

Plasma Levels of Anabolic Hormones in Suckling Lambs are Affected by Late Gestational Nutrition

Research Article

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ABSTRACT

In this study, the effects of late gestational undernutrition (LGU) on plasma concentration of insulin-like growth factor 1 (IGF-I), leptin, insulin and glucose in subsequent suckling lambs was investigated. Ten twin-bearing ewes were fed either restrictedly (Restricted) or adequately (Control) during the last six weeks of gestation and were fed *ad libitum* after parturition. Blood samples were taken from subsequent suckling lambs at birth day, 7, 17 and 35 days of age. The average growth rate of restricted lambs was significantly lower than control lambs (292 ± 21 vs. 216 ± 19 g/d) during first two weeks of suckling period. Lambs in both groups were hypoglycemic (2.5 ± 0.2 mmol/L) and had lowest level of leptin (0.6 ± 0.08 ng/mL) at birth. Plasma glucose and leptin increased sharply by 7 days of age to 5.9 ± 0.2 mmol/L and to 0.6 ± 0.08 ng/mL respectively. Late gestational undernutrition reduced plasma glucose in restricted (3.1 ± 0.2 mmol/L) at birth but not in control lambs (2.1 ± 0.3 mmol/L). Plasma insulin was the highest at 7 days of age (0.9 ± 0.08 ng/mL). Restricted lambs had highly significant lower insulin values (0.6 ± 0.1 vs. 1.3 ± 0.1 ng/mL) and significantly higher ratio of glucose/insulin than control lambs. Insulin-like growth factor I values at birth was not affected by LGU. However, during suckling period restricted lambs had lower IGF-I than control lambs. In conclusion, LGU decreased insulin plasma concentration, changed the ratio of glucose to insulin, and decreased the concentration of IGF-I in subsequent offspring. Results also confirm the finding that circulating glucose rather than insulin is a determinant of leptin secretion in suckling lambs.

KEY WORDS IGF-I, insulin, leptin, nutrient restriction, sheep.

INTRODUCTION

Endocrine hormones such as insulin, insulin-like growth factor 1 (IGF-I) and leptin plays important roles in fetal growth and regulation of interrelationships between mother and her fetus (Fowden *et al.* 2006). In undernourished pregnant ewes maternal plasma glucose and leptin concentrations are significantly decreased in comparison to well-fed ewes (Muhlhausler *et al.* 2002). Undernourishment causes hypoglycemia and low IGF-I level during late gestation and at parturition day in ewes (Kiani *et al.* 2011a). On the other hand, restriction in nutrient supply to the fetus

causes significant alterations in endocrine milieu in the fetus (Fowden *et al.* 2006). Fetal plasma glucose concentration decreases in the undernourished ovine fetus (Muhlhausler *et al.* 2002). In the fetus, concentration of IGF-I is affected by nutrient supply to the fetus and nutrient sensitive hormones. Nutrient restriction decreases concentration of anabolic hormones (e.g. insulin and IGF-I) in the fetus (Gicquel and Le, 2006). Additionally there are relations among this hormones in fetus for instance, insulin positively regulates IGF-I levels (Fowden and Forhead, 2004) probably by increasing glucose uptake and the cellular availability of glucose. Remarkably, the endocrine al-

terations in the fetus induced by undernutrition are reported to have not only short-term but also long-term effects even after birth. As an example of short term effect, a significant relationship between fetal weight and circulating level of IGF-I and leptin has been previously reported (Cetin *et al.* 2001). In long-term studies, we have shown that, late gestational undernutrition had long-term effects on plasma IGF-I during lactation even when ewes were adequately fed (Kiani *et al.* 2011a). In addition, intermediary metabolism in growing lambs is affected by late gestational maternal undernutrition (Kiani *et al.* 2011b; Husted *et al.* 2007). However, birth by itself causes metabolic adaptations and might affect endocrine changes induced by maternal undernutrition.

