Ewe size and nutrition during pregnancy

Effects on metabolic and productive performance of the offspring

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Danitsja Stephanie van der Linden

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Thesis supervisors Associate Professor P. R. Kenyon
Professor H. T. Blair
Dr C. M. C. Jenkinson
Dr S. W. Peterson

Thesis examiners Associate Professor D. D. S. Mackenzie
Massey University, Palmerston North
Associate Professor F. H. Bloomfield
University of Auckland
Dr M. P. Tygesen
Copenhagen University, Denmark

ABSTRACT

Exposure of the fetus to adverse conditions *in utero* may result in developmental adaptations that alter metabolism and postnatal growth of the offspring. This thesis investigated the effects of dam size and nutrition during pregnancy on growth, metabolic function and lactational and productive performance of the female offspring to two years of age. Four-hundred and fifty heavy (60.8 kg ± 0.18) and 450 light (42.5 kg ± 0.17) dams were randomly allocated to *ad libitum* or maintenance nutritional regimens from days 21 - 140 of pregnancy, under pastoral grazing conditions. From one week prior to lambing, all dams were fed *ad libitum* until weaning. After weaning, female progeny were managed and fed under pastoral conditions as one group. Maternal nutrition during pregnancy affected lamb growth to weaning, however, after weaning lamb growth was affected by dam size. Dam size had no effect on glucose metabolism, adrenal function or fat metabolism in 16-month-old female twin offspring. Dam nutrition during pregnancy had a minor effect on glucose metabolism and no effect on adrenal function or lipolysis, however, it did possibly affect gluconeogenesis and/or glycogenolysis, with increased glucose production in ewes born to maintenance-fed dams. Ewes born to dams fed maintenance showed greater milk production, lactose percentage, lactose and crude protein yield. Ewes born to heavy dams showed greater milk production and lactose yield. Dam size had no effect on reproductive performance of the female offspring. Being born to a larger dam showed no advantages over being born to smaller dams, for number of lambs born and weight of lambs at birth and weaning. 'Grand'dam maintenance nutrition increased lamb birth and weaning weight and lamb growth rates of the 'grand'offspring. Ewes born to maintenance-fed dams could have an advantage over ewes born to *ad libitum*-fed dams in physiological stressful situations in life as their liver may be able to supply more glucose to support their growing conceptus and milk production to increase the chances of survival of their offspring. These results indicate that it is possible to programme the offspring by feeding their dams differently during pregnancy under grazing conditions. With a better understanding of how offspring can be programmed through different maternal nutritional regimens, it may be possible to significantly increase the production potential of the New Zealand ewe population.

SAMENVATTING

Blootstelling van een ongeboren jong aan ongunstige omstandigheden *in utero* (baarmoeder), kan resulteren in veranderingen in de ontwikkeling van het metabolisme en de groei van het nageslacht. In dit proefschrift worden de effecten beschreven van het gewicht en het voedings niveau van de Nieuwe Zeelandse ooi tijdens de dracht op de groei, het metabolisme, de lactatie en het productie vermogen van haar vrouwelijke nageslacht tot twee-jarige leeftijd. Vierhonderdvijftig zware (60.8 kg ± 0.18) en 450 lichte (42.5 kg ± 0.17) ooien waren *ad random* verdeeld over twee groepen: een groep had toegang tot *ad libitum* gras en een groep werd gegraasd op onderhouds-niveau van dag 21 – 140 van de dracht. Alle ooien werden gehouden onder graas omstandigheden.Vanaf één week voor het lammeren, alle ooien hadden toegang tot *ad libitum* gras tot aan het spenen. Hierna werd het vrouwelijke nageslacht als één groep gemanaged en hadden *ad libitum* gras beschikbaar. Het voer niveau van de ooi tijdens de dracht beïnvloedde de groei van het nageslacht tot aan het spenen. Het gewicht van de moeder beïnvloedde de groei van de lammeren na het spenen, maar dit had geen effect op het functioneren van het glucose metabolisme, de bijnieren (adrenal) en ook niet op het vet weefsel metabolisme op een leeftijd van 16 maanden. Het voer niveau van de ooi tijdens de dracht had ook geen effect op het functioneren van het glucose metabolisme, de bijnieren en lipolyse (vetafbraak), maar het had mogelijk wel een positief effect op het proces van gluconeogenese (opnieuw vormen van glucose) en/of glycogenolyse (het process waarbij glycogeen wordt afgebroken en omgezet in glucose). Vrouwelijk nageslacht van ooien die op het onderhouds-niveau gevoerd werden, hadden een grotere glucose produktie. Nageslacht van ooien die onderhouds-niveau gevoerd werden, produceerden meer melk, lactose en eiwitten en hadden hogere lactose percentages in de melk. Nageslacht van zware ooien produceerden meer melk en lactose. Het gewicht van de ooi had geen effect op het reproductie vermogen van het nageslacht. Er waren geen verschillen gevonden tussen de zware en lichte groep in het aantal geboren lammeren (tweede generatie) en het gewicht van deze lammeren zowel bij de geboorte als bij

het spenen. Voeren van het onderhouds-niveau aan de (groot)moeder verhoogde het geboorte en speen gewicht en ook de groei van de tweede generatie lammeren. Vergeleken met het nageslacht van ooien die *ad libitum* gevoerd werden, had het nageslacht van ooien die het onderhouds-niveau gevoerd kregen een voordeel in fysiologische stressvolle situaties. Omdat hun lever mogelijk meer glucose kan produceren en waardoor er meer glucose beschikbaar is voor het groeiende jong tijdens de dracht en voor de daarop volgende melk productie is de overlevingskans voor dit nageslacht groter. De resultaten in dit proefschrift laten zien dat het mogelijk is om het nageslacht te ‘programmeren’ door de moeder verschillende niveaus te voeren tijdens de dracht. Door meer inzicht in het ‘programmeren’ van het nageslacht te krijgen, door middel van verschillende voer niveaus tijdens en na de dracht, is het mogelijk om het productie vermogen van de Nieuw Zeelandse schapen te vergroten.

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CHAPTER 1

INTRODUCTION

The ‘developmental origins of adult health and disease’ or the ‘Barker’ hypothesis states that adverse influences early in development and particularly during intrauterine life can result in permanent changes in physiology and metabolism persisting into later life (Armitage *et al.*, 2004; De Boo and Harding, 2006). A wide range of techniques (e.g. nutrition, carunclectomy, uterine and umbilical artery embolism) have been used to compromise the intrauterine environment and alter fetal development (Fowden *et al.*, 2006). Such experiments have been performed in a number of species (rats, guinea pigs, sheep, pigs, horses) and generated the perception that the intrauterine environment and the possible carry-over effects into early postnatal life could be of importance for altering production performance of the offspring.

Intrauterine as well as early postnatal programming of production related traits could have major effects for the New Zealand sheep industry. This phenomenon could become even more relevant in countries where animal production is extensive, such as New Zealand, where sheep are kept under grazing conditions year round. This could lead to suboptimal nutrition of the animals during various stages of life due to variation in pasture growth and herbage availability. Pregnant ewes could be exposed to suboptimal nutrition, especially during early pregnancy, due to either a shortage of pasture or through a feed management scheme, which aims to ‘save’ pasture for later stages of pregnancy and/or lactation. Previous work has shown that suboptimal nutrition negatively affects, for example, fetal mammary gland development (Jenkinson, 2003), postnatal growth (Clarke *et al.*, 2000; Ford *et al.*, 2007) and reproductive performance (Gunn, 1977; Borwick *et al.*, 1997; Rae *et al.*, 2002) of the offspring. The long-term effects of suboptimal maternal nutrition during pregnancy are not well understood and have potential to alter animal production. To date, there is no data in sheep which has examined the effects of suboptimal maternal nutrition on the lactational performance of the offspring. If maternal nutrition would affect lactational performance, it will potentially have major implications as most offspring are solely dependent on the mothers’ lactation performance in early life for survival and growth.

Apart from nutrition having an impact, the set of non-genetic and non-pathological influences by which the mother limits fetal growth, due to mature body size, may also affect the performance of the offspring and this is a reflection of the limited capacity of the mother and placenta to supply nutrients to the fetus(es) (Gluckman and Hanson, 2004). In New Zealand, most ewes are conceiving multiple lambs and, therefore, the size of the mother could compromise the intrauterine environment (Gootwine *et al.*, 2007) which in turn could affect the performance of the offspring in terms of e.g. growth, reproductive function. Additionally, there is currently a debate among New Zealand farmers regarding the efficiency of heavy/large versus light/small ewes (i.e. is it more profitable on a per hectare basis to have fewer relatively heavier ewes or more ewes that are lighter?). Thus, investigating the effects of maternal size, within breed, on the performance of the offspring could potentially result in better advice for farmers as to whether to select for heavy/large or light/small ewes.

The objectives of this thesis are to examine the effects of maternal constraint (ewe size being heavy or light) and nutritional regimen (maintenance requirements of non-pregnant ewes or *ad libitum*) from days 21 – 140 of pregnancy under New Zealand grazing conditions on:

- Postnatal growth and the onset of puberty of female offspring (Chapter 5).
- Glucose metabolism, fat metabolism and adrenal function of post-pubertal female twin offspring (Chapter 6).
- The relationships between early postnatal growth and metabolic function of 16 months old female offspring (Chapter 7).
- Fetal mammary gland development and lactational performance of offspring at their first lactation (Chapter 8).
- Reproductive and productive performance of two-year-old female offspring (Chapter 9).

In addition, four different milking techniques to estimate milk production in ewes were compared, to determine which technique would be the most accurate to investigate lactational performance in non-dairy animals (Chapter 3).

CHAPTER 2

REVIEW OF LITERATURE

As mentioned in the introduction, adverse influences (e.g. nutrition, maternal constraint) during intrauterine and/or postnatal life can result in permanent changes in physiology and metabolism persisting into later life (Armitage *et al.*, 2004; De Boo and Harding, 2006). Intrauterine and/or early postnatal life programming of postnatal metabolic and productive function of the offspring will be outlined here. Firstly, potential critical windows during pregnancy of dietary manipulations will be described followed by hypothesized mechanisms of (intrauterine) programming. Then the effects of maternal nutrition during pregnancy and the effects of maternal constraint on the offspring are described. Finally, the perspective and hypotheses of this thesis are described. The review will concentrate on studies using ovine models, however, rodent, bovine and human data will be included when no ovine data are available.

2.1 Potential critical windows of dietary manipulations affecting postnatal function of offspring

Dietary manipulations used in ovine models have included the periconceptional period (Edwards and McMillen, 2002; Bloomfield *et al.*, 2004; Jaquiery *et al.*, 2007; Rumball *et al.*, 2008a; Rumball *et al.*, 2008b), early gestation and mid gestation (Clarke *et al.*, 1998; Heasman *et al.*, 1999), and late gestation (Gunn *et al.*, 1995; Oliver *et al.*, 2001; Oliver *et al.*, 2002; Husted *et al.*, 2008), targeting different developmental periods of the growing fetus (Figure 2.1).

Glucose and adipose metabolism

Studies in rats have shown that calorie restriction during late gestation lowers pancreatic β-cell neogenesis and that protein restriction during late gestation reduces proliferation and increases apoptosis of islet cells in the pancreas. The net effect is a reduction of pancreatic β-cells in the islets at birth and these changes persist after birth, leading to lower pancreatic insulin content and poor insulin responses to glucose and amino acids (Fowden *et al.*, 2005). Therefore, the critical window for intrauterine programming of adult glucose intolerance appears to be in late gestation in rats. In sheep, identifying the critical window for development of the pancreas is more difficult (Figure 2.2), as pancreas islet formation and changing islet size and topography begin at an earlier stage of pregnancy and continue for a longer period after birth compared to rats (Fowden and Hill, 2001). However, studies conducted in sheep indicate that the critical window for glucose metabolism may be from mid to late gestation as maternal caloric restriction during mid (Ford *et al.*, 2007) and late gestation (Gardner *et al.*, 2005; Husted *et al.*, 2008) altered the glucose tolerance and insulin sensitivity of the offspring, while maternal caloric restriction during early gestation had no effect (Gardner *et al.*, 2005).

Adipose metabolism seems to be altered by maternal nutrition from mid to late gestation (Figure 2.1), as studies showed that offspring born to undernourished ewes during mid (Ford *et al.*, 2007) and late (Gardner *et al.*, 2005) pregnancy had increased adiposity, which was accompanied by glucose intolerance and insulin resistance at eight and twelve months, respectively.

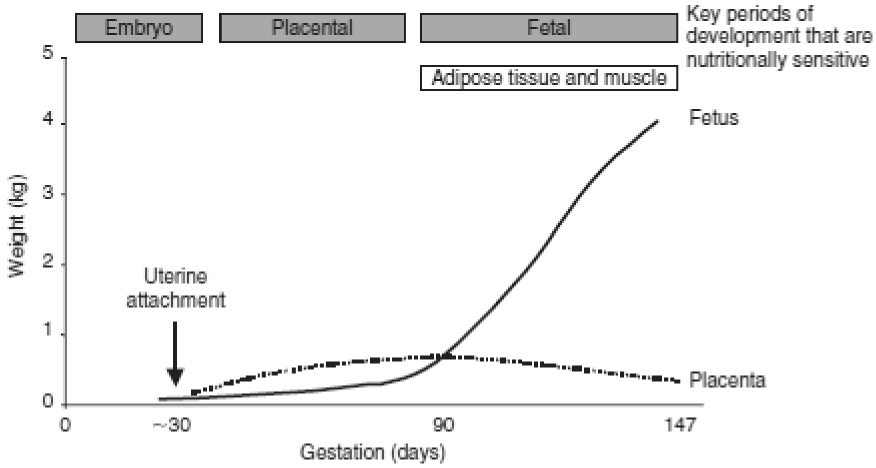

Figure 2.1. Summary of the main developmental windows during the reproductive period in sheep during which manipulations of the maternal diet significantly modulate placental and fetal development. Filled bars represent windows of developmental plasticity with respect to adipose tissue and muscle (Adapted from Symonds *et al.* (2007)).

