lambs from CON and HIGH ewes that did not differ ($P = 0.83$; Table 1). Moreover, male lambs had a greater ($P = 0.03$) EBW compared with female

Figure 3 Circulating concentrations of T_4 (A) and cortisol (B and C) in blood collected 24 h after birth in lambs born from ewes assigned to differing maternal nutritional intake treatments: RES (60% of control), CON (control; 100% requirements for gestating ewe lambs), and HIGH (140% of control) fed from day 40 to term (A and B). Lamb sex influenced cortisol concentrations (C). ^{ab}LSMean $\pm$ S.E.M. within a panel differs; $P < 0.05$.

Figure 4 An offspring sex $\times$ day of age interaction for T_3 (A), cortisol (B), and T_4 (C) determined on days 3, 7, 14, and 19. *Within a measure and day, concentrations differ; $P < 0.05$.

lambs (Table 2). Whereas brain weight was not affected ($P=0.78$) by maternal nutritional treatment, female lambs had decreased ($P=0.02$) brain weight compared with male lambs. When brain weight was divided by EBW, lambs from RES ewes had a greater ($P < 0.01$) brain

to EBW ratio compared with CON and HIGH ewes, which did not differ ($P=0.87$). Male and female lambs did not differ ($P=0.36$) in brain to EBW ratio. There was a main effect ($P \leq 0.03$; Table 1) of maternal nutritional intake on blood weight (g and g/g brain weight), where lambs born to ewes fed RES diets had less ($P \leq 0.03$) blood mass than lambs from CON and HIGH-fed ewes. When expressed as blood weight to EBW ratio, there was a maternal Se $\times$ nutritional intake interaction ($P=0.03$), with lambs from ASe-RES and ASe-HIGH ewes having a greater ($P < 0.05$) ratio compared with HSe-RES and HSe-HIGH respectively. Moreover, blood weight (g) was greater ($P=0.02$; Table 2) and blood weight proportional to brain weight tended ($P=0.08$) to be greater in male lambs compared with female lambs. There tended to be an interaction of maternal Se $\times$ nutritional intake on heart weight (g), in which lambs from HSe-RES had lighter ($P \leq 0.005$; Table 3) hearts than lambs from HSe-CON and HSe-HIGH. When heart weight was expressed proportionally to brain weight, a main effect of maternal nutritional intake ($P < 0.01$) was observed, in which lambs from RES-fed ewes had lighter hearts compared with lambs from CON- and HIGH-fed ewes. Females had lighter ($P=0.005$; Table 2) hearts (g) than males and tended ($P=0.10$) to have decreased heart weight as a proportion of brain weight. Maternal nutrition did not affect ($P \geq 0.20$; Table 1) neonatal lung mass (g and g/g brain weight). There was a tendency ($P=0.08$) for lambs from HIGH ewes to have a reduced ($P=0.03$) lung to EBW ratio compared with lambs from RES ewes. However, male lambs had heavier ($P \leq 0.01$; Table 2) lungs (g and g/g brain weight) than female lambs.

Maternal nutritional intake affected ($P \leq 0.03$; Table 1) total gastrointestinal, liver, and spleen masses (g and g/g brain weight), in that weights were less ($P \leq 0.04$) for lambs born to RES ewes compared with CON- and HIGH-fed ewes. There was a maternal Se $\times$ nutritional intake interaction ($P=0.02$; Table 3) for liver to EBW ratio. Within the lambs from the ASe-fed ewes, RES had a greater ($P \leq 0.05$) liver to EBW ratio compared with CON and HIGH, which did not differ ($P=0.67$). In lambs from HSe-fed ewes, CON had an increased ($P \leq 0.04$) liver to EBW ratio compared with RES and HIGH, which did not differ ($P=0.98$). Lambs from ASe-RES ewes had an increased ($P=0.04$) liver to EBW ratio compared with lambs from HSe-RES ewes, while lambs from ASe-CON ewes had a reduced ($P=0.05$) liver to EBW ratio compared with lambs from HSe-CON (Table 3). There was a tendency ($P \leq 0.09$; Table 2) for females to have a lighter gastrointestinal mass (g) and spleen weight (g/g brain weight) than males. Gastrointestinal mass as a proportion of brain weight was not affected by sex of offspring ($P=0.36$). Liver (g and g/g brain weight) and spleen weights (g) were lighter ($P \leq 0.03$; Table 2) in female lambs compared with male lambs. There was a maternal Se $\times$ offspring sex interaction ($P < 0.01$) for liver to EBW ratio (Table 4). While there was no difference

Table 1 Impacts of maternal nutrition on organ mass weight (g, g/g brain weight, and g/g EBW) in 19 day-old lambs.