At birth, metabolic adaptations occur to change of nutrition in order to maximize survival of the new born. For instance, gluconeogenesis is absent in the fetus and emerges after birth to reach adult values after 24 h (Girard, 1990). Accordingly, endocrine milieu changes after birth is critical both to enhance glucose level in the blood and most importantly to commence feeding. For instance in humans, leptin concentrations of newborns after birth reduced rapidly and was extremely low by approximately 6 days of life (Matsuda *et al.* 1999). At birth, there is a shift from IGF-II predominance to IGF-I predominance. IGF-I production then becomes GH-dependent, resetting the mechanisms regulating growth to ensure appropriate postnatal growth in the new nutritional environment (Gicquel and Le, 2006). Generally in new born ruminants, glucose and insulin levels very low. However, it is not fully known whether the endocrine hormones right after birth and during suckling period are affected by late gestational undernutrition. Thus, in this study, we proposed that alteration in anabolic hormones during fetal life induced by maternal undernutrition may transit to afterbirth life and continue to permanently alter concentrations of circulation hormones during suckling period. Therefore the aim of this study was to determine the effect of late gestational undernutrition in twin bearing ewes on blood concentrations of IGF-I, leptin, insulin and glucose in subsequent suckling lambs.

MATERIALS AND METHODS

Experimental animals and diets

All experimental procedures complied with the guidelines of and were approved by the National Committee on Animal Experimentation, Denmark. Ten twin pregnant Shropshire multiparous ewes were fed either adequately (control; 100% energy and protein requirements) or restrictedly (restricted; about 60% of energy and protein requirements) according to National Research Council (NRC, 1985).

The restricted ewes were fed only hay silage (58% DM, 10 MJ ME per kg DM, 8.2% crude protein (CP), 41.9% NDF, 9.2% ash and 1.6% fat) whereas the control ewes received hay silage supplemented with barley (88.7% DM, 10.4% CP, 2.2% ash and 2.3% fat) and protein supplement (89.5% DM, 45.4% CP, 5.1% ash and 5.7% fat). After parturition, all ewes were fed *ad libitum* with hay silage plus 1000 g barley and 200 g protein supplements. Ewes body weight and body condition score (BCS) were measured. In total, fourteen lambs were born; 8 lambs (5 females and 3 males) in restricted and 6 lambs (3 females and 3 males) in control group, respectively. All lambs were reared by their own dam and from three weeks of age lambs were offered commercial concentrate (Faremix, DLG, Denmark, 88.8% DM, 14.0% CP, 6.78% ash and 3.2% fat) *ad libitum*.

Blood sampling and assays

Blood samples were taken at birth day, 7, 17 and 35 days of age. Blood samples at birthday were taken within 2 hours after parturition in which lambs did not allow suckling their mothers. All other blood samples were taken at 10:00 a.m. All samples were taken by vein puncture of the jugular vein, and blood was collected in 10 mL heparin-flourine vacuum tube (Vacutainer™, Becton Dickinson Vacutainer System Europe, Meylan Cradex, France) and 10 mL EDTA vacuum tube (BD Vacutainer System, Preanalytical Solution Deliver Industrial Estate Plymouth PL6 7 BP, UK). Blood samples were immediately cooled on ice and then centrifuged at 1600 g for 15 min at 4 °C within 30 min after collection. Plasma samples were transferred to polystyrene tubes (Hounissen, Rossikov, Denmark) and frozen at -20 °C pending analysis.

Plasma concentrations of glucose were analyzed by commercially available spectrophotometric kit (17-25 Infinity™, Sigma Diagnostic® Inc, P.O. Box 14508, St. Louis, MO 63178, USA). Plasma insulin concentration was determined by a sandwich-type time-resolved fluoroimmunoassay (DELFIA) (Ingvarsen *et al.* 1999). Plasma samples for leptin were freeze dried and analyzed at the University of Western Australia, Perth. Leptin analyses were performed in duplicate by a double-antibody radioimmunoassay using ovine leptin raised against bovine leptin (Blache *et al.* 2000). The limit of detection was 0.07 ng/mL. Plasma IGF-I concentration was determined by double-antibody RIA with human recombinant IGF-I and antihuman IGF-I antiserum (Breier *et al.* 1991).

Statistical analysis

Normality of residuals was tested using Shapiro-Wilks test. Pearson's correlations were calculated using CORR procedure in SAS version V8.2. Repeated measurements of plasma hormones and metabolites were analyzed using the

following linear mixed model with the MIXED procedure in SAS version V8.2.

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ij}$$

Where:

μ : the population mean.

α_i : the fixed effect of late gestation maternal nutrition (control or restricted).

β_j : the fixed effects of sample time.

$(\alpha\beta)_{ij}$: the interactions between fixed effects.

e_{ijk} : the residual error (Littell *et al.* 2000).