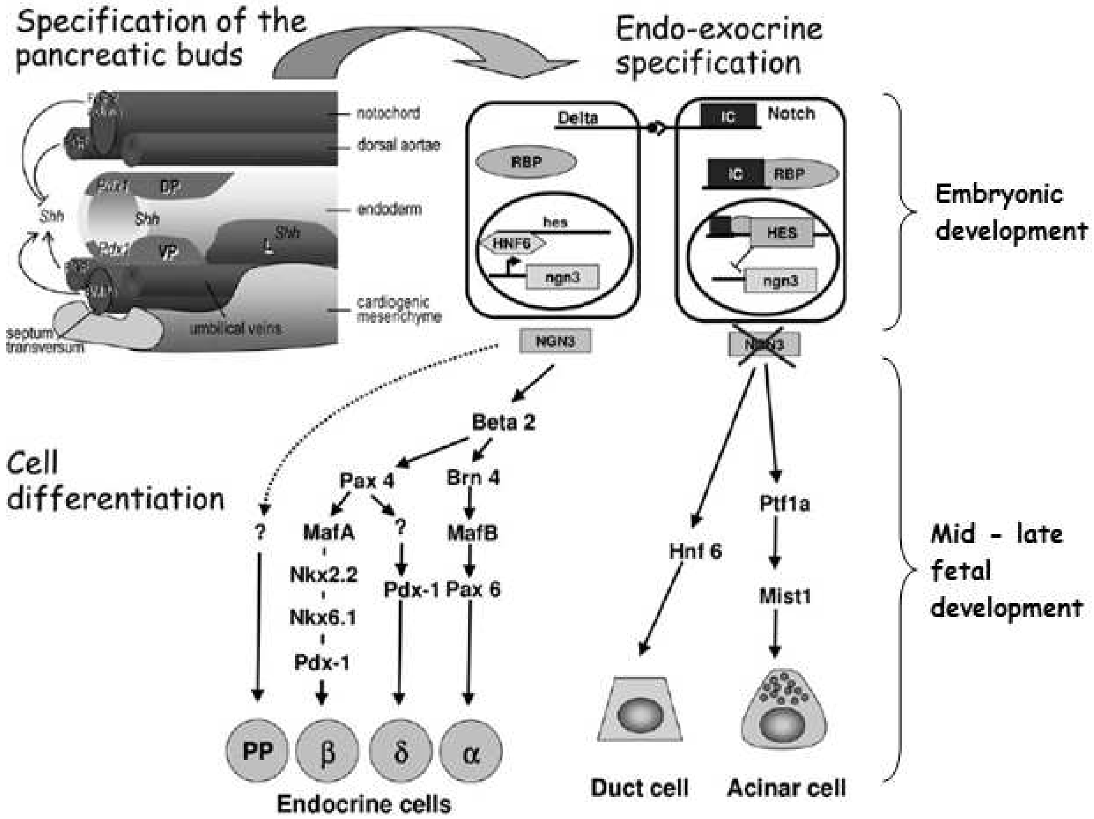

Figure 2.2. Schematic overview of pancreatic development. During early embryonic growth, the specification of the future dorsal (DP) and ventral (VP) pancreatic buds involves induction by similar morphogens from adjacent mesodermal structures, such as notochord, dorsal aorta, cardiogenic mesoderm and *septum transversum*, as noted. The exo-endocrine specification is controlled by the Notch/Hes signalling system, leading to the suppression of neurogenin-3, which determines the duct and acinar fate. From mid – late fetal growth, the endocrine cell differentiation is based on the succesive expression of transcription factors, some steps being presented in the figure. (Adapted from Remacle *et al*. (2007) and Fowden and Hill (2001)).

Hypothalamic-pituitary-adrenal (HPA)-axis

Studies conducted in sheep indicate that the critical window during which maternal undernutrition may act to alter the set point of the HPA-axis function is during early pregnancy and, in particular, during the periconceptional and the preimplantation periods (Hawkins *et al.*, 1999; Hawkins *et al.*, 2001; Edwards and McMillen, 2002; Bloomfield *et al.*, 2004).

Mammary gland / lactation

Organogenesis of the mammary gland starts in embryogenesis and continues into adult life (during puberty and pregnancy), when functional development and differentiation occur (Anderson, 1975; Masso-Welch *et al.*, 2000; Knight and Sorensen, 2001; Robinson, 2007). Therefore, potential critical windows to alter mammary gland development and subsequently the animal's lactation potential are during early gestation when the animal is still *in utero* (Jenkinson, 2003; Berry *et al.*, 2008), during the (peri)pubertal period (Sejrsen, 1994) and during the animals' own pregnancy (Peart, 1970; Tygesen *et al.*, 2008).

Reproductive axis

Maternal nutrition can influence development of the fetal reproductive systems at several stages of development involving many different physiological systems (Figure 2.3). However, undernutrition of the dam during early to mid gestation may have the greatest effect on reproductive performance of the offspring (Rae *et al.*, 2001; Da Silva *et al.*, 2002; Rae *et al.*, 2002). Nutritional effects on the process of tissue differentiation, gonad formation or the establishment of associated enzyme systems could possibly have a fundamental effect on the subsequent function of these organs (Rhind *et al.*, 2001; Rhind, 2004) and consequently affect the reproductive performance of the animals.

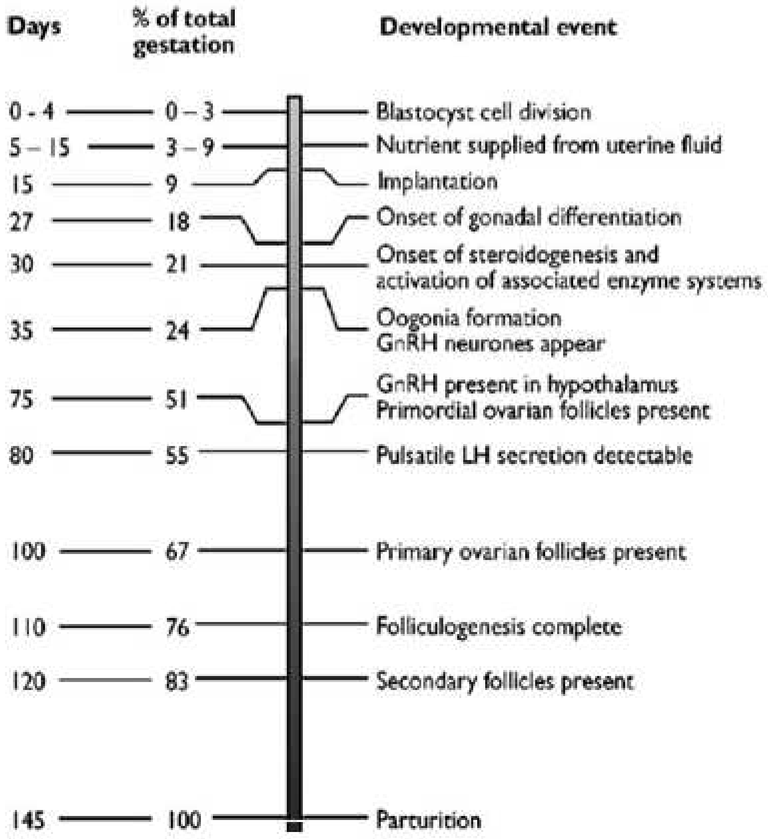

Figure 2.3. Approximate timing of reproductive development events in the sheep which may be sensitive to early life nutritional influences, expressed as days of gestation and percentage of gestation (Adapted from Rhind (2004)).

Growth

Muscle fibre formation begins in early pregnancy (approximately day 32) (Brameld and Daniel, 2008) and is completed *in utero* in precocious species such as sheep (Buttery *et al.*, 2000). Nutrient restriction in early gestation can cause a permanent reduction in muscle fibre number and maternal undernutrition in sheep can lead to offspring with fewer muscle fibres (Buttery *et al.*, 2000; Brameld and Daniel, 2008). Muscle fibre number is fixed soon after birth and postnatal growth is achieved by hypertrophy of the existing fibres, thus muscle fibre number is a critical determinant of muscle mass. Primary muscle fibres develop relatively early in gestation (Brameld and Daniel, 2008) and appear to be genetically determined. Secondary fibres, on the other hand, constitute the majority of the fibre population in developed muscle and are reduced in offspring born to nutritionally-restricted dams (Buttery *et al.*, 2000; Brameld and Daniel, 2008).

2.2 Hypothesised mechanisms of (intrauterine) programming

Programming can occur at any level within the affected physiological system and may involve structural and/or functional changes in genes, cells, tissues and even whole organs (Armitage *et al.*, 2004). However, the precise mechanism(s) of (intrauterine) programming is still not fully understood.

Several hypotheses have been formed in an attempt to explain the mechanism behind intrauterine programming. Neel (1962) introduced the concept ‘thrifty genotype’, meaning that this genotype was exceptionally efficient in the intake and/or utilization of food. He suggested that adaptations that allowed individuals to rapidly lay down fat in times of food surplus would have a survival advantage in the reciprocal periods of food shortages and famine (Neel, 1962). The ‘thrifty genotype’ has become widely adopted, although it remains imprecise and hard to identify (Prentice *et al.*, 2005).

The ‘thrifty’ phenotype hypothesis attempted to explain the relationship between poor fetal and infant growth, and increased risk of developing impaired glucose tolerance and metabolic syndrome in later life (Hales and Barker, 2001). The hypothesis proposed that environmental factors are the dominant cause of non-insulin dependent diabetes mellitus and the key elements are; poor development of the pancreatic β-cells mass and function and a link between poor early nutrition and later type II diabetes (Hales and Barker, 2001) (Figure 2.4).

The ‘fetal-insulin-hypothesis’ of Hattersley and Tooke (1999) suggested that low birth weight, insulin resistance, glucose intolerance, diabetes and hypertension in later life, could all be phenotypes of the same insulin-resistant genotype. Central to their fetal-insulin-hypothesis is the concept that insulin-mediated fetal growth will be affected by fetal genetic factors that regulate either fetal insulin secretion or the sensitivity of fetal tissues to the effects of insulin (Hattersley and Tooke, 1999), which possibly persists into later life.

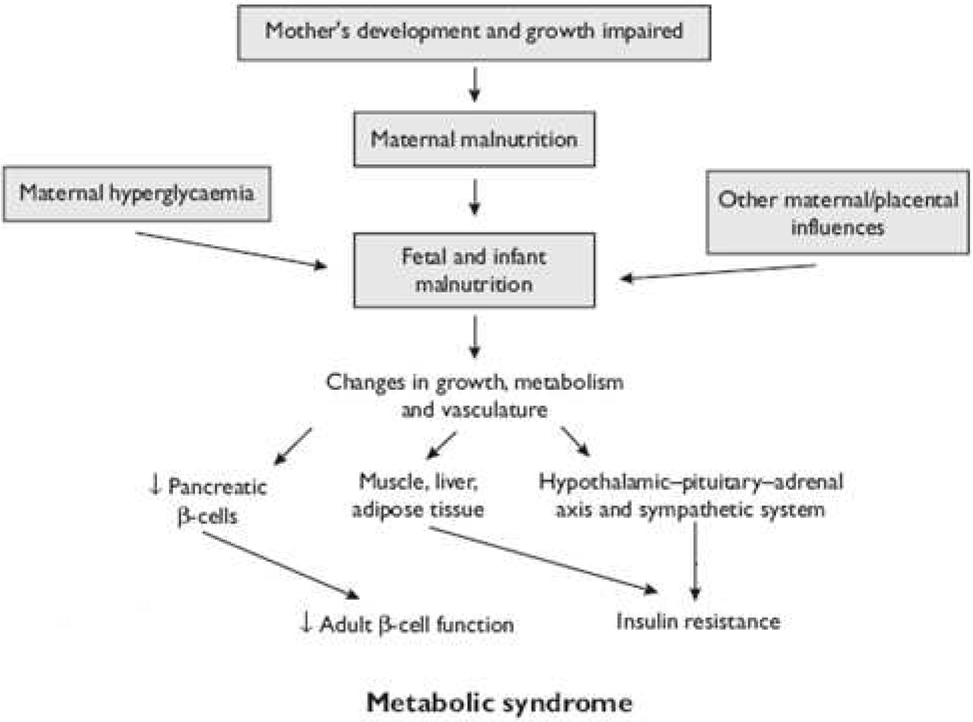

Figure 2.4. Diagram of the 'thrifty phenotype' hypothesis (Adapted from Hales and Barker (2001)).

Lee (1999) showed that mitochondrial DNA (mtDNA) was required for the regulation of glucose-stimulated insulin secretion and that under certain circumstances, abnormal mtDNA could cause insulin resistance (Lee, 1999). Thus, poor nutrition during early life would lead to mitochondrial changes that contributed to the development of type II diabetes and insulin resistance in adult life (mitochondrial-based model) (Lee *et al.*, 2005). However, the mechanism by which under nutrition in early life causes mutation and reduction of mtDNA is still unknown (Lee, 1999).

The predictive adaptive response was defined by Gluckman *et al.* (2005) as "a form of developmental plasticity that evolved as adaptive responses to environmental cues acting early in the life cycle, but where the advantages of the induced phenotype is primarily manifest in a later phase of the life cycle" in an attempt to explain intrauterine programming.

Recently, particular attention has focused on the role of epigenetic processes in intrauterine programming (Gicquel *et al.*, 2008). Epigenetics relates to stable and heritable patterns of gene expression that do not involve changes in DNA sequence (Gicquel *et al.*, 2008). In addition, epigenetic mechanisms play an important role in regulating gene expression and are required to achieve the stable expression or repression of genes at defined developmental stages and could possibly be one of the main mechanisms of *in utero* and/or early postnatal life programming.

2.3 Potential intrauterine growth restriction models

Intrauterine growth restriction (IUGR) can be induced in numerous ways in ovine pregnancies. Models to investigate IUGR in sheep are, for example, surgical models which include carunclectomy (Jones *et al.*, 1988; Rees *et al.*, 1998; Butler *et al.*, 2002; De Blasio *et al.*, 2007), uterine and umbilical artery embolism (Gagnon *et al.*, 1997; Bloomfield *et al.*, 2002) and uterine artery ligation (Brown and Vannucci, 1978). Another widely investigated model is the use of exogenous glucocorticoid administration during pregnancy, because of the known influence of glucocorticoids on fetal growth and development (Ikegami *et al.*, 1997; Dodic *et al.*, 1998; Jobe *et al.*, 1998; Sloboda *et al.*, 2000; Moss *et al.*, 2001; Sloboda *et al.*, 2002; Fowden *et al.*, 2006). Dam parity and the effect of age during pregnancy have also been used as a model for IUGR as adolescent or first parity ewes give birth to lighter lambs than second or third parity ewes (Gluckman and Hanson, 2004; Gardner *et al.*, 2007b; Gootwine *et al.*, 2007).

Dietary manipulations of the dam have also been the focus of many studies (Armitage *et al.*, 2004). In ovine models, the global caloric restriction model is predominantly used to investigate the effects of nutrition during pregnancy on postnatal life of the offspring. Effects of dietary manipulations are the focus of this thesis. Therefore, the following section will focus on known consequences of maternal nutrition on the resulting offspring.

2.4 Known effects of maternal nutrition on offspring

Growth

Animal studies have shown that alterations in maternal diet around the time of conception can change the fetal growth trajectory (Barker, 2003), which subsequently, can alter postnatal growth of the offspring.