Item	Nutrition treatments ^a			S.E.M.	P value ^b
	RES (n=23)	CON (n=27)	HIGH (n=26)		
Live wt (kg)	9.7*	11.3 [†]	11.6 [†]	0.4	<0.01
EBW (kg)	8.7*	10.2 [†]	10.3 [†]	0.4	<0.01
Brain (g)	71.9	70.5	71.2	1.4	0.78
g/g EBW	8.52*	7.19 [†]	7.11 [†]	0.34	<0.01
Blood (g)	538.9*	632.7 [†]	640.8 [†]	30.9	0.03
g/g Brain	7.5*	9.0 [†]	9.0 [†]	0.4	0.01
g/g EBW ^A	61.49	61.94	60.35	1.50	–
Heart (g) ^A	62.0	71.8	70.4	2.4	–
g/g Brain	0.87*	1.02 [†]	0.99 [†]	0.03	<0.01
g/g EBW	7.23	7.04	6.86	0.15	0.20
Lung (g)	197.7	213.2	191.5	11.2	0.35
g/g Brain	2.75	3.05	2.68	0.16	0.20
g/g EBW	23.18*	21.56* [†]	18.61 [†]	1.51	0.08
Total gastrointestinal ^c (g)	498.6*	560.4 [†]	572.2 [†]	21.7	0.03
g/g Brain	6.95*	8.02 [†]	8.04 [†]	0.30	0.01
g/g EBW	57.96	55.54	55.50	1.31	0.29
Liver (g)	272.6*	322.6 [†]	313.7 [†]	13.0	0.02
g/g Brain	3.80*	4.61 [†]	4.40 [†]	0.19	<0.01
g/g EBW ^A	31.46	31.64	29.93	0.70	–
Spleen (g)	26.6*	33.4 [†]	31.0 [†]	1.4	<0.01
g/g Brain	0.37*	0.48 [†]	0.44 [†]	0.02	<0.01
g/g EBW	3.06	3.30	3.01	0.11	0.14
Pancreas ^A	13.0	14.3	13.7	0.05	–
g/g Brain ^A	0.183	0.205	0.192	0.008	–
g/g EBW	1.56	1.44	1.34	0.08	0.14
Kidney (g) ^A	59.8	64.1	69.5	2.8	–
g/g Brain ^A	0.83	0.91	0.98	0.04	–
g/g EBW ^A	6.89	6.31	6.66	0.15	–
Adrenal (g)	1.04*	1.17 [†]	1.10* [†]	0.04	0.05
mg/g Brain ^A	14.7	16.7	15.5	0.05	–
g/g EBW	0.12	0.12	0.11	0.005	0.15
Visceral adiposity ^d (g)	220.0*	283.3 [†]	310.4 [†]	22.0	0.01
g/g Brain	3.05*	4.04 [†]	4.38 [†]	0.30	<0.01
g/g EBW ^A	24.57	26.98	28.99	1.49	–
Thyroid (g)	1.08	1.20	1.13	0.07	0.42
mg/g Brain	15.0	17.2	15.9	1.0	0.27
g/g EBW	0.13	0.12	0.11	0.007	0.23
Testes (g)	3.76	4.66	4.61	0.43	0.18
mg/g Brain	50.4*	67.2 [†]	62.3 [†]	6.0	0.10
mg/g EBW	0.41	0.43	0.42	0.03	0.89
Ovaries (g)	0.82	1.09	1.06	0.25	0.69
mg/g Brain	11.5	16.0	14.9	3.7	0.62
mg/g EBW	0.10	0.12	0.10	0.03	0.75
Uterus (g)	2.56	2.98	2.90	0.19	0.24
mg/g Brain	36.6	42.9	42.2	2.8	0.19
mg/g EBW	0.31	0.32	0.30	0.02	0.51

*^aLSMean $\pm$ S.E.M. within a measure differs; $P < 0.05$. EBW, empty body weight = live weight minus digesta weight.

^aMaternal nutritional treatments were RES (fed at 60% of CON), CON (control; 100% requirements for gestating ewe lambs), and HIGH (fed at 140% of CON). ^bThere was no effect of maternal Se supplementation ($P \geq 0.26$) or maternal Se by maternal nutritional intake ($P \geq 0.11$), maternal nutritional intake $\times$ offspring sex ($P > 0.14$), or maternal Se $\times$ maternal nutritional intake $\times$ offspring sex ($P > 0.15$) interactions for the measures presented in the table, except for ^Ablood wt (g/g EBW), heart wt (g), kidney wt (g, g/g brain, and g/g EBW), pancreas wt (g and g/g brain), liver wt (g/g EBW), visceral adiposity (g/g EBW), and adrenal wt (mg/g brain); see Tables 3, 4 and 5. ^cTotal gastrointestinal mass was calculated by summing the empty stomach, small and large intestinal masses. ^dVisceral adiposity was calculated by summing the omental, mesenteric, and perirenal fat masses.

($P = 0.61$) between males and females in ASe-fed ewes, males from HSe ewes had a greater ($P < 0.01$) liver to EBW ratio compared with females from HSe ewes. Moreover, females from ASe ewes had a greater ($P = 0.03$) liver to EBW ratio compared with females from HSe ewes. Pancreatic mass (g and g/g brain) tended ($P \leq 0.09$; Table 3) to be affected by maternal Se and was altered ($P \leq 0.03$; Table 5) due to maternal nutritional intake and offspring sex. There also was a tendency for

maternal Se $\times$ maternal nutritional intake interactions for pancreatic mass (g; Table 3), which was less ($P = 0.03$) in HSe-HIGH compared with HSe-CON and when expressed proportionally to brain weight was less in HSe-RES and HSe-HIGH lambs ($P \leq 0.04$) compared with HSe-CON offspring. The maternal nutritional intake $\times$ offspring sex interaction (Table 5) resulted in females having less ($P \leq 0.003$) pancreatic mass (g and g/g brain) compared with males within the CON-fed

Table 2 Impacts of offspring sex on organ mass (g, g/g brain weight, and g/g EBW) in 19 day-old lambs.