Samples within each lamb were declared as repeated measurements. The fixed effect of gender and its systematic interactions were also added to the model. If any of the systematic interaction effects did not reach significance ($P > 0.05$), it was eliminated from the model. Based on likelihood ratio test, the covariance structure of the repeated measurements was modeled as compound symmetry (CS), auto-regressive order 1 (AR1) or unstructured (UN) (Littell *et al.* 2000). Relative standard deviation (RSD) is presented unless otherwise mentioned. Comparison with $P < 0.01$ are declared highly significant, $P < 0.05$ significant and $0.05 < P < 0.10$ are considered as trends.

RESULTS AND DISCUSSION

At the beginning of the experiment, means of ewe's weight were 90 and 89 kg for control and restricted group, respectively. Late gestational nutrient restriction reduced body weight of restricted ewes about 7 kg whereas no decrease was observed in control ewes. After parturition means body weight was 82 and 72 kg and thereafter ewe's weights were constant. Nutrient restriction significantly reduced body condition score in restricted in comparison with control ewes. At parturition day, BCS in restricted ewes (3.3) was significantly ($P < 0.05$) lower than that in control ewes (4.2). Further results regarding to the dams have been previously presented (Kiani *et al.* 2001a).

No significant effect was found between female and male lambs in the parameters measured in this experiment except for IGF-I values, therefore data from both sexes were combined for analysis. Body weights of lambs are presented in Figure 1. Control and restricted lambs had similar birth weight with no significant differences. Restricted born lambs were lighter at 7 and 17 days of age but at 32 days of age there was no significant different of weight between two groups of lambs (Figure 1). The average growth rate (g/d) of lambs from birth until 32 days of age was 228 ± 27 and 222 ± 25 for control and restricted lambs with no significant differences. However, during the first two weeks of

life, lambs born in restricted group had significantly lower daily growth rate (216 ± 19 g) in comparison with lambs born in control group (292 ± 21 g). Restricted female lambs (187 ± 23 g) but not restricted males (244 ± 30 g) had significantly lower growth rate than both female (291 ± 30 g) and male lambs (292 ± 30 g) in control group. However, from 17 until 32 days of age, the daily growth rates of restricted lambs were similar to those in control lambs.

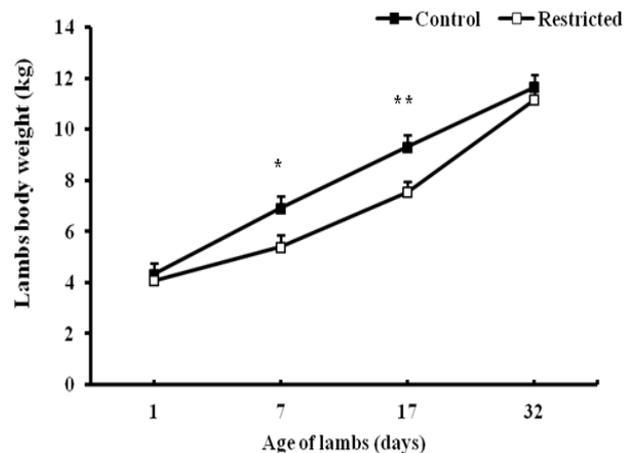


Figure 1 Body weight (kg) of suckling lamb whose mother were fed either 60% (Restricted; □) or 100% (Control; ■) of their energy and protein requirements during late gestation

* $P < 0.05$ and ** $P < 0.01$

Plasma concentration of glucose and leptin are shown in Figure 2. Lambs in both groups were hypoglycemic (2.5 ± 0.2 mmol/L) at birth. Remarkably, plasma glucose concentrations increased sharply by 7 days of age (5.9 ± 0.2 mmol/L) and remained constant thereafter (Figure 2). Maternal undernutrition reduced plasma glucose in restricted lambs (3.1 ± 0.2 mmol/L) at birth in comparison to control lambs (2.1 ± 0.3 mmol/L). However, no significant effect of maternal undernutrition on glucose level was seen during suckling period.

Plasma concentration of leptin like plasma glucose was notably low at birth (0.6 ± 0.08 ng/mL) and sharply increased by 7 days of age (1.5 ± 0.13 ng/mL) (Figure 2). Leptin concentration tend to decrease between 7 and 32 days of age from 1.5 to 1.3 ± 0.09 ng/mL ($P = 0.05$). Leptin concentration in plasma was not affected by late gestational undernutrition affected neither at birth nor during suckling period.