It can be observed from Table 2.1 (adapted from Kenyon (2008)) that undernutrition in early pregnancy is less likely to reduce birth weight and live weight later in the life of the offspring compared to undernutrition of the dam during later stages of pregnancy. In addition, growth after birth can be affected by maternal nutrition, without necessarily affecting birth weight. For example, while wether lambs born to dams fed 50% of their nutrient requirements from days 28 - 78 of pregnancy showed no differences in birth weight, daily liveweight gain from 60 - 120 days of postnatal age was greater and they remained heavier, compared to control offspring (Ford *et al.*, 2007). This increased liveweight gain during early postnatal life has been referred to as catch-up growth and has been shown to be an important contributor to an altered metabolic function in adult life (Ozanne and Hales, 2005). Within mismatched twins (difference in birth weight >25%), light lambs at birth grew slower during the first 6 months of life compared to their heavier twins without an impairment in glucose homeostasis. Thus, Clarke *et al*. (2000) hypothesised that the somatotrophic axis may be reset in light lambs, resulting in altered growth characteristics. Therefore, no catch-up growth was found in the light lamb of the twin-set. However, care must be taken when interpreting the effects of maternal nutrition on postnatal growth of the offspring as there can be a carry-over effect of dietary manipulation on the dam's subsequent milk production, especially during late gestation (Mellor and Murray, 1985). This carry-over effect may either mask or exacerbate any potential effects on the growth of the offspring.

In summary, maternal undernutrition during early stages of pregnancy is unlikely to affect the birth or later live weight of the offspring, however, undernutrition during later stages of pregnancy can negatively affect birth weight and later live weight of the offspring, which can be exacerbated if the lactational performance of the dam is affected.

Table 2.1. The effects of maternal dietary manipulation during different periods of gestation on birth and live weight of the offspring.

Time of dietary manipulation	Dietary manipulation	Effect on birth weight	Effect on live weight/growth after birth	Reference
60 days premating (d-60) – day 7 of pregnancy (d7) then d8 - d147	0.7 maintenance (M) vs. 1.0 M (four treatments)	No effect in singleton fetuses Twin fetuses were lighter in 1.0 M followed by 0.7 M	-	(Edwards and McMillen, 2002)
d-14 - d70	0.85 M vs. 1.0 M	No effect	No effect at 82-90 d of age	(Hawkins *et al.*, 2000a)
d1 - d30	0.5 M vs. 1.0 M	No effect	No effect	(Gardner *et al.*, 2006)
d0 - d95	0.5 M vs. 1.0 M	No effect	-	(Gopalakrishnan *et al.*, 2004)
d0 - d95	0.5 M vs. 1.0 M	No effect	No effect	(Rae *et al.*, 2002)
d0 - d30 or d110 - parturition	0.5 M vs. 1.0 M	No effect	No effect at 1 yr of age	(Gardner *et al.*, 2005)
d28 - d78	0.5 M vs. 1.0 M	No effect	No effect	(Gilbert *et al.*, 2005)
d28 - d78	0.5 M vs. 1.0 M	No effect	0.5 M: heavier at 280 d of age	(Ford *et al.*, 2007)
d28 - d78	0.5 M vs. 1.0 M	0.5 M: lighter fetuses at d78 of pregnancy	-	(Vonnahme *et al.*, 2003)
d30 - d96 vs. d50 - d96 vs. d75 - d96	High vs. low (10 vs. ~3.4 kg DM/week)	No effect	-	(McCrabb *et al.*, 1992)
d45 - parturition	High vs. low based on pasture quality	Low: lighter	Low: lighter until 18 mo of age, no effect thereafter	(Gunn *et al.*, 1995)
d105 - d115 (UN10) vs. d105 - d125 (UN20)	0.3 – 0.5 vs.13-15 MJ/d	UN20: lighter	UN20: lighter at weaning. No effect at 30 mo of age	(Oliver *et al.*, 2001)
d100 - parturition	0.7 M vs. 1.0 M	0.7 M: lighter	0.7 M: lighter at 14 wks of age	(Borwick *et al.*, 2003)
Last 6 wks of gestation	7 vs. 15 MJ ME/d	7 MJ: lighter	7 MJ: lighter at 11 mo of age, no effect thereafter	(Husted *et al.*, 2008)
Last 6 wks of gestation	0.6 M vs. 1.0 M	0.6 M: lighter	0.6 M: lighter at weaning No effect at 145 d of age	(Tygesen *et al.*, 2007)
d104 – parturition until 1 yr of age	High vs. low based on pasture quality	No effect	Low: lighter	(Gunn, 1977)
2 wks – 15wks of age followed into adult life	High vs. low based on pasture quality	No effect	Low early: lighter at 14 and 24 wks and 18 mo; Low adult: lighter at 30, 42 and 54 mo of age	(Rhind *et al.*, 1998)

d: days; yr(s): year(s); mo: months wks: weeks; UN: undernutrition: MJ ME/day: mega joules of metabolisable energy per day (Adapted from Kenyon (2008)).

Reproductive performance

Several studies have assessed the effects of nutritional programming of reproductive performance on the resulting offspring (Table 2.2; adapted from Kenyon (2008)). For example, global nutrient restriction by 50% during the first 95 days of gestation resulted in a reduction in ovulation rate in 20 month-old offspring (Rae *et al.*, 2002). However, no differences in luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations were found in response to a gonadotrophin-releasing hormone (GnRH) challenge, which could indicate that the difference in ovulation rate may be attributable to gonadotrophin-independent differences in ovarian development (Rae *et al.*, 2002). A possible explanation for the reduced ovulation rates could be that undernutrition retarded fetal folliculogenesis (Rae *et al.*, 2002). This hypothesis agrees with work by Da Silva-Buttkus *et al.* (2003), who found more primordial follicles, fewer primary follicles and no secondary follicles in piglets exposed to IUGR, which could result in reduced total number of follicles in the adult. These findings imply that a delay in follicular development had occurred in IUGR piglets, probably in the activation of primordial follicles (Da Silva-Buttkus *et al.*, 2003).

Gunn *et al.* (1995) compared the effects of high level nutrition during the last 100 days of gestation or first 100 days of lactation with naturally grazed controls. They showed that offspring born to ewes on a high nutritional level during late gestation and early lactation had increased fecundity over three successive parities. This was probably expressed through an effect on embryo or fetal loss index as there were no differences in live weight, body condition score or ovulation rate between offspring born to the high- or control-fed dams (Gunn *et al.*, 1995). A re-analysis of the data of Gunn *et al.* (1995) by Gardner *et al.* (2008) showed that the greatest effect of high level nutrition on mean lambing percentage, was during lactation. This is in agreement with subsequent work conducted by Rhind *et al.* (1998), who showed that undernutrition during the first month of life had a negative effect on the reproductive performance (fecundity) of the ewes compared to well-fed animals. Gunn (1977) showed, in a pasture based system, that offspring exposed to a low nutritional level during the rearing period and early life (birth - 12 months of age) had long-term negative effects on their ovulation rate and eventually litter size. This is in contrast to work by Allden (Allden, 1979), in which severe undernutrition during the first year of postnatal life didn’t result in reduced reproductive performance in adult Merino ewes.

A study in cattle showed that supplementation (0.45 kg/d of a 42% crude protein supplement) of the cow during the last trimester of pregnancy increased pregnancy rates of the heifer offspring compared with heifers born to non-supplemented cows (Martin *et al.*, 2007). However, supplementation had no effect on the age at which the heifers reached puberty. This is in agreement with work by Da Silva *et al*. (2001) using the ovine adolescent model. In this model, IUGR was induced by high maternal nutritional intakes throughout gestation (Da Silva *et al.*, 2001). The results showed that nutritionally induced growth restriction was insufficient to delay puberty in female lambs when they were fed *ad libitum* postpartum. These results support the accepted principle that if female lambs have reached a threshold body weight (energy balance) and when photoperiodic cues are appropriate, puberty commences, independent of any potential programming effect (Da Silva *et al.*, 2001). This is in contrast to work conducted by Adair (2001) in humans, who found a relationship between size at birth and age at puberty. Girls who were relatively long and light at birth reached puberty earlier compared to short and light girls at birth, even after adjusting for BMI and skin fold at 8 years of age. Additionally, the birth size effect was more pronounced among girls with increased postnatal growth (Adair, 2001).

In summary, maternal nutrition can affect reproductive performance of the resulting offspring during several critical windows ranging from early to late gestation and even into early postnatal life, however, the relative importance of each time period is not yet fully understood.

Table 2.2. The effects of maternal dietary manipulation during different periods of gestation on reproductive traits of the offspring.

Time of dietary manipulation	Dietary manipulation	Effect on reproductive traits	Reference
d1 - d35	0.5 M vs. 1.5 M	No effect on ovulation rate to two yrs of age	(Parr *et al.*, 1986)
d0 - d47	0.5 M vs. 1.5 M	0.5 M: negatively affects fetal ovarian development	(Borwick *et al.*, 1997)
d0 - d95	0.5 M vs. 1.0 M	0.5 M: reduced ovulation rate at 20 mo of age	(Rae *et al.*, 2002)
d0 - d30/d50; d31 - d50/d65; d66 - d110; d0 - d110	0.5 M vs. 1.0 M	0.5 M: delayed fetal follicle development	(Rae *et al.*, 2001)
d0 - d30/d50; d31 - d50/d65; d66 - d110; d0 - d110	0.5 M vs. 1.0 M	0.5 M: negatively affected ovarian development	(Lea *et al.*, 2006)
d30 - parturition	0.9 M vs. 1.1 M	0.9 M: reduced response to GnRH at 55 d of age No effect on female or male gonad weight at 55 d of age	(Deligeorgis *et al.*, 1996)
d45 - parturition and lactation	High vs. low based on pasture quality	Low: negatively affected reproductive life time performance.	(Gunn *et al.*, 1995)
d100 - parturition	0.7 M vs. 1.0 M	No effect on hypothalamic-pituitary function based on plasma LH and FSH concentrations	(Borwick *et al.*, 2003)
d104 - term until 1 yr of age	High vs. low based on pasture quality	Low: negatively affected ovulation rate	(Gunn, 1977)
2 wks - 15wks of age followed into adult life	High vs. low based on pasture quality	Low during early life: reduced life time reproductive performance	(Rhind *et al.*, 1998)

d: days; yr(s): year(s); mo: months wks: weeks; GnRH: gonadotrophin-releasing hormone; LH: luteinizing hormone; FSH: follicle stimulating hormone (Adapted from Kenyon (2008)).

Glucose metabolism

Several studies have investigated the effects of maternal undernutrition during pregnancy on glucose metabolism of offspring in sheep, with inconsistent results. For example, maternal undernutrition (50% vs. 100% of the nutrient requirements) from days 28 - 78 of pregnancy resulted in dysregulated glucose uptake in male offspring at both 63 and 250 days of age (Ford *et al.*, 2007). However, severe undernutrition of pregnant ewes for 10 (d105 - d115) or 20 (d105 - d125) days during late gestation, had little effect on glucose tolerance of the female offspring at 30 months of age once birth weight and weight at test-day were taken into account (Oliver *et al.*, 2002). On the other hand, when undernourishment was less severe for 30 days during late gestation (day 110 - term) compared to the study of Oliver *et al*. (2002) (0.5 M vs. 0.03 M, respectively), one-year-old male and female offspring born to undernourished dams showed glucose intolerance and insulin resistance after a glucose tolerance test (Gardner *et al.*, 2005). These results indicate that moderate undernutrition from late gestation through to term may have a greater impact on the glucose metabolism of the offspring than when severe undernutrition is followed by re-feeding before term.

Pregnant offspring, born to ewes fed 50% of the nutritional requirements during the last 6 weeks of pregnancy, showed no glucose intolerance or insulin resistance compared to pregnant control offspring (Husted *et al.*, 2008). However, when the pregnant offspring were exposed to a short period of undernutrition during late gestation, undernourished offspring were more glucose intolerant and insulin resistant compared to control offspring (Husted *et al.*, 2008).

Poulsen *et al*. (1997) showed an association between low birth weight and the risk of developing impaired glucose tolerance in discordant twins, such that the lighter baby of the twin-set had an increased risk of developing impaired glucose tolerance. Clarke *et al*. (2000) based their study on this association and investigated the effect of size difference at birth (> 25% weight difference) on glucose homeostasis between female twins. Light lambs were more glucose and insulin tolerant than the heavy and control lambs at one and six months of age, however, no differences were found at three and twelve months of age. One possible explanation for not finding a difference at three months of age is because this period coincides with endocrine changes in response to feeding and at the same time maturation of the islet of Langerhans within the pancreas occurs, in conjunction with the onset of growth hormone (GH) dependent growth (Clarke *et al.*, 2000).

The inconsistent results between studies could be due to differences in dam live weight and body condition, the timing within pregnancy, duration, level and type of the nutritional manipulation, postnatal management of the young offspring, and the timing and nutritional levels prior to the period of investigating the offspring (Mellor, 1983; Robinson *et al.*, 1999b; Wu *et al.*, 2006; Symonds, 2007; Kenyon, 2008). Besides all these factors, most of the studies have been carried out under controlled non-commercial conditions and it would be of merit to examine if the metabolic function of offspring born to dams fed suboptimally during pregnancy, due to, for example, shortage of grass under commercial grazing conditions, has been affected.

HPA-axis

Studies have been undertaken to investigate the effects of undernutrition during pregnancy on the HPA-axis functioning in ovine prenatal and some postnatal offspring (McMillen and Robinson, 2005), as it is believed that altered HPA-axis function is related to impaired glucose metabolism and insulin resistance (Phillips *et al.*, 1998).

Undernutrition (UN) during the periconceptional period (60 days prior to mating until 30 days after) resulted in a up-regulation of the HPA-axis in fetuses at day 128 of pregnancy compared to control fetuses (Bloomfield *et al.*, 2004). UN-fetuses showed a decrease in cortisol concentrations and an increase in adrenocorticotrophic hormone (ACTH) and 11-deoxycortisol concentrations, after a metyrapone challenge. This positive response to the metyrapone challenge in UN-fetuses indicated that 11β-hydroxylase was successfully inhibited, shown by the fall in cortisol concentration, a rise in 11-deoxycortisol, followed by a rise in ACTH concentration (Bloomfield *et al.*, 2004). This intact negative feedback loop in UN-fetuses could indicate that the HPA-axis was more mature than the HPA-axis of control fetuses (Bloomfield *et al.*, 2004).