Item	Sex		S.E.M.	P value ^a
	Female (n=47)	Male (n=29)		
Live wt (kg)	10.3	11.4	0.4	0.03
EBW (kg)	9.2	10.2	0.3	0.03
Brain wt (g)	69.3	73.0	1.2	0.02
g/g EBW	7.78	7.43	0.3	0.36
Blood wt (g)	562.4	645.8	27.3	0.02
g/g Brain	8.09	8.92	0.37	0.08
g/g EBW	60.17	62.35	1.3	0.19
Heart wt (g)	64.3	71.9	2.1	< 0.01
g/g Brain	0.93	0.99	0.03	0.10
g/g EBW	6.99	7.09	0.13	0.55
Lung wt (g)	180.2	221.4	9.8	< 0.01
g/g Brain	2.61	3.04	0.14	0.01
g/g EBW	20.07	22.16	1.33	0.22
Total gastrointestinal wt (g)	520.2	567.4	19.0	0.06
g/g Brain	7.51	7.83	0.27	0.36
g/g EBW	56.68	55.99	1.16	0.64
Liver (g)	280.0	325.9	11.5	< 0.01
g/g Brain	4.04	4.50	0.16	0.03
g/g EBW ^A	30.24	31.78	0.61	–
Spleen wt (g)	28.4	32.3	1.2	0.01
g/g Brain	0.41	0.45	0.02	0.09
g/g EBW	3.07	3.18	0.10	0.43
Pancreas ^A	12.8	14.6	0.4	–
g/g Brain ^A	0.185	0.202	0.007	–
g/g EBW	1.43	1.46	0.07	0.72
Kidney	61.0	67.9	2.4	0.03
g/g Brain	0.88	0.94	0.03	0.17
g/g EBW	6.59	6.64	0.13	0.76
Adrenal	1.08	1.13	0.03	0.21
mg/g Brain ^A	15.6	15.6	0.4	–
g/g EBW	0.12	0.11	0.004	0.33
Visceral adiposity	276.4	266.0	19.4	0.67
g/g Brain	3.98	3.67	0.27	0.37
g/g EBW	28.68	25.02	1.32	0.03
Thyroid	1.12	1.15	0.06	0.76
mg/g Brain	16.2	15.9	0.9	0.80
g/g EBW	0.12	0.11	0.006	0.31

EBW, empty body weight=live weight minus digesta weight

^aThere was no effect of maternal Se by offspring sex ($P \geq 0.17$), maternal nutritional intake by offspring sex ($P > 0.14$), or maternal Se $\times$ maternal nutritional intake $\times$ offspring sex ($P > 0.15$) interactions for the measures presented in the table, except for ^Apancreas wt (g and g/g brain) and adrenal wt (mg/g brain), see Table 5; and liver (g/g EBW), see Table 4.

ewes. Pancreatic mass (g) within the HIGH-fed ewes was less ($P=0.01$) in females than in males. Male offspring from RES-fed ewes had decreased ($P \leq 0.04$) pancreatic mass (g) compared with those from CON- and HIGH-fed ewes; however, when expressed as a proportion of brain weight, male offspring from RES-fed ewes were less than ($P=0.002$) those from CON-fed ewes.

Maternal Se and maternal nutritional intake tended ($P=0.07$; Table 3) to affect kidney weight (g) and its proportion to brain weight ($P=0.05$) and EBW ($P=0.03$). Within HSe-fed ewes, kidney weight (g and g/g brain weight) was least ($P \leq 0.03$) in lambs from RES compared with those from CON- and HIGH-fed ewes. When expressed as kidney/EBW, there was no impact

($P > 0.35$) of maternal intake in HSe ewes; however, lambs from ASe-RES ewes had an increased ($P < 0.02$) ratio compared with ASe-CON and ASe-HIGH, which did not differ ($P=0.14$). Moreover, lambs from ASe-RES ewes had an increased ($P=0.05$) kidney to EBW ratio compared with lambs from HSe-RES ewes. Kidney weight (g) was less ($P=0.03$; Table 2) in females compared with males; however, when expressed proportionally to brain weight, it did not differ between the sexes ($P=0.17$). There was a main effect of maternal nutritional intake ($P=0.05$; Table 1) on absolute adrenal gland mass (g), in which adrenal glands from CON lambs were heavier ($P=0.02$) than those from RES lambs, and those from HIGH lambs were intermediate. Adrenal gland mass (g) was not affected by offspring sex ($P=0.21$; Table 2). For adrenal gland weight expressed per unit of brain weight (mg/g), a tendency for a maternal nutritional intake $\times$ offspring sex interaction ($P=0.09$; Table 5) was detected. Although female adrenal gland mass (mg/g brain weight) did not differ ($P > 0.29$) due to maternal nutritional intake, male lambs from CON ewes had heavier adrenals ($P \leq 0.02$) than did lambs from RES and HIGH ewes. Visceral adiposity (g and g/g brain weight) was less ($P \leq 0.04$) in offspring from RES compared with offspring from CON- and HIGH-fed ewes and was not altered ($P > 0.36$) due to offspring sex. When visceral adiposity was expressed per EBW, there tended to be a maternal Se $\times$ nutritional intake interaction ($P=0.08$; Table 3). While maternal intake within ASe ewes did not influence ($P > 0.15$) lambs' adiposity, within the HSe ewes, RES had less ($P < 0.04$) adiposity to EBW ratio compared with CON and HIGH, which did not differ ($P=0.47$). Furthermore, female lambs had a greater ($P=0.03$) visceral adiposity to EBW ratio compared with male lambs (Table 2).