Insulin plasma concentration and glucose / insulin values in lambs at birth and during suckling period are shown in Figure 3.

Insulin concentration in plasma was the highest at 7 days of age (0.9 ± 0.08 ng/mL). Plasma level of insulin at birth, 7 and 32 days of age were not significantly different.

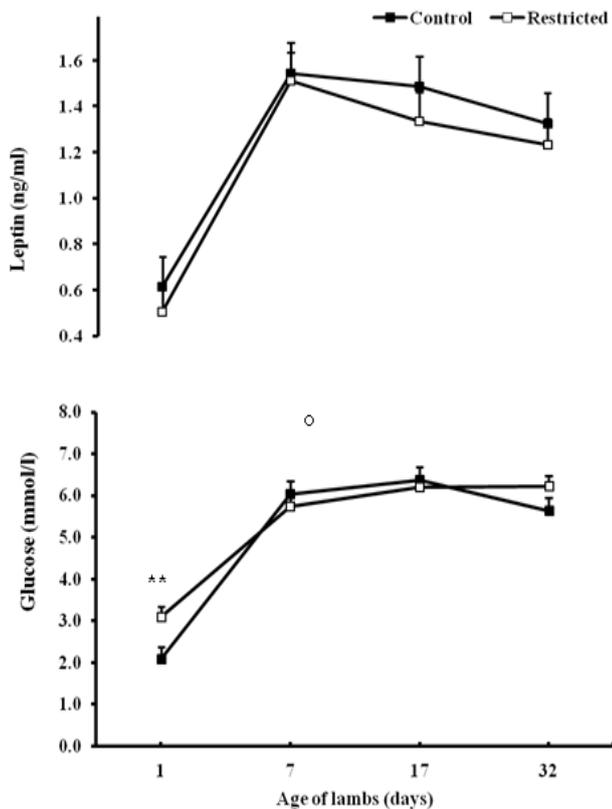


Figure 2 Plasma glucose (mmol/L) and leptin (ng/mL) concentrations in lambs whose dams were fed either 60% (Restricted; □) or 100% (Control; ■) of their energy and protein requirements during late gestation
* $P < 0.05$ and ** $P < 0.01$

Regardless of the time of sampling, restricted lambs (0.3 ± 0.05 ng/mL) had significantly lower insulin concentration than control lambs (0.6 ± 0.06 ng/mL). Remarkably, at 7 days of age, restricted lambs showed highly significant lower insulin than control lambs (0.6 ± 0.1 vs. 1.3 ± 0.1 ng/mL). When ratio of glucose to insulin was calculated, the difference in plasma insulin between restricted and control lambs became more obvious (Figure 3). Restricted lambs had highly significant higher ratio of glucose/insulin than control lambs at birth and at 32 days of age.

IGF-I concentration levels at birth and during suckling period are shown in Figure 4. Male lambs had higher blood concentration of IGF-1 than female lambs (136 ± 9.3 vs. 100 ± 7.8 ng/mL). IGF-I concentration in plasma tended to increase with the age from birth (69 ± 10.5) until 32 days of age (155 ± 11.5 ng/mL). Even though, restricted and control lambs had similar plasma concentration of IGF-I at birth, restricted lambs had significantly lower IGF-I than control lambs at 7 (93 ± 15.0 vs. 137 ± 17.3 ng/mL, $P = 0.06$), 17 (107 ± 16.2 vs. 159 ± 17.3 ng/mL, $P = 0.04$) and 32 (129 ± 15.0 vs. 182 ± 17.3 ng/mL, $P = 0.03$) days of age (Figure 4).

Late gestation is the time that most of fetal growth occurs under hormonal regulations. Anabolic hormones such as insulin, insulin-like growth factors (IGF-I and IGF-II) and

leptin play particularly important roles in fetal growth. Environmental conditions such as availability of nutrients and maternal nutrition affect the plasma level of anabolic hormones in the fetus (Fowden *et al.* 2006).