Undernutrition from conception until day 70 of pregnancy (85% of the requirements, followed by 100% of the requirements) down-regulated the HPA-axis of fetuses in late gestation as shown by their response to a corticotrophin-releasing hormone/arginine vasopressin (CRH/AVP) challenge (Hawkins *et al.*, 1999) and acute hypoxaemia (Hawkins *et al.*, 2000b). However, lambs born to dams undernourished during early gestation (d0-d70) showed an up-regulated HPA-axis in response to a CRH/AVP challenge at 80 days of age (Hawkins *et al.*, 2000a). The UN-lambs showed greater cortisol and ACTH concentrations in response to the challenge than did control lambs (Hawkins *et al.*, 2000a). This finding of an up-regulated HPA-axis in postnatal life was also found in offspring born to dams undernourished during late gestation (Bloomfield *et al.*, 2003). Undernutrition for 10 days during late gestation (d105-

d115) resulted in greater ACTH responses to a CRH/AVP and insulin tolerance test in offspring at 30 months of age compared to control offspring, however, no differences in cortisol concentrations were found between groups in response to the challenges (Bloomfield *et al.*, 2003). No effect on cortisol concentrations in response to an insulin tolerance test were found in offspring born to dams grazed at 2 cm vs. 6 cm sward from days 64 – 132 of pregnancy (Corner *et al.*, 2005), however, no ACTH concentrations were measured, thus it is unknown if the HPA-axis was up-regulated at hypothalamic or pituitary level.

In summary, undernutrition during early and mid gestation resulted in a down-regulation of the fetal HPA-axis and an up-regulation of the axis during postnatal life. The down-regulated fetal HPA-axis might be an adaptive mechanism of the undernourished fetuses, as suggested by Bloomfield *et al*. (2003), to postpone delivery so that the fetuses increase their chance of survival. Hawkins *et al*. (2000a) suggested that the up-regulated HPA-axis in postnatal life of offspring born to undernourished dams might be due to a reduction of glucocorticoid receptor numbers in the pituitary and therefore, corticoid-mediated inhibition of the HPA-axis is reduced, resulting in elevated concentrations of glucocorticoids. In humans, high concentrations of cortisol have been associated with raised cholesterol concentrations and increased incidence of type II diabetes (Matthews, 2002).

Fat metabolism

Children of women who experienced the Dutch famine in 1945 during early gestation had an artherogenic lipid profile (Roseboom *et al.*, 2000) and increased risk of abdominal obesity in adult life (Ravelli *et al.*, 1999). Adolescent male offspring born to ewes undernourished (0.5 M vs. 1.0 M) from mid (days 28 – 78) or late (days 100 – parturition) gestation showed increased adiposity compared to those born to control-fed ewes in combination with glucose intolerance and insulin resistance (Gardner *et al.*, 2005; Ford *et al.*, 2007). This was found in combination with a significant increase in protein expression for the insulin receptor β-subunit and p110β-subunit of phosphoinositide 3-kinase (PI3-kinase) but a decrease in glucose transporter-4 (GLUT-4) protein expression in perirenal fat (Gardner *et al.*, 2005). The proposed mechanism for this disturbed glucose-insulin homeostasis appears to be altered adipose tissue glucose uptake, through reduced cellular expression of GLUT-4 (Gardner *et al.*, 2005). Adipocytes from male adolescent rats born to dams fed a low protein (LP) diet (8% vs. 20% protein) throughout pregnancy and lactation, showed greater basal and insulin stimulated glucose uptake compared to adipocytes from their control-fed counterparts (Ozanne *et al.*, 1999). In addition, the adipocytes of the LP-offspring showed increased insulin receptor number and insulin receptor substrate-1 (IRS-1) associated PI 3-kinase activity, which could explain the increased glucose uptake found by Ozanne *et al.* (1999) and is in agreement with the findings in sheep by Gardner *et al*. (2005). However, the cellular expression of GLUT-4 was not measured in the adipocytes of the male rats, therefore, it is unknown if the increased glucose uptake found in LP-offspring is due to a greater number and activity of insulin receptors to counteract for the possible reduction in GLUT-4, as was observed in sheep (Gardner *et al.*, 2005), or due to possible increased GLUT-4 in adipose tissue. Maternal low-protein diet during pregnancy in rats has shown that the lipid metabolism homeostasis can be altered in the offspring, showing greater plasma triacylglycerol and non-esterified fatty acids (NEFA) concentrations, increased expression of hepatic peroxisome proliferator-activated receptor-α (PPARα) and decreased expression of PPARγ in adipose tissue (Burdge *et al.*, 2004) and increased gene expression of lipogenic enzymes in the liver (Maloney *et al.*, 2003). However, the effects of the LP-diet on plasma triacylglycerol and NEFA in rat offspring is not consistent, as Desai *et al.* (1997) and Lucas *et al.* (1996) showed that the concentrations can be unaltered or decreased compared to control rat offspring. In addition, Ozanne *et al.* (1999) showed that adipocytes of the LP-rat offspring were more sensitive to catecholamines and showed resistance to the anti-lipolytic action of insulin. In humans, obese subjects showed impaired catecholamine-induced lipolysis and this may contribute to the development or maintenance of increased adipose tissue stores and obesity in humans (Jocken and Blaak, 2008). However, there is no information on the effects of undernutrition during pregnancy on the fat metabolism of the offspring in response to catecholamines in sheep. Therefore, data obtained from, for example, an epinephrine challenge to investigate if offspring born to undernourished dams are more “thrifty” by protecting their adipose tissue from lipolysis, would make a significant contribution to the programming literature.

Mammary gland development and lactation

Many environmental factors applied postnatally are known to affect milk production of the dam (Walker *et al.*, 2004; Pulina *et al.*, 2006) however, to date, little is known about the effects of *in utero* conditions on an animal's subsequent lactational performance. Jenkinson (2003) has shown that fetuses carried by dams fed maintenance from days 19 - 140 of pregnancy had smaller duct areas compared to fetuses carried by a dam fed 1.5 times maintenance. Knight and Sorensen (Knight and Sorensen, 2001) suggested that deficient ductular development in early fetal life may affect secretory tissue mass because the secretory cells proliferate on the ducts. Since all the dams in the study of Jenkinson (2003) were euthanized during gestation, it is unknown if the smaller fetal mammary glands would have resulted in impaired milk production in adult life. Nevertheless, maternal undernutrition during the last 6 weeks of pregnancy had no effect on milk yield and composition of offspring (Husted *et al.*, 2008). Research in dairy cattle showed that offspring born to high-yielding dams, have reduced milk production in their first and third lactation (Banos *et al.*, 2007; Berry *et al.*, 2008). Therefore, it is possible that postnatal mammogenesis may be affected by prenatal intrauterine conditions (Berry *et al.*, 2008). The negative maternal effect on the offspring's milk production observed by Berry *et al*. (2008) and Banos *et al*. (2007) is in contrast to the work of Koch (1972) in beef cattle, who suggested that the environmental covariance correlation between offspring and dam is negative. Thus, offspring born to and reared by dams with low milk production would have high milk production when rearing their offspring, which would then result in greater weaning weights. The work of Koch (1972) would suggest a 'critical window' during early postnatal life that affects the offspring's milking and rearing ability.

Offspring born to rats fed a LP-diet during gestation and lactation had reduced mammary gland growth compared with control offspring (Fernandez-Twinn *et al.*, 2007). Subsequently, the mammary glands of LP-offspring showed rapid compensatory development, specifically in epithelial density, for up to two weeks after weaning (Fernandez-Twinn *et al.*, 2007).

These reported intrauterine effects on milk yield are potentially at odds with the view that prenatal mammary development is autonomous and, therefore, not likely to be affected by external factors (Robinson *et al.*, 1999a). However, based on the above studies it is not possible to clearly identify whether the cyclical pattern of milk production is due to factors in the fetal period, the early postnatal period or a combination of both, as nutrition of the dam during pregnancy will affect her subsequent milk production. Thus, the offspring could potentially be affected *in utero* and/or in early life.

Intergenerational effects on birth weight and growth

Emanuel (1986) defined intergenerational factors as those factors, conditions, exposures and environments experienced by one generation that relate to the health, growth and development of the next generation. The potential mechanism behind these intergenerational factors is epigenetics (Gicquel *et al.*, 2008). To date, most attention has been given to the ‘first generation’ of offspring exposed to a manipulation *in utero* and early postnatal life, however, there is growing evidence from epidemiologic studies in humans and experimental studies in rodents that the so-called ‘programmed phenomena’ can be perpetuated in later generations (Drake and Walker, 2004).

Human evidence from the Dutch famine studies suggested that maternal exposure to the famine had no significant effect on the birth weight of their grand offspring (second generation), independent of stage of pregnancy (Stein and Lumey, 2000). Data from the national birth cohort of British births (National Child Development Study) showed that the height of the grandmother is positively associated with the birth weight of the grandchildren (Emanuel *et al.*, 1992).

Rodent studies have shown that offspring born to dams injected with dexamethasone during pregnancy gave birth to lighter second generation offspring (G_2) than offspring born to control dams (Drake *et al.*, 2005). However, this effect was not observed in the third generation (G_3) (Drake *et al.*, 2005). Birth weights of rats whose ‘grand’ dams were fed a protein restricted diet

(8% vs. 20%) during pregnancy and lactation, were not affected by the diet of the ‘grand’ dam (Benyshek *et al.*, 2006). This observation is in contrast to other rodent protein restriction models, which found that protein restricted nutritional regimens of the ‘grand’ dam were associated with lower birth weights and poorer growth of the ‘grand’ offspring (Zambrano *et al.*, 2005; Pinheiro *et al.*, 2008).

To date, no data are available in sheep on the intergenerational effects of maternal nutrition on birth weight or growth of the second generation of offspring. It would be of interest to examine intergenerational effects of nutrition during pregnancy, as it could negatively affect the productive performance (number and weight of lambs weaned) of ewe offspring kept as replacements of the breeding flock.

2.5 Other long-term effects of dietary manipulations on offspring

Epidemiological studies in humans have shown that impaired growth *in utero* and low birth weight relative to genetic potential in particular, was associated with hypertension, ischemic heart disease, glucose intolerance, insulin resistance, type II diabetes, hyperlipidemia, hypercortisolemia, obesity, obstructive pulmonary disease and reproductive disorders in adult life (Elias *et al.*, 2005; Painter *et al.*, 2005; Fowden *et al.*, 2006).

Dietary manipulation by global caloric restriction of the dam has been the focus of a large body of work in ovine models (Armitage *et al.*, 2004) and has been shown to affect cardiovascular function (Hawkins *et al.*, 2000a; Gardner *et al.*, 2004; Gopalakrishnan *et al.*, 2004), reduce nephron number in the kidney (Gilbert *et al.*, 2005), increase adiposity (Gardner *et al.*, 2005; Ford *et al.*, 2007), glucose intolerance and insulin resistance (Oliver *et al.*, 2002; Gardner *et al.*, 2005), HPA-axis function (Hawkins *et al.*, 2000a; Bloomfield *et al.*, 2003) and animal production traits (Gunn *et al.*, 1995; Rhind *et al.*, 2001) with or without affecting birth weight from its relative genetic potential. These results are important for furthering our understanding of how maternal nutrition affects the performance and health of the offspring, to be able to improve production and animal well-being in animal production systems.

2.6 Effects of maternal constraint on offspring

Maternal constraint describes the set of non-genetic and non-pathological influences by which the mother limits fetal growth; this is a reflection of the absolute limitation on the capacity of the mother and placenta to supply nutrients to the fetus(es) (Gluckman and Hanson, 2004). IUGR occurs naturally in animals carrying multiple fetuses and the inverse relationship between the number of fetuses and the mean birth size of the litter is well described (Gluckman and Hanson, 2004; Gardner *et al.*, 2007b; Gootwine *et al.*, 2007). Therefore, litter size models are good models to study naturally occurring IUGR. Other natural approaches to induce IUGR are, for example, exposing dams to different altitudes (Parraguez *et al.*, 2005) or heat stress (Cartwright and Thwaites, 1976; McCrabb *et al.*, 1993) during pregnancy.

Maternal constraint due to body size of the dam is the main focus of this thesis as body size is important in terms of efficiency of the animal from an agricultural production perspective. For example, Morel and Kenyon (2006) have shown that unless a larger animal is more productive, it is less efficient. Although, few data are available on possible long-term effects of maternal constraint, due to dam size, on the offspring's metabolic function and/or production performance, the long-term effects of dam size will be reviewed.

Growth

The heritability of birth weight through to adult weight in sheep is shown in Table 2.3 (adapted from Safari *et al.* (2005). These data indicate that larger ewes are more likely to have larger adult offspring. In addition, mating weight of the dam is positively associated with the birth weight of twin lambs (Kenyon *et al.*, 2004b). In polytocous species, the inverse relationship between the number of fetuses and the mean birth size of the litter is well described (Gluckman and Hanson, 2004; Gardner *et al.*, 2007b; Gootwine *et al.*, 2007) and this is most likely due to the limited maternal uterine space (Gardner *et al.*, 2007b). In addition, fetal growth is matched to maternal size, otherwise normal birth could not occur (Barker, 2003). Embryo transfer and cross-breeding experiments have shown in large and small breeds of sheep (Dickinson *et al.*, 1962; Gootwine *et al.*, 1993; Jenkinson *et al.*, 2007; Sharma *et al.*, 2009), horses (Walton and Hammond, 1938; Allen *et al.*, 2002) and pigs (Wilson *et al.*, 1998) that there is an interaction between fetal and maternal factors, which determines the extent of fetal growth and that the maternal environment alone does not determine or support maximal fetal growth (Gootwine *et al.*, 2007). This is supported by the negative genetic correlation for birth weight shown in Table 2.3. Thus, a small dam will constrain the growth of her offspring more than a big dam and this constraining effect is exacerbated if a small dam carries multiple offspring, resulting in lighter offspring at birth. Lower birth weight is associated with reduced postnatal growth rate, however, Greenwood *et al.* (1998) showed that very low-birth-weight lambs could reach similar growth rates compared to heavy-birth-weight lambs under artificial rearing conditions. Lambs born as triplets and quadruplets are lighter at birth and have lower growth rates than that of lambs born as singletons, suggesting that litter-size dependent factors (e.g. milk yield) are also responsible for the relatively low postnatal growth (Gootwine *et al.*, 2007)

Thus, based on these data, it could be hypothesised that offspring born to larger ewes have an advantage over offspring born to smaller ewes by being born heavier, especially when the dam is carrying multiple lambs.

Table 2.3. Weighted means (± S.E.) of estimates for direct (h^2) and maternal (m^2) heritability and correlation between direct and maternal genetic effects (r_{am}) for growth traits in sheep

Trait	h^2 ± S.E.	m^2 ± S.E.	r_{am} ± S.E.
Birth weight	0.19 ± 0.02	0.18 ± 0.02	-0.08 ± 0.06
Weaning weight	0.16 ± 0.01	0.10 ± 0.01	0.34 ± 0.04
Post-weaning weight	0.28 ± 0.03	0.04 ± 0.01	-0.07 ± 0.13
Adult weight	0.40 ± 0.06	0.06 ± 0.03	-0.16 ± 0.29

Estimates are for dual purpose breeds (Adapted from Safari *et al.* (2005)).