There was no effect ($P > 0.23$; Tables 1 and 2) of maternal nutritional intake or offspring sex on thyroid weight (g, mg/g brain weight and mg/g EBW). Maternal nutritional intake did not affect ($P=0.18$; Table 1) testes weight (g); however, there was a tendency ($P=0.10$) for maternal nutritional intake to affect testes weight, with testes weight (g/g brain weight) of males from RES-fed ewes being lesser ($P \leq 0.10$) than males from CON- and HIGH-fed ewes. There were no effects ($P > 0.18$; Table 1) of maternal nutrition on ovarian weight (g, mg/g brain weight and mg/g EBW) or uterine weight (g, mg/g brain weight and mg/g EBW).

Discussion

Previous work from our laboratory has demonstrated that maternal Se supplementation much above the requirements may help to offset some of the negative impacts of maternal dietary restriction, which in our model leads to IUGR (Reed *et al.* 2007, Meyer *et al.* 2010). In this study, our focus was how neonatal growth, T_3 , T_4 , and cortisol concentrations would be impacted by maternal diet, as

Table 3 Impacts of maternal Se supplementation^a and maternal nutritional intake on organ mass weight (g, g/g brain weight and g/g EBW) in 19 day-old lambs.

Item	ASe			HSe			S.E.M.	Se×Nut P value
	RES (n=13)	CON (n=14)	HIGH (n=13)	RES (n=10)	CON (n=13)	HIGH (n=13)		
Blood wt (g)	583.4	597.0	654.7	494.4	668.3	626.9	45.7	0.15
g/g EBW	64.49*	59.77*,†	61.78*	58.50†	64.11*,†	58.93†	2.27	0.03
Heart wt (g)	65.0*,†	68.0*,†,‡	68.4†,‡	59.1*	75.5†	72.5†,‡	3.5	0.10
g/g Brain	0.90	0.97	0.95	0.84	1.08	1.04	0.05	0.11
Pancreas	12.9*	13.5*,†	14.5*,†	13.2*,†	15.1†	12.9*	0.7	0.06
g/g Brain	0.179*	0.192*,†	0.201*,†	0.187*	0.219†	0.184*	0.011	0.09
Liver (g)	292.6	305.8	309.4	252.5	339.5	318.0	19.3	0.11
g/g EBW	32.89*	30.37†	29.81†	30.03†	32.91*	30.05†	1.01	0.02
Visceral adi- posity (g)	244.6	263.4	290.1	195.4	303.2	330.7	32.4	0.22
g/g EBW	26.64*,†	25.08*,†	27.06*,†	22.49*	28.88†,‡	30.92†	2.19	0.08
Kidney (g)	64.1*,†,‡	60.3*,†	67.0†,‡	55.5*	67.9†,‡	72.0†	4.1	0.07
g/g Brain	0.88*,†	0.86*,†	0.92†,‡	0.78*	0.97†,‡	1.03†	0.05	0.05
g/g EBW	7.16*	6.08†	6.51†,‡	6.61†	6.55†,‡	6.81*,‡	0.21	0.03

*,†,‡LSMean±S.E.M. within a measure differs; $P<0.05$. EBW, empty body weight=live weight minus digesta weight.

^aMaternal Se supplementation treatments were 11.5 µg Se/kg of body weight (adequate Se; ASe) or 77.0 µg Se/kg of body weight (high Se (HSe)) throughout gestation. Maternal nutritional intake treatments were RES (60% of control), CON (control; 100% requirements for gestating ewe lambs), and HIGH (140% of control) fed from day 40 to term. There was no effect of maternal Se supplementation ($P\geq 0.24$) and no maternal Se×maternal nutritional intake×offspring sex interactions were significant ($P>0.15$) for blood, heart, kidney, and pancreas measurements. There was a maternal Se supplementation×offspring sex interaction for liver (g/g EBW) as depicted in Table 4.

well as how neonatal organ mass may have been influenced by their uterine environment. In the current study, lambs were removed immediately from their dams in order to more closely assess the impacts of the maternal environment during gestation. We have previously reported that maternal diet (both nutritional intake and Se supplementation) can impact colostrum/milk components as well as colostrum/milk volumes (Swanson *et al.* 2008, Meyer *et al.* 2011). Maternal Se supplementation throughout gestation did impact the growth patterns of offspring born from dams fed differing levels of nutrition from mid-to-late gestation. Two interesting responses observed on percentage weight change were the decreased weight change in lambs born to ASe-HIGH-fed ewes and the increased percentage weight change in lambs born to HSe-HIGH-fed ewes. Fry *et al.* (2010) demonstrated that jejunal cell proliferation was greater in lambs born to ASe-HIGH-fed ewes than HSe-HIGH-fed ewes. However, no differences in jejunal morphology suggested increased cell turnover and thus increased nutrient usage by ASe-HIGH. This may have

diverted nutrients from whole-body growth contributing to decreased percentage weight change in lambs from this treatment.

Se supplementation did not appear to impact the growth of offspring in this study if the dams were fed adequately throughout gestation. It is interesting to think that supranutritional Se may be protective when nutritional intake is not ideal (Meyer *et al.* 2010). When ewes were supplemented with HSe, there was also a reduction in lamb cortisol concentrations at birth. Within 24 h, this effect ceased and only nutritional intake impacted cortisol concentrations, with increased offspring cortisol as maternal intake increased. Dietary Se supplementation has been shown to enhance humoral immune function and increase IgG absorption (Knight & Tyznik 1990, Kamada *et al.* 2007). Our laboratory has suggested that maternal Se supplementation and nutritional intake level during pregnancy can affect neonatal health and its ability to obtain passive immunity via IgG (Hammer *et al.* 2011). In a study similar to the current experiment (note, this study used a

Table 4 Impacts of maternal selenium^a and offspring sex on organ mass weight (g and g/g EBW) in 19 day-old lambs.