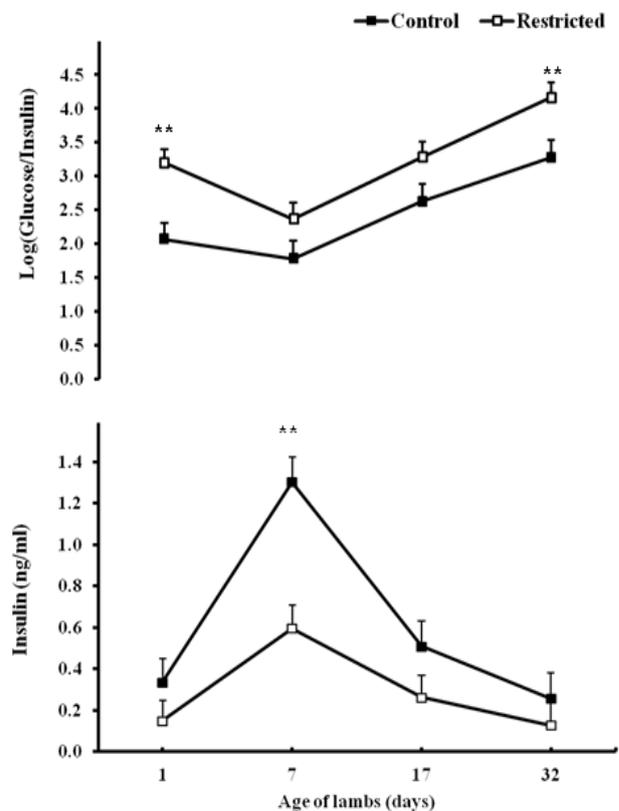


Figure 3 Plasma insulin (ng/mL) concentration and glucose/insulin ratio in lambs whose dams were fed either 60% (Restricted; □) or 100% (Control; ■) of their energy and protein requirements during late gestation.
* $P < 0.05$ and ** $P < 0.01$

Plasma level of insulin, IGF-I and leptin are reduced in both mother and fetus in restricted nourished ewes (Muhlhausler *et al.* 2002; Gicquel and Le 2006). In the present study, blood samples at birth could be compared to those of fetus values with some cautions. The values of 69 ± 10.5 ng/mL for IGF-I concentration at birth are in agreements with reported values of IGF-I (50-100 ng/mL) in ovine fetus plasma (Fowden, 2003).

Restricted born lambs had significantly higher glucose and ratio of glucose / insulin and a tendency towards lower insulin at birth in comparison to control lambs. Higher ratio of glucose/insulin ratio might be as a sign of diabetes or insulin resistance.

These results are in consistent with the finding that maternal undernutrition during late gestation affects intermediary metabolism, in particular, glucose-insulin homeostasis in the offspring (Gardner *et al.* 2005; Kiani *et al.* 2011b; Husted *et al.* 2007).

All Lambs were born with hypoglycemia and hypoleptinemia at birth regardless to the maternal nutrition.

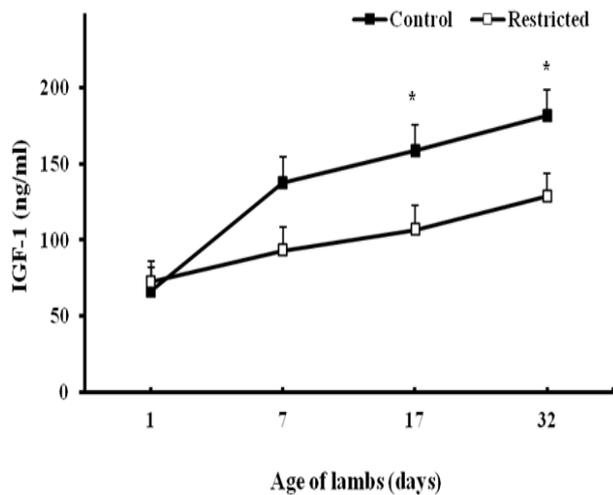


Figure 4 Plasma insulin-like growth factor (ng/mL) concentrations in lambs whose dams were fed either 60% (Restricted; □) or 100% (Control; ■) of their energy and protein requirements during late gestation
* $P < 0.05$ and ** $P < 0.01$

These observations were consistent with those of Tokuda (2003) who used five lambs to determine plasma concentration of leptin in pre and post weaning lambs (Tokuda 2003) and those of Long *et al.* (2011) who observed a leptin peak in lambs between days 6 and 9 of postnatal life (Long *et al.* 2011). Presumably, the endocrine adaptations at birth occur to change of nutrition in order to maximize survival of the new born lamb. Similar to sheep, in humans also leptin concentrations of newborns declines rapidly after birth and are extremely low by approximately 6 days of life (Matsuda *et al.* 1999).