Reproductive performance

The direct effects of ewe size on ovulation rate and fecundity have been well studied (reviewed by Michels *et al.* (2000)). It has been shown that large Merino ewes had more multiple ovulations than smaller ewes and there was a significant linear relationship between ovulation rate and ewe weight or body size (Michels *et al.*, 2000). However, Rutherford *et al.* (Rutherford *et al.*, 2003) showed that there is a different relationship between ovulation rate and number of lambs with ewe live weight at breeding for small- and large- framed mixed-aged Coopworth ewes. A positive relationship was found between ovulation rate and ewe live weight at breeding for small-framed ewes, however, no significant relationship was found in large-framed ewes, which indicates a likely upper limit to the relationship for large-framed ewes (Rutherford *et al.*, 2003). This is in agreement with work by Kenyon *et al.* (2004a) who also found an upper limit in the relationship between ewe live weight and reproductive performance.

Thus, size of the dam has direct positive effects on the reproductive performance of the dam itself (given the upper limit of the relationship has not been reached), which in turn can be inherited by the offspring (Table 2.4; adapted from Safari *et al.* (2005)). Therefore, offspring born to a larger dam could have the potential to give birth to and wean more lambs than offspring born to smaller dams.

Table 2.4. Weighted means (± S.E.) of estimates for direct (h^2) heritability of reproduction traits and weighted means (95% confidence interval) for genetic correlations between adult weight and reproduction traits in sheep.

Trait	h^2 ± S.E.[1]	Adult weight
Ovulation rate	0.15 ± 0.02	-
Lambs born / ewe mated	0.10 ± 0.01	0.15 (-0.38 – 0.61)
Lambs weaned /ewe mated	0.07 ± 0.01	0.33 (0.16 – 0.48)
Weight weaned / ewe mated	0.13 ± 0.03	0.70 (0.30 – 0.89)

[1] Estimates are for dual purpose breeds (Adapted from Safari *et al.* (2005))

Glucose and fat metabolism

Rams born to dams with low body condition score during pregnancy showed impaired glucose tolerance at 72 weeks of age compared to rams born to dams with high body condition score

(Cripps *et al.*, 2008). These findings are in agreement with data obtained from human adults born between 1948 to 1954 in Beijing, China. Adults born to mothers with a low maternal body mass index in early and late gestation showed elevated concentrations of plasma glucose, insulin and triglycerides (Mi *et al.*, 2000). Even though the mothers were of short stature, the poor condition of these women was most likely due to undernutrition, because during the period of 1948 to 1954 most of the population was chronically malnourished (Mi *et al.*, 2000).

The constraining effect of litter size showed that light-born sows (< 10th percentile) had greater glucose intolerance at six months of age and during late pregnancy compared to their normal-birth-weight counterparts (Corson *et al.*, 2009). The effects of dam size within the same population/breed (small vs. large) on metabolic function of the offspring has been given little attention to date in sheep. It would be of value to determine if maternal constraint affects metabolic function in the offspring in a similar way as maternal nutrition does.

HPA-axis

The direct effects of maternal constraint due to dam size within the same population or breed (small vs. large) have received little investigation to date, however, some relationships between body size and HPA-axis measures have been found. For example, BMI of men aged 66-77 years and who were small at birth, showed a linear increase with total urinary cortisol metabolite excretion (r = 0.19) (Reynolds *et al.*, 2001). In addition, increased lean body mass was also associated with greater total urinary cortisol metabolites in men born small (r = 0.23) (Reynolds *et al.*, 2001). This latter association is in agreement with work conducted in nine-year-old children who were light at birth, in which urinary glucocorticoid metabolite excretion was positively related to weight (Clark *et al.*, 1996). These data indicate that in humans, who were born small, the HPA-axis is up-regulated (increased cortisol metabolites) when weight or BMI in later life increases. However, in sheep, the opposite relationship was found. Area under the cortisol curve in response to an insulin tolerance test was negatively related to live weight at the time of the test in 30-month-old sheep, irrespective of maternal nutritional treatment during pregnancy (Bloomfield *et al.*, 2003).

Therefore, it would be of considerable value to determine if the size of the ewe affects the HPA-axis function of the offspring and what level of the axis is affected as the HPA-axis can affect behaviour and is believed to play a role in the onset of impaired glucose metabolism and insulin resistance (Phillips *et al.*, 1998).

Mammary gland development and lactation

It is well known that the survival and growth of lambs is dependant on their dams' milk supply in the first three to four weeks of life (Peart, 1967, 1968). To date, the direct effects of dam live weight and body condition on her milk production have been well studied (Peart, 1968, 1970; Berry *et al.*, 2006). It is believed that larger dams have more body capacity to consume more feed, which in turn, might allow the dam to produce greater volumes of milk. However, McGloughin and Crowley (1970) compared milk yields of four breeds of sheep and found no significant relationship between milk yield and ewe body weight among the breeds. Therefore, they suggested that if average body weight differences among the breeds were taken into consideration, the smaller breeds would be more efficient at producing milk (McGloughlin and Crowley, 1970). This finding is in agreement with work in dairy cows, within the same breed. Swali and Wathes (2006) investigated if maternal age and milk yield during pregnancy could alter birth size and if this birth size effect would alter subsequent milk production. They found that older cows (≥ 3 lactations) with a high peak milk yield (> 42 kg/day) were more likely to produce light calves at birth, which remained light throughout their life, compared to calves born to cows in their first or second lactation producing less milk. However, despite the smaller size at birth, there were no differences in milk production between high-, average- or low-birth-weight offspring in this study (Swali and Wathes, 2006). In addition, Holstein cows from two selection lines (large vs. small body size) differing in live weight, body dimensions and birth weight of calves, did not differ in milk production (Hansen *et al.*, 1999).

The long-term effects of being born to either a large dam or small dam on milk production have received relatively minor attention, especially in sheep. Information on the effects of ewe size on the subsequent lactation performance of her offspring would give a better understanding in the efficiency of ewes rearing their lambs, which could lead to more specific advice to farmers whether to select for large or small ewes.

Intergenerational effects on birth weight and growth

The constraining effect of litter size in pigs showed that sows born light (L) (< 10th percentile) gave birth to piglets with a significant increased variation in birth weight in contrast to sows born in the normal birth weight range. These sows had litters with little variation in birth weight (Corson *et al.*, 2009). However, the piglets born to L-sows showed no difference in weight after day 21 of age. After weaning, the small piglets born to L-sows showed reduced growth rates compared to the larger piglets born to L-sows, but Corson *et al*. (2009) suggested this might be due to that these animals were lower in social hierarchy in the post-weaning groups and therefore had reduced access to food. The effects of dam size on birth weight and growth of the ‘grand’ offspring would be of interest to determine, as this would give more insight in the efficiency of lamb production between large and small dams.

2.7 Perspective and hypotheses

Nutritional regimen during pregnancy could have important consequences for the offspring and for farmers. Therefore, it is of high interest to know how maintenance nutrition (due to, for example, shortage of feed due to slow pasture growth following low temperatures and reduced day light) during pregnancy could affect the performance of the offspring, especially those kept for replacing breeding ewes. In addition, there is still an ongoing debate among farmers regarding the efficiency of heavy/large versus light/small ewes and whether the size of the ewe could have implications for the performance of her offspring. The effects of ewe size and nutritional regimen during pregnancy under commercial grazing conditions on the long-term performance of offspring have not been thoroughly investigated.

Objectives and hypotheses

The objectives of this thesis are to examine the effects of maternal constraint (ewe size being heavy or light) and nutritional regimen (maintenance requirements of non-pregnant ewes or *ad libitum*) from days 21 – 140 of pregnancy under New Zealand grazing conditions on growth, metabolic function, reproductive and lactational performance of the first generation of offspring and birth and weaning weights of the next generation of offspring.

Based on the data available to date, it is hypothesised that a smaller versus a larger dam and maintenance versus *ad libitum* nutrition during pregnancy, would negatively affect:

- birth weight and early postnatal growth of the offspring
- metabolic function of the offspring
- reproductive and lactational performance of the offspring
- birth and weaning weight of the second generation of offspring

CHAPTER 3

COMPARISON OF FOUR TECHNIQUES TO ESTIMATE MILK PRODUCTION IN EWES

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Abstract

Accurate estimates of milk production or milk intake are difficult, as all methods interfere to some degree with the natural behaviour of the dam and her young, and potentially alter milk yield itself. The present study compared milk yield obtained by the "oxytocin" method, udder dimensions (UD), the isotope dilution method, and liveweight change of the lamb, in an attempt to select the most accurate and convenient way of measuring milk production in non-dairy sheep. In addition, the study investigated which of the three milk-estimation techniques was an accurate predictor of lamb growth rates. Thirty-seven singleton-bearing-and-rearing ewes were milked once a week, for seven consecutive weeks, using the "oxytocin" method. Prior to each afternoon milking, the external dimensions of the ewe's udder were measured. Lambs were weighed weekly for the first seven weeks of life and liveweight change was calculated. The deuterium oxide (D_2O) dilution technique was used to estimate milk intake of the lambs and was performed at approximately 7 days post-partum and finishing on approximately day 14. Pearson's correlation coefficients and multiple regression coefficients among techniques were calculated. The UD-models at d7 ($R^2 = 0.35$), d35 ($R^2= 0.36$) and d42 ($R^2 = 0.34$), were the best models explaining variation in milk yield (concordance correlation coefficient (CCC) = 0.49; 0.53; 0.51; for d7, d35 and d42, respectively). The lamb liveweight-change model explained the variation in milk yield best at d28 ($R^2 = 0.32$; CCC = 0.49), at d35 ($R^2 = 0.22$; CCC = 0.36) and at d42 ($R^2 = 0.28$; CCC = 0.44). At d14, the intake of milk by lambs as measured by the D_2O technique, did not explain the variation in milk yield. In conclusion, udder dimensions, lamb liveweight change and lamb milk intake are relatively poor estimators of the milk yield of singleton-rearing ewes obtained by the "oxytocin" method. Additionally, udder dimensions, milk yield and lamb milk intake do not give an accurate prediction of growth rates of singleton lambs. These results emphasize that there is a difference between ewe milk production potential and lamb milk intake, which need to be considered when estimating milk production in non-dairy animals.

Introduction

Milk is the sole source of nutrients for the new-born mammal, thus, its survival and potential to reach maturity is directly dependent upon the success of its dam's lactation (Macfarlane *et al.*, 1969). Accurate estimates of milk production or milk intake are difficult, as methods used have the potential to interfere with the natural behaviour of the dam and her young (Treacher and Caja, 2002) or with milk yield itself. Studies determining milk production, and milk intake of pre-ruminants, have usually been based either on the weigh-suckle-weigh method or hand or machine milking of the dam after oxytocin administration ("oxytocin" method) (Robinson *et al.*, 1968; Aboul-Naga *et al.*, 1981; Dove, 1988).

There are potential errors with these methods. The weigh-suckle-weigh method can underestimate lamb milk intake, if handling and disturbance of the animals interferes with normal suckling behaviour or milk ejection of the dam (Robinson *et al.*, 1968; Dove, 1988). Milking of the dam can lead to an overestimation of lamb milk intake when compared to the weigh-suckle-weigh method, as milking could result in a greater degree of udder emptying than that achieved by the offspring (Robinson *et al.*, 1968), especially if it is a singleton. In addition, both methods have the disadvantage of estimating milk yield or intake during a period of disturbance and extrapolating this to an undisturbed grazing situation (Dove, 1988).

Macfarlane *et al.* (1969) developed a technique to estimate lamb milk intake based on the total water turnover, utilising isotopically labelled water. They suggested that this technique offers several advantages over other methods, as milk intake is estimated in naturally suckling offspring between, rather than during, periods of disturbance (Macfarlane *et al.*, 1969). Another method of estimating milk yield of the dam is to measure the growth rate of the lamb. Lamb growth rates were found to be highly correlated with milk production, particularly during early lactation (Robinson *et al.*, 1968). In addition, the size of the udder could be used as a predictor for ewe milk production and lamb growth (Mellor and Murray, 1985; Snowder *et al.*, 2001).

However, no studies have compared the various techniques in one study. Therefore, the present study compared four methods of estimating milk yield ("oxytocin" method, udder dimensions, isotope dilution method and liveweight change of the lamb) in an attempt to select the most accurate and convenient way of measuring milk production in non-dairy sheep. In addition, the study investigated which of the three milk-estimation techniques is the most accurate predictor of lamb growth rates.

Materials and Methods

The study was conducted at the Massey University Keeble Sheep and Beef farm, 5 km south of Palmerston North, New Zealand. The study and all animal handling procedures were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Ewe milk production – "oxytocin" method

Thirty-seven ewes bearing and rearing singleton lambs were milked once a week, for seven consecutive weeks, using the "oxytocin" method (Morgan *et al.*, 2006). The first milking commenced at a mean day 7 (range of 5 to 9 days) after parturition. Ewes were divided into two milking groups, based on time of parturition. On each milking occasion, ewes were initially milked by machine followed by hand-stripping, after an intravenous injection of 1 IU synthetic oxytocin (Oxytocin V, 10 IU/mL, PhoenixPharm, Auckland, New Zealand). The time when the udder was empty was recorded. Animals were milked again (machine and hand-stripping) approximately five hours later, when the time and weight of the milk were recorded. Lambs were separated from the ewes and bottle fed as required during the intervening period. Daily milk yield was calculated using the formula:

$$\frac{24\ hours}{\text{[illegible]}} \times milk\ \ yield\ \ at\ 2^{nd}\ \ milking\ .$$

Ewes were drenched (Matrix Low Mineral (1 g/l abamectin, 40 g/l oxfendazole and 22.7 g/l levamisole) Ancare New Zealand Ltd.) at the first two weekly milkings followed by drenching every fortnight, to ensure milk production would not be affected by gastrointestinal nematode infections, which was confirmed by zero faecal egg counts.

Lamb growth rates and liveweight change

Lambs were ear tagged, identified to their dam and weighed within 24 h after birth. Lambs were weighed weekly for the first seven weeks of life.

Lamb growth rates (LWG: g/day) were calculated as:

$$\frac{(LW_{d+7} - LW_{d}) \times 1000}{days}$$

d = day; LW = live weight (kg).

Lamb liveweight change (LWC: kg) was calculated as:

$$LW_{d+n} - LW_{d7}$$

LW_{d7} = live weight at d7 (kg); LW_{d+n} = live weight at day_{n}.

Udder dimensions

Prior to each afternoon milking for seven weeks, the udders of the ewes were measured, based on a technique previously described by Mellor and Murray (1985). Four dimensions (A, BLR, BTB and C) were measured by following the contours of the udder with measuring tape (Figure 3.1). Dimension A was the mean of three measurements from the posterior margin to the anterior margin of the udder along the midline and parallel to the midline immediately medial to each teat. Dimension BLR was the distance between the left and right lateral edges of the udder immediately anterior to the teats. Dimension BTB was the distance between the posterior margin to the anterior margin of the udder along the midline. Dimension C was the circumference of the gland at the base. The ewe was turned over, resting on her rump and held in a semi-upright position during measurements.