Item	ASe		HSe		S.E.M.	P value
	Female (n=22)	Male (n=17)	Female (n=24)	Male (n=12)		
Liver (g)	280.2	324.9	279.8	326.9	17.4	0.93
g/g EBW	31.30*	30.75*,†	29.18†	32.81*	0.92	<0.01

*,†LSMean±S.E.M. within a measure differs; $P<0.05$. EBW, empty body weight=live weight minus digesta weight.

^aMaternal Se supplementation treatments were 11.5 µg Se/kg of body weight (adequate Se; ASe) or 77.0 µg Se/kg body weight (high Se; HSe) throughout gestation. For liver (g/g EBW), there was a main effect of sex (see Table 2), and an effect of maternal Se×maternal nutritional intake was significant ($P=0.02$) and is presented in Table 3.

Table 5 Impacts of maternal nutritional intake and offspring sex on organ mass weight (g and g/g brain weight) in 19 day-old lambs^a.

Item	RES		CON		HIGH		S.E.M.	Nut×Sex P value
	Female (n=12)	Male (n=11)	Female (n=20)	Male (n=7)	Female (n=15)	Male (n=11)		
Pancreas (g)	13.2* [†]	12.9*	12.7*	16.0 [†]	12.5*	14.9* [†]	0.9	0.03
g/g Brain	0.191*	0.175*	0.182*	0.228 [†]	0.182*	0.203* [†]	0.011	0.01
Adrenal wt (g)	1.04	1.05	1.11	1.24	1.10	1.10	0.06	0.41
mg/g Brain	15.0*	14.3*	15.8* [†]	17.6 [†]	15.9* [†]	15.0*	0.9	0.09

*[†],[†]LSMean±S.E.M. within a measure differs; $P<0.05$.

^aMaternal nutritional intake treatments were RES (60% of control), CON (control; 100% requirements for gestating ewe lambs), and HIGH (140% of control) fed from day 40 to term. There was no effect of maternal Se×maternal nutritional intake×offspring sex interactions ($P>0.15$) for measures presented in the table.

different Se source), maternal Se and nutritional intake impacted lamb serum IgG concentrations at 24 h after birth (Hammer *et al.* 2011; Exp. 1). In animals from the current study (Hammer *et al.* 2011, Exp. 2), lambs from RES ewes had greater serum IgG than CON or HIGH, suggesting increased IgG uptake postnatally. Moreover, female lambs had increased times of medical treatment (i.e. indicator of morbidity; Hammer *et al.* 2011). By 24 h *postpartum*, we observed (this study) that cortisol concentrations are elevated in females and in lambs from HIGH-fed ewes. While the exact role of stress or cortisol on gut closure and IgG uptake in ruminants is still questionable (Stott 1980), we find our associations between cortisol and IgG patterns of interest. The capacity to take up immunoglobulins may associate with factors such as fetal age and plasma cortisol levels (Johnston & Stewart 1986). Sangild *et al.* (1999) observed no significant correlation between cortisol and IgG 15 h after birth in premature or mature calves. Changes in morphology and function of the tissues have been associated with glucocorticoids, which activate many biochemical processes. Changes in tissue function may have little or no function *in utero* but could be essential for neonatal survival (reviewed by Fowden & Forhead (2004)).

Maternal dietary Se supplementation had no effect on neonatal serum T₃ and T₄ concentrations. However, lambs born to RES-fed ewes had decreased T₃ concentrations compared with those born to CON- and HIGH-fed ewes. In addition, male lambs had more T₃ than female lambs. Conversely, T₄ was greater in lambs born to RES- and CON-fed ewes compared with those born to HIGH-fed ewes. Thyroid hormone concentrations from fetuses are reduced during hypoxemic conditions associated with IUGR (Fowden & Forhead 2004). DeBlasio *et al.* (2006) suggested that placental restriction and small size at birth may increase activation of T₄ to T₃ and sensitivity of soft tissue to thyroid hormones, which may contribute to the accelerated growth following IUGR. Moreover, Mellor & Pearson (1977) demonstrated a positive correlation between lamb BW and concentrations of T₄ at birth, which could be related to effects of T₄ on fetal growth. Both T₃ and T₄ seem to influence synthesis and secretion of GH as well as insulin-like

growth factor 1 (IGF1) and IGF binding proteins. Schneider *et al.* (2002) suggested that conversion of inactive T₄ to active T₃ by hepatic deiodinase is controlled by GH. An interesting finding in the current study was that maternal Se supplementation did not affect T₃ and T₄ concentrations even though Se is being known to participate in the conversion of T₄ to T₃ (Beckett *et al.* 1987) through selenoprotein type 1 iodothyronine 5-deiodinase (Arthur *et al.* 1990). The lack of Se effect may be that dams were offered adequate and HSe intake rather than a restricted Se intake, which is where selenoproteins seem to differ (i.e. during inadequate Se intake). The fetal thyroid gland in humans becomes functional after mid gestation; therefore, thyroid hormone transfer from the dam to the conceptus through the placenta is very important (Contempre *et al.* (1993) as cited by Poppe & Ginoer (2003)). Development of the fetal central nervous system (CNS) during the second third of pregnancy largely depends on maternal thyroid hormones. In this study, lambs born to RES-fed ewes had lighter brain weight compared with lambs born to CON- and HIGH-fed ewes, while there was no difference in thyroid gland weight of offspring.