Accordingly endocrine milieu changes after birth is critical both to enhance glucose level in the blood and most importantly to commence feeding. In lambs, the rapid loss of uncoupling protein-1 mRNA, which occurs within the first few days of life, appears to be accelerated by leptin administration, possibly stimulating the development of white adipose tissue and generation of body heat through mechanisms other than non-shivering thermogenesis by uncoupling protein-1 in brown adipose tissue (Mostyn *et al.* 2001).

Thus, it could be concluded that hypoleptinemia and hypoglycemia at birth may be a motive to make the move for feed searching and a signal to initiate feeding. In the present study, unfortunately milk intake were not measured thus it is not informative to discuss about postnatal growth. However, the postnatal growth, during the first two weeks of life, was slower in restricted lambs in particular in female lambs. However, from 17 until 32 days of age, the daily growth rates of restricted lambs were similar to those in

control lambs in spite of lower plasma IGF-I level and higher glucose / insulin ratio. The potential application of hypoleptinemia at birth and lower plasma IGF-I at early life to postnatal growth remains an exciting possibility both in animal production and human health.

In the present study, a strongly positive relationship between leptin and glucose was found which confirms the finding that circulating glucose rather than insulin is a determinant of leptin secretion (Tokuda, 2003). Furthermore, in restricted lambs leptin was correlated to insulin whereas in control lambs leptin was correlated to IGF-I. Long *et al.* (2011) reported that the leptin peak in lambs born to control ewes was not clearly related to any changes in plasma cortisol, insulin, triiodothyronine, IGF-I or glucose (Long *et al.* 2011).

Body weights of lamb were positively correlated to IGF-I, Glucose, and leptin regardless to the late gestational nutrition. Presumably, because of that body weight of control lambs was strongly related to IGF-I. Such relationship did not found in restricted lambs. Body weight of restricted lambs but not control lambs were correlated to IGF-I. In fact, only in control lambs IGF-I was positively correlated to body weight.

Plasma concentration of IGF at birth was not affected by late gestational undernutrition in the present study. These observations are not consistent with the finding that fetal IGF-I concentration is affected by placenta supply nutrient to the fetus (Fowden, 2003). However, present study showed that IGF-I increased as lambs became older and that nutrient restriction during fetal life reduced IGF-I concentration in restricted born lambs during suckling period. This finding is consistent with previously studies in which it has been reported that plasma IGF-I levels increased rapidly after birth (Breier *et al.* 1988). It might be primarily as a result of the onset of growth hormone (GH) stimulated IGF-I production by the liver (Li *et al.* 1999; Gluckman 1995). Lower plasma IGF-I in restricted lambs might be related to lower insulin concentration in these lambs. Insulin positively regulates IGF-I levels in fetus (Fowden and Forhead, 2004) but again such a relationship were not observed in the present study. The dams of the restricted lambs had lower IGF-I during late gestation and at parturition.

In spite of been adequately fed during lactation, restricted ewes showed lower IGF-I at 35 days of lactation but not sooner than that (Kiani *et al.* 2011a). Similarly, it has been shown that intermediary metabolism in growing lambs is affected by late gestational maternal undernutrition (Kiani *et al.* 2011b; Husted *et al.* 2007). Together these findings showed that late gestational undernutrition had long-term effects on glucose-insulin homeostasis and IGF-I concentration in subsequent offspring.

CONCLUSION

In conclusion, results of this study showed that late gestation maternal nutrition may decrease concentration of insulin in offspring as it happens during fetal life. Late gestational nutrition may also change the ratio of glucose to insulin and decrease the concentration of IGF-1 in suckling lambs. Results also confirm the finding that circulating glucose rather than insulin is a determinant of leptin secretion.