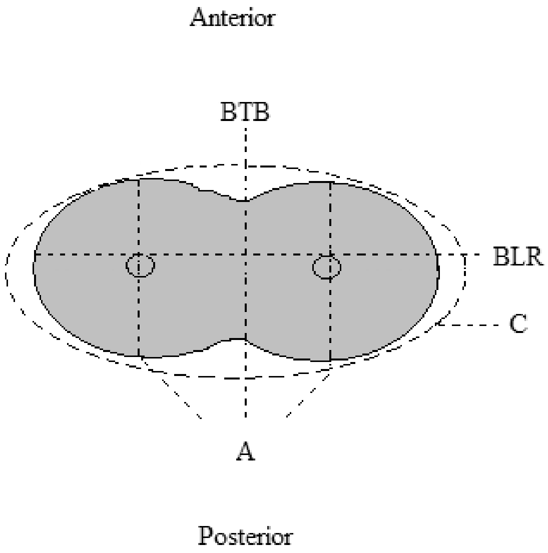

Figure 3.1. Overview of the udder (ventral view) and the dimensions (A, BLR, BTB and C) measured. Dimension A was the mean of three measurements from the posterior margin to the anterior margin of the udder along the midline and parallel o the midline immediately medial to each teat. Dimension BLR was the distance between the left and right lateral edges of the udder immediately anterior to the teats. Dimension BTB was the distance between the posterior margin to the anterior margin of the udder along the midline. Dimension C was the circumference of the gland at the base.

D2O dilution technique

The deuterium oxide (D_2O) dilution technique was used to estimate lamb milk intake of the singleton-born and -reared lambs (Auchtung *et al.*, 2002). This procedure was performed in lambs starting at approximately 7 days of age (range of 5 to 9 days of age) and finished on approximately day 14 (range 12 to 16 days of age), when lambs were functionally monogastric and solely dependent on milk for nutrients. It is assumed, that at this age, the main intake of water is via milk from the mother (Geenty and Sykes, 1983). Therefore, milk ingestion can be estimated by measuring total body turnover of water using D_2O dilution. D_2O is a stable isotope of water that is rapidly uniformly distributed in total body water. Therefore, the dilution of D_2O over time, allows the estimation of water intake.

At the start of the procedure, which coincided with the first milking of the ewes, lambs were weighed and baseline samples of blood (5 mL) were collected from the jugular vein of the lamb into sterile heparinized vacutainers (BD Vacutainer Systems, UK) and placed on ice. Lambs were then given an intravenous injection of 300 mg of D_2O/kg LW (99.9%, Sigma-Aldrich Co., St Louis, MO, USA). D_2O was salinized (60% D_2O solution) with 0.9% sodium lactate (Compound Sodium Lactate, Baxter). Time and duration of injections were recorded. Syringes were weighed before and after injections to the nearest 0.1 g to gravimetrically determine the actual dose administered. The D_2O was allowed to equilibrate with body water for 2 h, before a blood sample was collected. Blood samples were collected at 24, 96 and 168 h post D_2O injection. The time at which each sample was collected was recorded to the nearest minute. Following the 2-h equilibration, lambs were bottled fed 100 – 250 mL, as the start of the D_2O procedure coincided with the first milking of their dams, as described above, and therefore dams would return to their lambs with an empty udder. On each of the subsequent days of blood collection, lambs were removed from their dams for the duration of the blood sampling only. Lambs were weighed on the day of the 168-h blood sample. All blood samples were centrifuged at 3000 rpm for 15 minutes at 4°C and the plasma collected and frozen at -20 ̊C until analysis.

Plasma samples were analysed for D_2O concentration using Nuclear Magnetic Resonance Spectroscopy (NMR). The NMR spectrometer (Bruker DRX-400, Bruker BioSpin, Karlsruhe, Germany) was fitted with a 10-mm broadband probe. Acquisition and analysis was carried out using *XwinNMR 3.5*. The spectrometer was locked using DMSO then the lock power was turned down and the lock cable removed. Either 2 mL of plasma or standard D_2O solution was added to a 10 mm NMR tube and a 5-mm NMR tube containing the internal standard (1% C_6D_6 in C_6H_6) was suspended in it. Samples were run in non-spinning mode at room temperature with the sweep off. Initial shimming was carried out on the proton-free induction decay to optimise the field for each sample, then the deuterium spectrum was recorded (32 scans). The integral of the D_2O signal was measured relative to that of C_6D_6 for both standards (0-1.0 mg/mL) and unknown samples. The concentration of D_2O in the unknowns was determined from the calibration curve. The D_2O determinations were carried out over a 6-week period with an inter-assay coefficient of variation of 3.5% in the slope of the standard curves and 3.2% in the average blank reading.

For each duplicate sample analysed, a D_2O calibration curve was produced. Estimated milk intake was computed from the disappearance curve of D_2O in plasma in each lamb.

Dose of D_2O (mg) administered was calculated as:

$$\frac{Final\ syringe\ weight - Initial\ syringe\ weight\ (g) \times 0.60}{1000}$$

0.60: amount of D_2O in dose is 60%

Milk intake (mL h^{-1}) was calculated as:

$$\frac{Dose\ D_2O\ (mg) \times \kappa}{Net\ concentration\ D_2O\ at\ time\ 0\ (mg\ mL^{-1})}$$

κ = exponential decay of D_2O over time

Statistical analysis

Repeated measures analysis of milk yield, udder dimension traits and lamb growth rates were undertaken using the MIXED procedure of SAS (2006) with a linear model that included the fixed effect of time and the data are presented as least square means ± S.E. Mean lamb milk intake per day was calculated and presented as mean ± S.E.

Estimates of Pearson’s correlation coefficients between milk yield , udder dimensions (A, BTB, BLR and C), LWG over seven consecutive weeks, and lamb milk intake (second week of lactation only), were obtained using the CORR procedure of SAS (2006).

Multiple regression analyses of udder dimensions (A, BTB, BLR and C) on milk yield over seven consecutive weeks were carried out using the REG procedure of SAS (2006). Linear, quadratic and interactions effects of the independent traits were considered in the model. The stepwise method was used to select the udder traits that better explained the variation in milk yield. Similarly, over seven weeks, multiple regression analyses of udder dimensions (A, BTB, BLR and C) on lamb growth rates (LWG), milk yield on LWG, milk yield and udder measurements on LWG and lamb milk intake (second week of lactation only) on LWG were carried out using the REG procedure of SAS (2006). Linear, quadratic and interactions effects of the independent traits were considered in the model. The stepwise method was used to select milk yield and/or udder traits that better explained the variation in LWG. Variables with a significant effect at a probability of less then 0.15 were selected in the regression models.

Similarly, multiple regression analyses of lamb live weight (LW) and lamb liveweight change (LWC) on milk yield over seven weeks were carried out using the REG procedure of SAS (2006). The selected independent effects of LW and LWC were used in the model to explain the variation in milk yield.

To evaluate the measure of fitness of the equations developed, the relative prediction error (RPE) and the concordance correlation coefficient (CCC) (Lin, 1989) were calculated using the following equations:

$$Relative\ prediction\ error\ (RPE)\ = MPE/\hat{A}$$

$$Concordance\ correlation\ coefficient\ (CCC) = \frac{2S_{AP}}{S_A^2 + S_P^2 + (\hat{A} - \hat{P})^2}$$

where P_i is the predicted value as calculated by the multiple regression equation and A_i is the observed value of ewe i. Means, standard deviations and covariances of A_i and P_i were calculated using the following equations:

$$Mean\ prediction\ error\ (MPE) = \sqrt{MSPE}$$

$$Mean\ square\ prediction\ error\ (MSPE) = \frac{1}{n}\sum_{i}^{n}(P_i - A_i)^2$$

$$\hat{A} = \frac{1}{n}\sum_{i}^{n} A_i; \qquad \hat{P} = \frac{1}{n}\sum_{i}^{n} P_i$$

$$S_A^2 = \frac{1}{n}\sum_{i}^{n}(A_i - \hat{A})^2; \qquad S_P^2 = \frac{1}{n}\sum_{i}^{n}(P_i - \hat{P})^2; \qquad S_{AP} = \frac{1}{n}\sum_{i}^{n}\left(A_i - \hat{A}\right)\left(P_i - \hat{P}\right)$$

Results

Milk yield, udder dimensions and lamb growth rates over the 7-week period and lamb milk intake between days 7-14 of lactation are presented in Table 3.1.

Table 3.1. Least square means (± S.E.) of milk yield (MY; kg/day), udder dimensions (A, BTB, BLR, and C; cm), and lamb growth rates (LWG; g/day) during the first 49 days of lactation and the mean (± S.E.) lamb milk intake (L/day) between days 7-14 of lactation.

Days in lactation (growth period)	MY	A	BTB	BLR	C	LWG	Lamb milk intake
7 (birth-L7)	1.99 ± 0.07	24.5 ± 0.35	25.4 ± 0.36	29.3 ± 0.35	59.6 ± 0.77	343 ± 13.8	-
14 (L7-L14)	1.79 ± 0.06	23.3 ± 0.33	24.3 ± 0.37	27.5 ± 0.77	57.0 ± 0.60	373 ± 9.7	1.39 ± 0.09
21 (L14-L21)	1.86 ± 0.05	22.8 ± 0.35	23.8 ± 0.39	29.9 ± 0.37	57.0 ± 0.56	401 ± 6.2	-
28 (L21-L28)	1.89 ± 0.07	22.7 ± 0.36	23.4 ± 0.47	30.9 ± 0.38	57.9 ± 0.50	295 ± 15.4	-
35 (L28-L35)	1.81 ± 0.06	22.9 ± 0.38	23.2 ± 0.39	30.1 ± 0.85	56.7 ± 0.46	323 ± 13.1	-
42 (L35-L42)	1.69 ± 0.06	21.8 ± 0.32	22.6 ± 0.37	28.0 ± 0.31	53.4 ± 0.53	411 ± 12.7	-
49 (L42-L49)	1.71 ± 0.04	21.0 ± 0.34	21.4 ± 0.43	27.0 ± 0.43	53.5 ± 0.62	340 ± 14.3	-

A: mean of three measurements from the posterior to the anterior margin of the udder along the midline and parallel to the midline immediately medial to each teat; BLR: distance between the left and right lateral edges of the udder immediately anterior of the teats; BTB: distance between the posterior margin to the anterior margin of the udder along the midline; C: the circumference of the gland at the base.

Correlations with milk yield

Estimates of Pearson correlations between milk yield and udder dimension traits, between milk yield and lamb growth rates over the 7-week period and between milk yield and lamb milk intake between days 7-14 of lactation are presented in Table 3.2.

Table 3.2. Estimates of the Pearson's correlation coefficients between milk yield (kg), udder dimensions (A, BTB, BLR and C; cm), lamb growth rates (LWG; g/day) during the first 49 days of lactation and lamb milk intake (L/day) between days 7-14 of lactation.

Days in lactation (growth period)	A	BTB	BLR	C	LWG	Lamb milk intake
7 (0 - 7)	0.37^{*}	0.38^{*}	0.59^{***}	0.18	$0.28^{\dagger}$	-
14 (7 - 14)	0.37^{*}	0.21	0.06	0.25	0.26	0.13
21 (14 - 21)	0.24	$0.30^{\dagger}$	0.38^{*}	$0.28^{\dagger}$	0.34^{*}	-
28 (21 - 28)	0.22	0.13	0.36^{*}	0.33^{*}	0.51^{***}	-
35 (28 - 35)	0.56^{***}	0.56^{***}	0.22	0.46^{**}	0.35^{*}	-
42 (35 - 42)	0.43^{**}	0.35^{*}	0.19	0.43^{**}	$0.28^{\dagger}$	-
49 (42 - 49)	0.34^{*}	0.16	0.13	0.38^{*}	0.38^{*}	-

* $P < 0.05$; ** $P < 0.01$; $^{***}P < 0.001$; † $P < 0.10$; A: mean of three measurements from the posterior to the anterior margin of the udder along the midline and parallel to the midline immediately medial to each teat; BLR: distance between the left and right lateral edges of the udder immediately anterior of the teats; BTB: distance between the posterior margin to the anterior margin of the udder along the midline; C: the circumference of the gland at the base.

At d7, milk yield was significantly positively correlated with udder dimensions A, BTB and BLR. In addition, milk yield tended to be positively correlated with LWG during the first week. At d14, milk yield was positively correlated with udder dimension A, however, no significant correlation was found between milk yield and the other udder dimensions, LWG or lamb milk intake. At d21, milk yield was significantly positively correlated with udder dimensions BLR and LWG and tended to be positively correlated with udder dimensions BTB and C. At d28, milk yield was significantly and positively correlated with udder dimensions BLR and C, and LWG. At d35 and d42, milk yield was significantly positively correlated with udder dimensions A, BTB and C, and LWG (tendency at d42). At d49, milk yield was positively correlated with udder dimensions A and C, and LWG.

Correlations with lamb growth rates

Estimates of Pearson correlations between lamb growth rates and udder dimension traits over the 7-week period and between lamb growth rate and lamb milk intake between days 7-14 of lactation are presented in Table 3.3.

Table 3.3. Estimates of the Pearson's correlation coefficients between lamb growth rates (g/day), udder dimensions (A, BTB, BLR and C; cm) during the first 49 days of lactation and lamb milk intake (L/day) between days 7-14 of lactation.

Days in lactation	A	BTB	BLR	C	Lamb milk intake
7 (0 - 7)	0.09	0.18	0.07	0.02	-
14 (7 - 14)	0.17	0.16	0.42^{**}	$0.28^{\dagger}$	$0.31^{\dagger}$
21 (14 - 21)	0.03	0.02	0.24	0.10	-
28 (21 - 28)	-0.16	-0.20	0.002	0.25	-
35 (28 - 35)	0.41^{**}	0.34^{*}	-0.12	$0.29^{\dagger}$	-
42 (35 - 42)	0.21	$0.28^{\dagger}$	-0.10	-0.15	-
49 (42 - 49)	0.15	0.21	-0.05	0.16	-

* $P < 0.05$; ** $P < 0.01$; † $P < 0.10$; A: mean of three measurements from the posterior to the anterior margin of the udder along the midline and parallel to the midline immediately medial to each teat; BLR: distance between the left and right lateral edges of the udder immediately anterior of the teats ; BTB: distance between the posterior margin to the anterior margin of the udder along the midline; C: the circumference of the gland at the base.