In the current study, lambs born to RES-fed ewes had lighter live weight, as well as several organ masses, compared with lambs born to CON- and HIGH-fed ewes at 19 day. In several cases, when organ masses are expressed per EBW, these relative organ masses are not different from the CON-fed animals. However, because live BW and EBW are influenced by maternal diet, we based organ mass on an organ that was not impacted by maternal diet, the brain. The majority of the organs that were influenced by maternal dietary intake were also influenced when organ mass was expressed per brain weight. Other researchers have used the brain as an indicator to determine asymmetric vs symmetric growth restriction, as the brain is spared during the time of maternal nutrient restriction used in this study (Anthony *et al.* 2003). Symmetric IUGR is often associated with genetic abnormalities or infections that may occur early in pregnancy (Anthony *et al.* 2003). Asymmetric IUGR is more common and often associated with placental insufficiency (Anthony *et al.* 2003), potentially due to

redistribution of blood flow to the fetus, allowing for brain sparing (Newton *et al.* 1987, Morrison 2008). Asymmetric IUGR has been documented in several mammalian species including sheep (Jensen *et al.* 1999, Anthony *et al.* 2003, Lang *et al.* 2003), swine (Widdowson 1971, Bauer *et al.* 1998), guinea pigs (Saintonge & Rosso 1981), and humans (Pearce & Campbell 1985). As we believe that our nutritional restriction model is due to differences in placental function, independent of size (Lekatz *et al.* 2010, 2011), basing offspring organ mass data per unit brain (i.e. a denominator that is consistent regardless of maternal nutritional intake) may be advantageous. While our model of overnutrition (from mid-to-late pregnancy) appears not to impact neonatal organ weights, a model of overnutrition from conception through parturition (Wallace *et al.* 2000) resulted in decreased brain, lung, heart, liver, kidneys, pancreas, spleen, and thyroid weights. While our study did use young, primiparous ewe lambs, they may have been slightly different in age (i.e. number of estrous cycle before conception) than those used in Wallace *et al.*'s (2000) study. We hypothesize that the differences between this model (Wallace *et al.* 2000) of overnutrition and the one in the current study is the level and duration of the overnutrition. Data from this study show only limited impacts of maternal Se on organ mass at 21 day. The alterations in organ masses in the current study were due to maternal diet, or sex of offspring, and could be linked to offspring cortisol concentrations. Glucocorticoids can impact the differentiation of several tissues including the lungs, liver, kidneys, muscle, fat, and gastrointestinal tract (reviewed by Fowden & Forhead (2004)).

In conclusion, despite similar postnatal diets, maternal Se and nutritional intake during gestation can impact offspring percentage weight change during the first 19 days of life. Organ mass by 19 days of life appears to be more influenced by maternal nutritional intake and sex of the offspring. However, it appears that maternal diet has a greater impact on cortisol and thyroid hormone concentrations for the first 24 h. The first hours postnatal are critical for immunoglobulin absorption, and cortisol and thyroid hormone concentrations may be essential for proper neonatal survival. It is still unclear how altered thyroid hormone and cortisol levels in the first 24 h impact the differential growth patterns that were observed in offspring from this study. Moreover, future studies are needed to determine whether these maternal diet-induced changes in hormones can impact physiological functions of the offspring.

Materials and Methods

Institutional Animal Care and Use Committees at North Dakota State University (NDSU), Fargo, and the USDA, ARS, U.S.

Sheep Experiment Station (USSES; Dubois, ID, USA) approved the animal care procedures used for this study.

Animal management and diets

Gestation

At the USSES, 178 Rambouillet ewe lambs (initial age = 240 $\pm$ 17 day; initial BW = 52.1 $\pm$ 6.2 kg) were synchronized for estrus and divided into six groups for breeding (n = 29 or 30/group). Ewe lambs were synchronized using intravaginal sponges (60 mg medroxyprogesterone acetate; Veramix, Pharmacia & Upjohn, Orangeville, ON, Canada) and PGF $_{2\alpha}$ (15 mg; Lutalyse, Pharmacia & Upjohn, Kalamazoo, MI, USA) over a 10 days period. More specifically, on day 0, sponges were inserted; on day 7, two injections of PGF $_{2\alpha}$ (7.5 mg/injection) were administered $\sim$ 4 h apart; on day 10, sponges were removed. On days 11–16, ewes were exposed to Rambouillet rams (n = 12, two/pen). Marking paint was placed on the rams' briskets to facilitate identification of ewes that the rams had attempted to breed. 'Marked' ewes were then stratified by BW and assigned randomly to one of two treatment pens. After rams were removed from the breeding pens, each pen was randomly assigned to Se treatments, which were ASe (3.5 μ g Se/kg BW per day) or HSe (65 μ g Se/kg BW per day). Se was provided to HSe ewes in the form of Se-enriched wheat mill run, which resulted from on-site processing of wheat grown in a seleniferous region near Pierre, SD, USA, whereas the sole source of Se for ewes on the ASe treatment was the feedstuffs provided. Ewes were fed a total mixed ration (ASe: 43% alfalfa hay, 19% dehydrated beet pulp, 18% whole corn, 8% barley straw, 6% soybean meal, and 6% condensed separator byproduct; HSe: 43% alfalfa hay, 25% Se-enriched wheat mill run, 17% dehydrated beet pulp, 8% barley straw, and 6% condensed separator byproduct; both 2.45 kcal metabolizable energy (ME)/kg and 10.4% metabolizable protein on a dry matter (DM) basis) during this period to achieve an ADG of 135 g/day (NRC, 1985). Pregnancy was determined 31 days after breeding via transrectal ultrasonography (Aloka, Wallingford, CT, USA). Eighty-four pregnant ewes (day 36 of gestation, n = 42 per treatment) were then shipped from the USSES to the Animal Nutrition and Physiology Center at NDSU (1584 km; $\sim$ 14.5 h transit time) where they were housed for the remainder of the experiment.