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REFERENCES

- Blache D., Tellam R.L., Chagas L.M., Blackberry M.A., Vercoe P.E. and Martin G.B. (2000). Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. *J. Endocrinol.* **165**, 625-637.
- Breier B.H., Gallaher B.W. and Gluckman P.D. (1991). Radioimmunoassay for insulin-like growth factor-I: solutions to some potential problems and pitfalls. *J. Endocrinol.* **128**, 347-357.
- Breier B.H., Gluckman P.D. and Bass J.J. (1988). Plasma concentrations of insulin-like growth factor-I and insulin in the infant calf: ontogeny and influence of altered nutrition. *J. Endocrinol.* **119**, 43-50.
- Cetin I., Radaelli T., Taricco E., Giovannini N., Alvino G. and Pardi G. (2001). The endocrine and metabolic profile of the growth-retarded fetus. *J. Pediatr. Endocrinol. Metab.* **14**(6), 1497-1505.
- Fowden A.L. (2003). The insulin-like growth factors and fetoplacental growth. *Placenta.* **24**, 803-812.
- Fowden A.L. and Forhead A.J. (2004). Endocrine mechanisms of intrauterine programming. *Reproduction.* **127**, 515-526.
- Fowden A.L., Giussani D.A. and Forhead A.J. (2006). Intrauterine programming of physiological systems: causes and consequences. *Physiology.* **21**, 29-37.
- Gardner D.S., Tingey K., Van Bon B.W., Ozanne S.E., Wilson V., Dandrea J., Keisler D.H., Stephenson T. and Symonds M.E. (2005). Programming of glucose-insulin metabolism in adult sheep after maternal undernutrition. *Am. J. Physiol. Regul. Integ. Comp. Physiol.* **289**, R947-R954.
- Gicquel C. and Le B.Y. (2006). Hormonal regulation of fetal growth. *Horm. Res.* **65**(3), 28-33.
- Girard J. (1990). Metabolic adaptations to change of nutrition at birth. *Biol. Neonate.* **58**(1), 3-15.
- Gluckman P.D. (1995). Clinical review 68: the endocrine regulation of fetal growth in late gestation: the role of insulin-like growth factors. *J. Clin. Endocrinol. Metab.* **80**, 1047-1050.
- Husted S.M., Nielsen M.O., Tygesen M.P., Kiani A., Blache D. and Ingvarsten K.L. (2007). Programming of intermediate metabolism in young lambs affected by late gestational maternal undernourishment. *Am. J. Physiol. Endocrinol. Metab.* **293**, 548-557.
- Ingvarsten K.L., Munksgaard L., Nielsen V.K.M. and Pedersen L.J. (1999). Responses to repeated deprivation of lying down on feed intake, performance and blood hormone concentration in growing bulls. *Acta Agric. Scand. Section. Anim. Sci.* **49**, 260-265.
- Kiani A., Nielsen M.O. and Chwalibog A. (2011a). Late gestational undernutrition alters plasma IGF1 concentration during subsequent lactation in ewe. *Agric. Consp. Scientif.* **76**, 361-364.
- Kiani A., Nielsen M.O., Tauson A.H., Tygesen M.P., Husted S.M. and Chwalibog A. (2011b). Long-term effects of foetal undernutrition on intermediary metabolism in growing lambs. *Arch. Anim. Nutr.* **65**, 46-54.
- Li J., Gilmour R.S., Saunders J.C., Dauncey M.J. and Fowden A.L. (1999). Activation of the adult mode of ovine growth hormone receptor gene expression by cortisol during late fetal development. *FASEB J.* **13**, 545-552.
- Littell R.C., Pendergast J. and Natarajan R. (2000). Tutorial in biostatistics: modelling covariance structure in the analysis of repeated measures data. *Stat. Med.* **19**, 1793-1819.
- Long N.M., Ford S.P. and Nathanielsz P.W. (2011). Maternal obesity eliminates the neonatal lamb plasma leptin peak. *J. Physiol.* **589**, 1455-1562.
- Matsuda J., Yokota I., Iida M., Murakami T., Yamada M., Saijo T., Naito E., Ito M., Shima K. and Kuroda Y. (1999). Dynamic changes in serum leptin concentrations during the fetal and neonatal periods. *Pediatr. Res.* **45**, 71-75.
- Mostyn A., Keisler D.H., Webb R., Stephenson T. and Symonds M.E. (2001). The role of leptin in the transition from fetus to neonate. *Proc. Nutr. Soc.* **60**, 187-194.
- Muhlhauser B.S., Roberts C.T., McFarlane J.R., Kauter K.G. and McMillen I.C. (2002). Fetal leptin is a signal of fat mass independent of maternal nutrition in ewes fed at or above maintenance energy requirements. *Biol. Reprod.* **67**, 493-499.
- NRC. (1985). Nutrient Requirements of Sheep. 6th. Ed. National Academic Press, Washington, DC.
- Tokuda T. (2003). Plasma leptin concentration in pre- and post-weaning lambs. *Anim. Sci.* **76**, 221-227.