At d7, d21, d28 and d49, lamb growth rates were not significantly correlated with any udder dimension. At d14, LWG was significantly positively correlated with udder dimension BLR and tended to be positively correlated with udder dimension C and lamb milk intake. At d35, LWG was significantly positively correlated with udder dimensions A and BTB and tended to be positively correlated with udder dimension C. At d42, LWG tended to be positively correlated to BTB.

Milk yield predicted by udder dimensions

At d7, the best model explaining variation in milk yield included the intercept and the quadratic effect of BLR (R^2 = 0.35) (Table 3.4). The best model explaining variation in milk yield at d14, included the intercept and the quadratic and linear effect of BTB and A (R^2 = 0.21). At d21 and at d28, the best model explaining variation in milk yield included the intercept and the linear effect of BLR (R^2 = 0.19; for both d21 and d28). At d35, d42 and d49, the best model explaining variation in milk yield included the intercept and the linear effect of the interaction C by A (R^2= 0.36; 0.34; 0.15; for d35, d42 and d49, respectively).

Overall, the models at d7, d35 and d42, were the best models explaining variation in milk yield (CCC = 0.49; 0.53; 0.51; for d7, d35 and d42, respectively).

Table 3.4. Multiple regression coefficients (± S.E.) of udder dimensions (A, BTB, BLR and C; cm) on milk yield (MY; kg) during the first 49 days of lactation of ewes bearing and rearing singleton lambs.

Days in lactation	Independent variables selected						Measure of fitness		
	Intercept	BLR^2	BTB	A^2	C×BLR	C×A	R^2	RPE	CCC
7	0.545 ± 0.360	1.78×10^{-3} $\pm 4.13 \times 10^{-4}$	-	-	-	-	0.35	16%	0.49
14	2.071 ± 0.671	-	-79.3×10^{-3} $\pm 47.3 \times 10^{-3}$	2.99×10^{-3} $\pm 1.13 \times 10^{-3}$	-	-	0.21	16%	0.34
21	0.556 ± 0.459	-	-	-	7.65×10^{-4} $\pm 2.67 \times 10^{-4}$	-	0.19	16%	0.32
28	0.091 ± 0.630	-	-	-	10.00×10^{-4} $\pm 3.49 \times 10^{-4}$	-	0.19	20%	0.32
35	0.172 ± 0.368	-	-	-	-	12.60×10^{-4} $\pm 2.80 \times 10^{-4}$	0.36	15%	0.53
42	-0.143 ± 0.432	-	-	-	-	15.70×10^{-4} $\pm 3.67 \times 10^{-4}$	0.34	17%	0.51
49	0.910 ± 0.293	-	-	-	-	7.10×10^{-4} $\pm 2.58 \times 10^{-4}$	0.15	14%	0.30

A: mean of three measurements from the posterior to the anterior margin of the udder along the midline and parallel to the midline immediately medial to each teat; BLR: distance between the left and right lateral edges of the udder immediately anterior of the teats; BTB: distance between the posterior margin to the anterior margin of the udder along the midline; C: the circumference of the gland at the base; RPE: relative prediction error (%); CCC: concordance correlation coefficient.

Milk yield predicted by lamb liveweight change

The models explaining variation in milk yield included the intercept and the linear effects of LW and LWC for all seven weeks of lactation (Table 3.5).

The model explained the variation in milk yield best at d28 (R2 = 0.32; CCC = 0.49), at d42 (R2 = 0.28; CCC = 0.44) and at d35 (R2 = 0.22; CCC = 0.36).

Table 3.5. Multiple regression coefficients (± S.E.) of lamb live weight (LW; kg) and lamb liveweight change ($LWC_{LWn-LW7}$; kg) on milk yield (MY; kg) during the first 42 days of lactation and the multiple regression equation of lamb birth weight (LW_0; kg) and total lamb live weight change during the first 49 days of life (LWC_{0-49}; kg) on accumulated milk yield over the first 49 days of lactation ($AccMY_{0-49}$; kg) of ewes bearing and rearing singleton lambs.

	Independent variables selected			Measure of fitness		
Days in lactation	Intercept	LW	LWC	R^2	RPE	CCC
7	1.925 ± 0.542	0.085 ± 0.051	-0.254 ± 0.157	0.12	18%	0.21
14	0.765 ± 0.590	0.044 ± 0.044	0.098 ± 0.118	0.09	17%	0.16
21	0.616 ± 0.558	0.025 ± 0.039	0.120 ± 0.070	0.09	16%	0.25
28	-0.489 ± 0.637	-0.005 ± 0.047	0.253 ± 0.083	0.32	18%	0.49
35	0.293 ± 0.561	-0.051 ± 0.054	0.193 ± 0.081	0.22	17%	0.36
42	0.449 ± 0.513	-0.077 ± 0.043	0.192 ± 0.060	0.28	18%	0.44
$AccMY_{0-49}$	42.23 ± 18.26	2.48 ± 2.39	1.95 ± 0.96	0.18	12%	0.31

RPE: relative prediction error (%); CCC: concordance correlation coefficient.

Milk yield predicted by lamb milk intake

At d14, lamb milk intake could not explain the variation in milk yield (data not shown).

Lamb growth rates predicted by milk yield

At d7, d14, d21, d35 and d42, the best model explaining variation in lamb growth rates included the intercept and the quadratic effect of MY (R^2 = 0.08; 0.08; 0.12; 0.12; 0.09; for d7, d14, d21, d35 and d42, respectively) (Table 3.6).

Table 3.6. Multiple regression coefficients (± S.E.) of milk yield (MY; kg) on lamb growth rates (LWG; g/day) during the first 49 days of lactation and the multiple regression coefficients of accumulated milk yield during the first 49 days of lactation($AccMY_{0-49}$; kg) on total lamb growth rates during the first 49 days of life of ewes bearing and rearing singleton lambs.

	Independent variables selected			Measure of fitness		
Growth period (days)	Intercept	MY	MY^2	R^2	RPE	CCC
0 - 7	286 ± 35.1	-	13.9 ± 7.92	0.08	23%	0.15
7 - 14	327.9 ± 28.2	-	13.8 ± 8.02	0.08	19%	0.10
14 - 21	365.9 ± 17.2	-	9.7 ± 4.54	0.12	18%	0.08
21 - 28	83.0 ± 62.3	112.5 ± 32.3	-	0.26	31%	0.06
28 - 35	251.6 ± 34.9	-	21.0 ± 9.61	0.12	24%	0.15
35 - 42	354.8 ± 32.3	-	18.7 ± 10.01	0.09	27%	0.06
42 - 49	127.2 ± 87.6	124.4 ± 50.55	-	0.15	27%	0.03
0 – 49	299 ± 19.6	-	6.64×10^{-3} $\pm 2.21 \times 10^{-3}$	0.20	9%	0.34

RPE: relative prediction error (%); CCC: concordance correlation coefficient.

The best model explaining variation in lamb growth rates at d28 and d49 included the intercept and the linear effect of MY (R^2 = 0.26; 0.15; for d28 and d49, respectively). The best model explaining variation in total lamb growth rates during the first 49 days of life included the intercept and the quadratic effect of accumulated MY ($R^2 = 0.20$).

Overall, the models explaining lamb growth rates for each individual week are poor (CCC < 0.15), however, the model including accumulated milk yield is the best model explaining total lamb growth rates (CCC = 0.34).

Lamb growth rates predicted by udder dimensions

At d7, d21 and d49, none of the udder dimension traits could explain the variation in lamb growth rates (Table 3.7). At d14, the best model explaining variation in lamb growth rates included the intercept and the linear effect of the interaction C by BLR ($R^2 = 0.22$). The best model explaining variation in lamb growth rates at d28 included the intercept and the linear and quadratic effect of C ($R^2 = 0.14$). At d35, the best model explaining variation in lamb growth rates included the intercept, the quadratic effect of BLR and the linear effect of the interaction A by C ($R^2 = 0.25$). The best model explaining variation in lamb growth rates at d42 included the intercept and the linear effect of BTB ($R^2 = 0.08$).

Overall, the models at d14 and d35 were the best models explaining lamb growth rates (CCC = 0.36; 0.40; for d14 and d35, respectively).

Lamb growth rates predicted by lamb milk intake

At d14, the best model explaining variation in lamb growth rates included the intercept and the linear effect of lamb milk intake (LWG = 251.5 (± 65.08) + 12.6 (± 6.62) × milk intake (L); $R^2 = 0.10$; RPE = 32%; CCC = 0.007).

Table 3.7. Multiple regression coefficients (± S.E.) of udder dimensions (A, BTB, BLR and C; cm) on lamb growth rates (LWG; g/day) during the first 49 days of lactation of ewes bearing and rearing singleton lambs.

Growth period (days)	Independent variables selected							Measure of fitness		
	Intercept	BTB	C	C^2	BLR^2	C×BLR	C×A	R^2	RPE	CCC
0 - 7	-	-	-	-	-	-	-	-	-	-
7 - 14	229.1 ± 46.1	-	-	-	-	0.09 ± 0.03	-	0.22	14%	0.36
14 - 21	-	-	-	-	-	-	-	-	-	-
21 - 28	-7317.0 ± 4179.0	-	255.1 ± 143.9	-2.1 ± 1.24	-	-	-	0.14	44%	0.14
28 - 35	92.9 ± 94.2	-	-	-	-0.11 ± 0.06	-	0.25 ± 0.08	0.25	21%	0.40
35 - 42	196.0 ± 125.0	9.47 ± 5.50	-	-	-	-	-	0.08	18%	0.15
42 - 49	-	-	-	-	-	-	-	-	-	-

A: mean of three measurements from the posterior to the anterior margin of the udder along the midline and parallel to the midline immediately medial to each teat; BLR: distance between the left and right lateral edges of the udder immediately anterior of the teats; BTB: distance between the posterior margin to the anterior margin of the udder along the midline; C: the circumference of the gland at the base; RPE: relative prediction error (%); CCC: concordance correlation coefficient.

Table 3.8. Multiple regression coefficients (± S.E.) of milk yield (MY; kg) and udder dimensions (A, BTB, BLR and C; cm) on lamb growth rates (LWG; g/day) during the first 49 days of lactation of ewes bearing and rearing singleton lambs.

Growth period (days)	Independent variables selected								Measure of fitness		
	Intercept	MY	MY^2	A^2	C^2	BLR^2	C×BLR	C×A	R^2	RPE	CCC
0 - 7	285.8 ± 35.1	-	13.9 ± 7.92	-	-	-	-	-	0.08	23%	0.15
7 - 14	229.1 ± 46.1	-	-	-	-	-	9.21×10^{-2} $\pm 2.89 \times 10^{-2}$	-	0.22	14%	0.36
14 - 21	365.9 ± 17.2	-	9.77 ± 4.54	-	-	-	-	-	0.12	18%	0.08
21 - 28	196.8 ± 82.3	125.4 ± 31.6	-	-0.26 ± 0.13	-	-	-	-	0.34	25%	0.50
28 - 35	92.9 ± 94.2	-	-	-	-	-0.11 ± 0.06	-	0.25 ± 0.08	0.25	21%	0.40
35 - 42	548.9 ± 100.6	-	27.7 ± 10.6	-	-0.07 ± 0.04	-	-	-	0.19	17%	0.29
42 - 49	127.2 ± 87.6	124.4 ± 50.55	-	-	-	-	-	-	0.15	27%	0.03

A: mean of three measurements from the posterior to the anterior margin of the udder along the midline and parallel to the midline immediately medial to each teat; BLR: distance between the left and right lateral edges of the udder immediately anterior of the teats ; BTB: distance between the posterior margin to the anterior margin of the udder along the midline; C: the circumference of the gland at the base; RPE: relative prediction error (%); CCC: concordance correlation coefficient.

Lamb growth rates predicted by milk yield and udder dimensions

At d7, d21 and d49, the best model explaining variation in lamb growth rates included the intercept and the linear and quadratic effects of MY, as previously described (Table 3.6 and 3.8). At d14 and d35, the best model explaining variation in lamb growth rates included the intercept and the linear and quadratic effects of the udder dimensions as previously described (Table 3.7 and 3.8). At d28, the best model explaining variation in lamb growth rates included the intercept, the linear and quadratic effects of MY and A (R^2 = 0.34). The best model explaining variation in lamb growth rates at d42 included the intercept and the quadratic effects of MY and C (R^2 = 0.19).

Overall, the models at d28, d35 and d14 were the best models explaining variation in lamb growth rates (CCC = 0.50; 0.40; 0.36; for d28, d35 and d14, respectively).

Discussion

Milk yield

The present study showed correlations among the different techniques, to allow for comparison with previous work. Milk yield was found to be positively correlated with lamb growth rates and the highest correlation coefficients were observed from d21 through to d49 of lactation. This finding is in agreement with previous work (Snowder and Glimp, 1991), although, the correlations found in the study of Snowder and Glimp (1991) between milk yield and lamb growth rate were much higher (*r* 0.7) than the correlation coefficients found in the present study. A possible explanation for this difference could be the number of animals studied, which was greater in the study of Snowder and Glimp (1991). Even though significant correlations were found between milk yield and lamb growth rates in the present study, milk yield was poorly predicted by the lamb liveweight change model during the first 21 days of lactation and was only moderately predicted from d28 onwards.

Previous work has shown that the D_2O dilution technique is a good predictor of actual milk intake (Macfarlane *et al.*, 1969; Dove, 1988; Auchtung *et al.*, 2002). Lamb milk intake, as calculated using the D_2O dilution technique, did not predict ewe milk yield at d14 of lactation and in addition, milk yield was poorly correlated with lamb milk intake between days7-14 of lactation. It is possible that the singleton-bearing ewe produced greater milk yields than the lamb’s capacity to consume all the milk produced during early lactation (Table 3.1). In addition, milking could result in a greater degree of udder emptying than is achieved by the offspring, particularly with singleton offspring. Robinson *et al.* (1968) stated that milk intake of the lamb may be more closely related to the appetite of the lamb than the true yield potential of the ewe. Although in the present study the first day of the D_2O procedure, coincided with the first milking of the ewes (for practical reasons), and the lambs were fed a known amount of milk (100-250 mL) on this day as the ewes would return to their lambs with an empty udder, the authors do not believe that this relative small amount of milk would have confounded the milk intake of the lambs, as milk intake was measured over a 7-day period and the average intake per day was 1.39 L.

The udder dimensions A and C were best correlated with milk yield from d35 onwards, which is in disagreement with previous work, in which the udder dimension BLR was found to be best correlated with milk yield in late lactation (Peterson *et al.*, 2006), however, that work was conducted in twin- and triplet-bearing ewes. Relatively low correlations were observed between milk yield and udder dimension BLR (r = 0.06) and BTB (r = 0.13) at d14 and at d28, respectively and these do not fit the trend of the greater and significant correlations observed for those udder dimensions on the previous and subsequent days of lactation. However, the authors have no explanation for this finding; neither can the authors explain the relatively flat lactation curve observed for the singleton-bearing ewes. Although significant correlations were found between milk yield and the udder dimensions, only 15-35% of the variation in milk yield was explained when udder dimensions were regressed on milk yield, indicating that udder dimensions are poor predictors of milk yield in singleton-rearing ewes. In addition, measuring the external dimension of the udder is time consuming and labour intensive as prior to each udder measurement, the udder needs to be milked out, so all ewes will start from the same “baseline”.