At NDSU, ewes were individually housed in 0.91 $\times$ 1.2 m pens with tenderfoot flooring (i.e. no bedding) in a temperature-controlled (12–21 $^{\circ}$ C), ventilated facility for the duration of the study. Lighting within the facility was automatically timed to mimic daylight patterns. Ewes remained on their Se treatments (actual intakes: ASe, 11.5 μ g Se/kg BW per day; HSe, 77.0 μ g Se/kg BW per day) and on day 40 of gestation were assigned randomly to one of three nutritional intake treatments supplying 60% (RES), 100% (CON), or 140% (HIGH) of NRC (1985) requirements for all nutrients except for Se.

Ewes had free access to water and a trace-mineralized salt block containing no additional Se (99% maximum, 96% minimum NaCl, 2000 mg/kg Mn, 1000 mg/kg Fe, 1000 mg/kg Mg, 500 mg/kg S, 250 mg/kg Cu, 100 mg/kg Co, 80 mg/kg Zn,

and 70 mg/kg I; Roto Salt Company, Penn Yan, NY, USA). During gestation, diets were fed once daily at 0800 h in a complete pelleted ration (0.48-cm diameter), and three pellet formulations (ASe pellet, HSe pellet, and concentrated Se pellet; as previously described by Meyer *et al.* (2010)) were blended to meet Se and ME levels as dictated by Se and nutritional intake treatments for each ewe. As for diets fed during early gestation, the Se source of the HSe pellet was the previously described Se-enriched wheat mill run, and the Se source of the ASe pellet was feedstuffs used in the diet. ASe or HSe pellets were fed to ewes in the ASe and HSe treatments, respectively, to meet the ME level appropriate for their nutritional intake treatment. The HSe pellet was used in combination with the ASe pellet as needed to meet the targeted HSe and ME levels for each ewe. When the HSe pellet could not meet the HSe level for RES- or CON-fed ewes without exceeding the desired nutritional intake, a concentrated Se pellet with purified selenomethionine as its Se source was used to augment the Se supply. Nutrient requirements were based on NRC (1985) recommendations as described by Swanson *et al.* (2008), and diets were individually adjusted for BW and BW gain for each 14 day interval of gestation. Feed refusals were collected daily to calculate intake (feed offered minus feed refused), but ewes generally ate the feed offered daily and rarely left refusals. As ewes were housed indoors, and lambs would never be exposed to direct sunlight, all ewes were injected i.m. with vitamin A and D (2.0 ml/ewe; 500 000 IU vitamin A and 75 000 IU vitamin D₃/ml; Duravet, Blue Springs, MO, USA) on day 109 of gestation as a preventative measure for skeletal related issues.

Parturition and lamb management

Ewes were closely monitored during lambing, and lambs were removed immediately after parturition, before being allowed to suckle, so they could be reared on a common diet. Immediately after birth, lamb BW was measured (this was designated as initial BW or BW at birth), and an initial jugular blood sample was taken for hormone analysis. Lambs were then s.c. administered a vaccination against *Clostridium perfringens* types C and D and tetanus (Ultrabac CD; Pfizer Animal Health, New York, NY, USA), injected i.m. with vitamins A and D (0.5 ml/lamb; 500 000 IU vitamin A and 75 000 IU vitamin D₃/ml; Duravet), and had their navels clipped and dipped in a 7% iodine tincture.

Lambs were fed artificial colostrum (45% crude protein; 15% crude fat; 0.15% crude fiber; 10% ash; 0.40% minimum, 0.90% maximum Ca; 0.30% minimum, 0.50% maximum NaCl; 1.0% minimum, 1.5% maximum Na; 0.40% P; 0.75 mg/kg Se; 110 000 IU/kg vitamin A; 2200 IU/kg vitamin D; and 440 IU/kg vitamin E (DM basis); Acquire Colostrum Replacement; APC, Inc., Ankeny, IA, USA) within 30 min of birth and at six additional times by 20 h *postpartum* to achieve 10.64 g IgG/kg BW (19.1 ml colostrum/kg BW for the first two feedings and 25.5 ml colostrum/kg BW in subsequent feedings; Meyer *et al.* 2010, Hammer *et al.* 2011). This dosage of IgG was calculated to provide 50 g IgG/4.7 kg BW based on the previous reports using a similar product (Quigley *et al.* 2002).

At 24 h, a blood sample was collected via jugular venipuncture, and lambs were transitioned to milk replacer

(24% crude protein; 30% crude fat; 0.10% crude fiber; 6.5% ash; 25% lactose; 0.50% minimum, 1.0% maximum Ca; 0.65% P; 2 mg/kg minimum, 6 mg/kg maximum Cu; 0.3 mg/kg Se; 66 000 IU/kg vitamin A; 22 000 IU/kg vitamin D₃; 330 IU/kg vitamin E (DM basis); Super Lamb Instant Milk Replacer; Merrick's, Inc., Middleton, WI, USA) fed from a bottle until a strong suckling response was observed (Meyer *et al.* 2010, Neville *et al.* 2010, Hammer *et al.* 2011). Once this occurred, the lambs were moved to a pen with free access to water and creep feed and adapted to a teat bucket system for *ad libitum* access to milk replacer (Meyer *et al.* 2010, Neville *et al.* 2010, Hammer *et al.* 2011). Tails were not docked, and male lambs were not castrated to prevent any decrease in performance caused by these procedures. Jugular blood samples were collected on days 3, 7, 14, and 19. On day 19, final weights and jugular blood samples were obtained from all lambs before necropsy. Percentage of BW change was calculated as follows: ((Final BW – Initial BW)/Initial BW) × 100, where initial BW was BW at birth.

Necropsy

On day 20.6 ± 0.9 (range: 19–21), lambs were killed by stunning. All internal organs, including the brain, liver, heart, lungs, total gastrointestinal complex, kidney, adrenal glands, gonads, uterus, thyroids, and spleen, were weighed using procedures similar to those described in Reed *et al.* (2007). Organ weight was divided by brain weight to assess asymmetric vs symmetric growth restriction, as the brain is spared during the time of maternal nutrient restriction used in this study (Anthony *et al.* 2003). For comparison, organ weight was also divided by EBW (live weight minus digesta weight), as our laboratory has previously reported (Reed *et al.* 2007, Swanson *et al.* 2008, Meyer *et al.* 2010, Neville *et al.* 2010).

Cortisol analysis

Lamb serum samples were analyzed for cortisol concentration as described previously (Lekatz *et al.* 2010). Briefly, serum samples (10 µl) were assayed in duplicate by the chemiluminescence immunoassay (Immulite 1000; Siemens, Los Angeles, CA, USA). Within each assay, lesser, medium, and greater cortisol pools were assayed in duplicate (49.8 ± 1.4, 134.2 ± 3.3, and 343.5 ± 5.2 ng/ml, mean ± S.E.M. for lesser, medium, and greater cortisol pools respectively). The sensitivity of the assay is 2 ng/ml. The intra- and interassay coefficients of variation (CV) were 6.2 and 6.4% respectively.

Thyroid hormone analysis

T₄ and T₃ concentrations were determined by the chemiluminescence immunoassay using the Immulite 1000 (Siemens) as we have described (O'Neil *et al.* 2009). Within each assay, T₄ and T₃ pools were assayed in duplicate (24.5 ± 0.1, 78.8 ± 0.1, and 105.9 ± 0.2 ng/ml and 0.88 ± 0.07, 1.53 ± 0.05, and 2.98 ± 0.05 ng/ml, mean ± S.E.M. for lesser, medium, and greater pools for T₄ and T₃ respectively). Fifteen microliter and 25 µl serum samples were assayed in duplicate for T₄ and T₃ respectively. The sensitivity of the T₄ and T₃ assays is 4.0

and 0.35 ng/ml respectively. The intra-assay CV was 3.4 and 3.7% for T_4 and T_3 , respectively, and the interassay CV was 7.8 and 13.4% for T_4 and T_3 respectively.

Statistical analysis

Twins ($n=3$ sets) were removed from the data set (one set each for HSe-RES, ASe-HIGH, and HSe-HIGH). One ewe (ASe-RES) was not pregnant and removed from the study. Five lambs died between birth and necropsy, and therefore, only BW at birth was included in the data set for these animals. Thus, the final lamb numbers used for each treatment were as follows: ASe-RES (13; six females and seven males), ASe-CON (14; ten females and four males), ASe-HIGH (13; seven females and six males), HSe-RES (13 at birth; eight females and five males; ten at necropsy; six females and four males), HSe-CON (14 at birth; 11 females and three males; 13 at necropsy; ten females and three males), and HSe-HIGH (13; eight females and five males).

Data were normally distributed and analyzed using the MIXED procedure of SAS version 9.1 (SAS Institute, Inc., Cary, NC, USA). Ewe gestational dietary Se supplementation (ASe vs HSe), nutritional intake (RES vs CON vs HIGH), and all interactions were used as fixed effects in the model. As maternal nutritional treatments can influence phenotypes of males and females differently, offspring sex was included in the model as a random effect, and interactions with sex were tested. When appropriate (i.e. for percentage weight change and hormone data), day of age was also included in the model. For the variables' percentage weight change and T_4 (from days 3 to 19), the first-order autoregressive covariance structure was used. For the variables' cortisol and T_3 (from days 3 to 19), the unstructured covariance structure was used. Interactions were removed from the model when $P>0.15$ but were retained in the model if they were of interest. Means were separated using least significant differences and considered significant when $P\leq 0.05$ or tendencies when $0.05<P<0.10$. In the absence of interactions ($P>0.10$), main effects ($P<0.10$) are reported; otherwise interactive means are discussed.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This project was partially supported by National Research Initiative Competitive Grant no. 2005-35206-15281 from the USDA Cooperative State Research, Education, and Extension Service to J S Caton, D A Redmer, and K A Vonnahme and by a LEAP grant to K A Vonnahme from National Science Foundation Grant #HRD-0811239 to the NDSU Advance FORWARD program. We would like to thank members of the Reproductive Physiology and Ruminant Nutrition laboratories for their assistance with animal care and data collection.

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Received 16 August 2011

First decision 16 September 2011

Revised manuscript received 28 March 2012

Accepted 18 April 2012