Lamb growth

Lamb growth rates were poorly predicted by milk yield during the first 14 days of lactation (explaining 8% - 12% of variation), followed by a moderate prediction of lamb growth rates by milk yield from d21 through to d28 (explaining 26% of variation). After d35, lamb growth rates were poorly predicted by milk yield (explaining 9% - 15% of variation), which is in agreement with the prediction of milk yield by the lamb liveweight change model. The poor prediction of lamb growth by milk yield during the first 14 days may be explained by the ewes having greater milk production than the singleton lamb could consume during early lactation (Robinson *et al.*, 1968; Snowder and Glimp, 1991) and subsequently partition to growth. This is in agreement with the poor prediction of lamb milk intake between days 7-14 of lactation for lamb growth. However, when the lamb gets older, it is able to consume more milk and consequently more variation in lamb growth was explained by milk yield. Nevertheless, the rumen develops rapidly as soon as the lamb starts consuming herbage such that at three to four weeks of age, it is considered to have the equal capacity as the adult to digest feed (Geenty and Sykes, 1983). Therefore, the variation in lamb growth is most likely poorly explained by milk yield after four weeks of age, as the lamb is no longer solely dependent upon the energy obtained by milk consumption.

In conclusion, this study showed that for singleton-rearing ewes, udder dimensions, lamb liveweight change and lamb milk intake did not explain a significant proportion of the variation in milk yield (< 36% of variation), even though positive correlations were found. In addition, the variation in growth rates of singleton-born lambs was poorly explained by udder dimension traits, lamb milk intake and milk yield, explaining no more than 35% of the variation. Therefore, udder dimensions, lamb liveweight change and lamb milk intake do not give an accurate prediction of the milk yield of singleton-rearing ewes. Additionally, udder dimensions, milk yield and lamb milk intake do not give an accurate prediction of growth rates of singleton lambs. These results emphasize that there is a difference between the milk production potential of singleton-rearing ewes and lamb milk intake, which needs to be considered when estimating milk production in non-dairy animals.

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CHAPTER 4

BACKGROUND INFORMATION DAMS

This chapter describes the treatment of the dams, to which the offspring studied in this thesis were born, in more detail (adapted from Kenyon *et al*. (2009). Only a brief description of the maternal treatments is given in later chapters.

Dam treatments

The heaviest 450 (heavy (H), 60.8 kg ± S.E. 0.18, body condition score (CS; scale 1-5 (Jefferies, 1961)) 3.02 ± 0.03) and lightest 450 (light (L), 42.5 kg ± 0.17, CS 1.97 ± 0.03) multiparous Romney ewes (3-5 years of age) were selected from a commercial flock of 2900 ewes, 69 days prior to artificial insemination (d-69) and managed under commercial grazing conditions as one group. At d-14, ewes had progesterone controlled internal drug release devices (CIDR, 0.3g progesterone, Pharmacia & UpJohn, Auckland, New Zealand) inserted vaginally. On d-2 half the dams, including individuals from each of the dam size groups had their CIDRs removed. The following morning (d-1), the remainder of the dams had their CIDRs removed. On d0 those dams which had their CIDRs removed on d-2 were artificially inseminated, via intra-uterine laproscopy using semen from one of four Suffolk rams, randomly allocated to each dam. On d1 the remaining dams underwent the same procedure and then both cohorts of dams were merged. At d4**,** eight crayon-harnessed entire Suffolk rams were introduced to the dams and dams were managed under commercial conditions.

At d21 the Suffolk rams were removed as were any dams displaying harness marks indicating returns to service. The remaining dams (*n* = 612), were randomly allocated to one of two nutritional regimes until d140 (maintenance (M) vs. *ad libitum* (A)) under pastoral grazing conditions. The average pre- and post-grazing covers during the period d21 to d140 were 1330 kg DM/ha ± 140.0 and 804.0 kg DM/ha ± 133.4 for the maintenance regimen and 2304.0 kg DM/ha ± 156.8 and 1723.3 kg DM/ha ± 149.7 for the *ad libitum* regimen.

The aim of the maintenance nutritional regimen was to ensure that total dam live weight increased in pregnancy at a level similar to that of the expected conceptus mass (Rattray *et al.*, 1974). The aim of the *ad libitum* nutritional regimen was to provide *ad libitum* grazing conditions. Within each feeding regimen singleton- and twin-bearing dams were not separated.

Therefore, from d21 until d140 the treatment groups included; heavy-*ad libitum* (*n* = 151), heavy-maintenance (*n* = 153), light-*ad libitum* (*n* = 155) and light-maintenance (*n* = 153) including singleton- and twin-bearing dams. To achieve these feeding regimens dams were rotationally grazed. The live weight and body condition of the dams prior and during pregnancy are shown in Figure 4.2.

At d141 all dams were set stocked for lambing and offered a minimum cover of 1200 kg DM/ha. During the period from ten days after the mid-point of lambing (L10) until L100 dams were managed in three groups under commercial grazing conditions with a minimum cover of 1200 kg DM/ha, with each group containing individuals from each treatment.

The present study began when the offspring were born, however, only the female offspring were studied until two years of age.

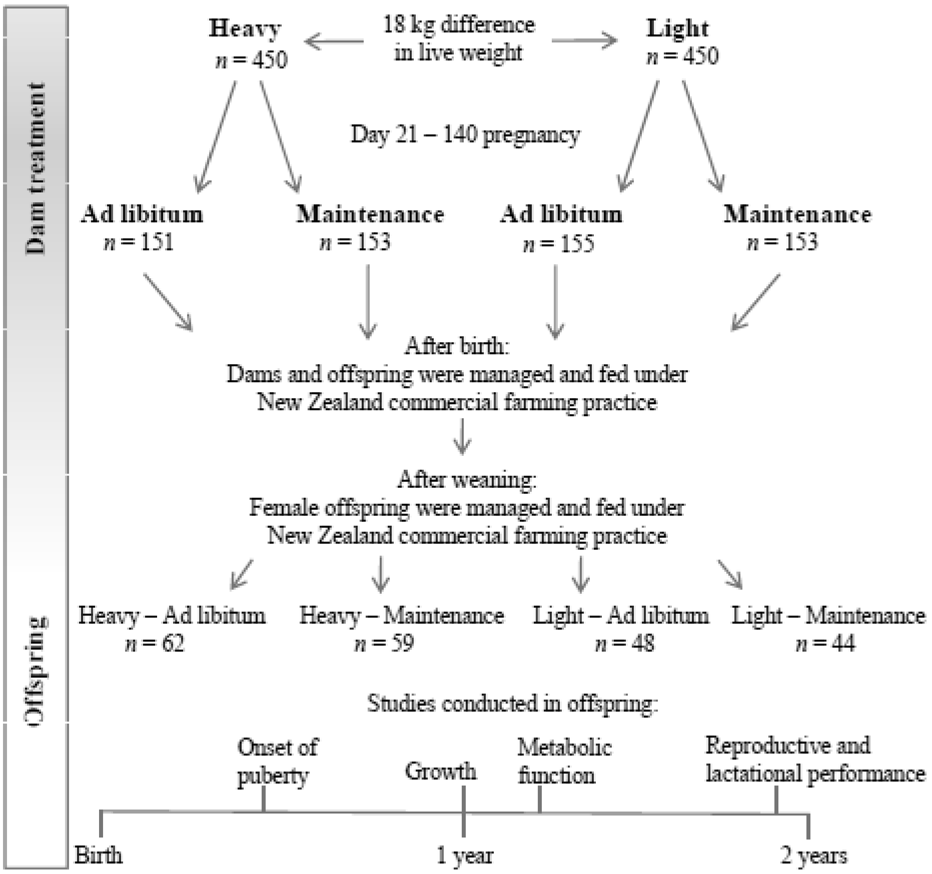

Figure 4.1. Study design and overview of studies conducted in the female offspring.

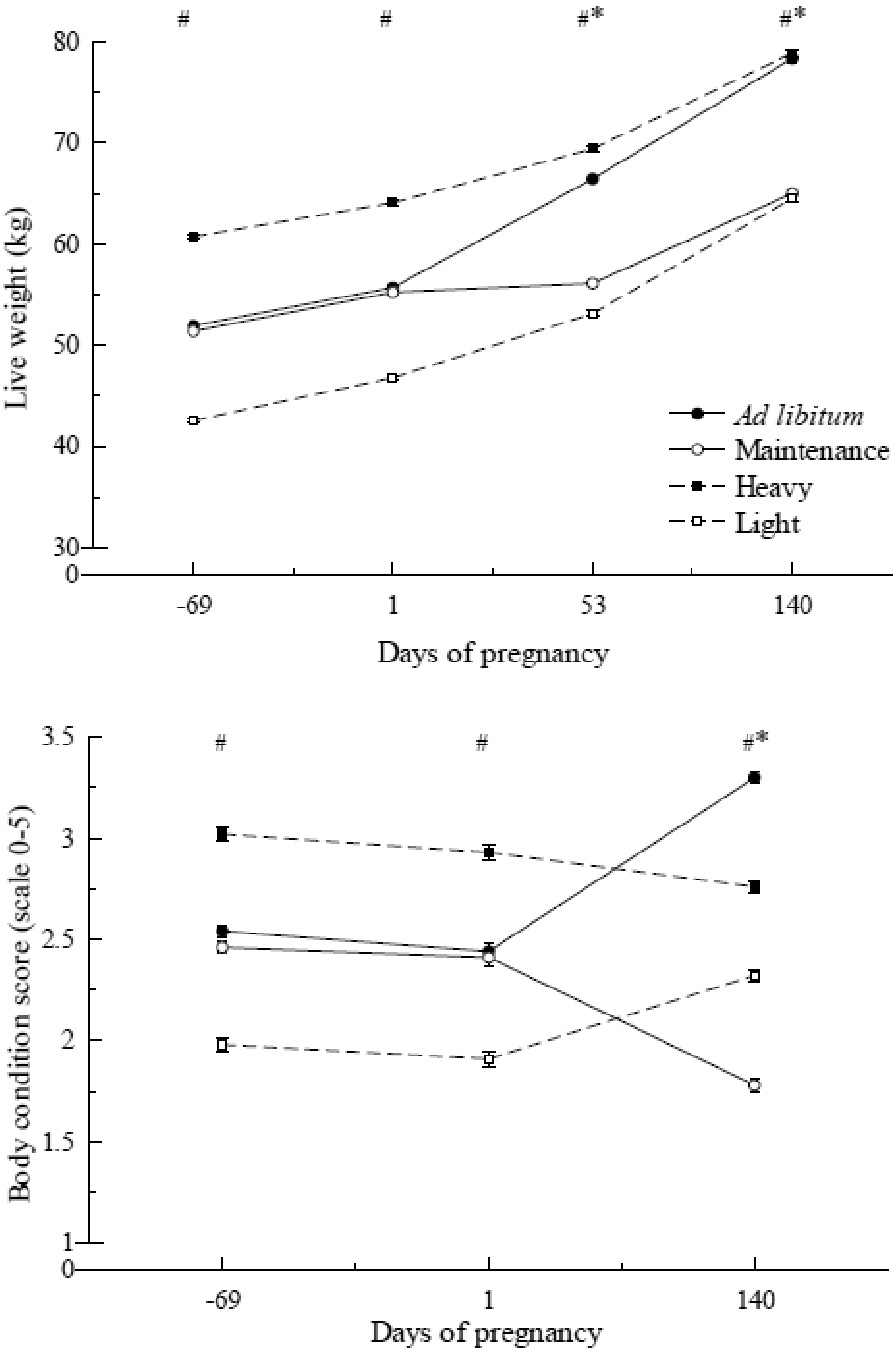

Figure 4.2. Live weight (kg) and body condition score (scale 0-5) of heavy (*n* = 255) and light (*n* = 255) dams fed either *ad libitum* (*n* = 242) or maintenance (*n* = 268) from days 21 - 140 of pregnancy at day -69, day 1, day 53 and day 140 of pregnancy * nutrition effect ($P < 0.05$), # size effect ($P < 0.05$) (Data adapted from Kenyon *et al*. (2009)).

CHAPTER 5

THE EFFECTS OF EWE SIZE AND NUTRITION DURING PREGNANCY ON GROWTH AND ONSET OF PUBERTY IN FEMALE PROGENY

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Abstract

In 2005, heavy and light Romney ewes were selected, synchronized and artificially inseminated. Half of the ewes in each weight group were fed *ad libitum* and the other half were restricted to maintenance from days 21 - 140 of pregnancy, resulting in four groups: heavy-maintenance (HM), heavy-*ad libitum* (HA), light-maintenance (LM) and light-*ad libitum* (LA). The aim of the present study was to investigate the effects of ewe size and nutrition during pregnancy on growth and onset of puberty in female progeny. Lamb birth weights were recorded. After weaning, the progeny were managed as one flock and weighed monthly. Crayon-harnessed teaser rams were joined with the lambs to investigate the onset of puberty. Maternal nutrition during pregnancy affected live weight of lambs from 22 days of age until weaning (100 days of age) ($P < 0.05$). During the first 22 days of life, the growth rate of lambs born to LM-ewes was lower ($P < 0.05$) compared with the other groups (215 g/day ± 8.2, 261 g/day ± 8.0, 250 g/day ± 7.9, 275 g/day ± 7.3 grams/day, for lambs born to LM-, LA-, HM- and HA-ewes, respectively). From weaning until one year of age lambs born to LM-dams were lighter than lambs born to HM-dams ($P<0.05$). No differences in the age of onset of puberty were found among the four groups. Singletons reached puberty earlier than twins ($P < 0.01$). At 13 months of age, no long-term effect of maternal nutrition on the live weight of resulting ewe offspring was found, although dam size had a significant effect on live weight and growth rate. However, neither dam nutrition nor size had any effect on the age of onset of puberty.

Introduction

Both human and sheep studies have shown that level of nutrition in fetal and early postnatal life can effect growth, development and physiology in later life (Gunn, 1977; Gunn *et al.*, 1995; Borwick *et al.*, 1997; Ravelli *et al.*, 1998; Ravelli *et al.*, 1999; Roseboom *et al.*, 2000; de Rooij *et al.*, 2006). Being smaller at birth, due to either under nourishment while *in utero* or because of the size of the dam, has consequences on the offspring's postnatal growth. Greenwood *et al.* (1998) showed that small, new-born lambs had lower growth rates compared with their larger new-born counterparts. Undernutrition during pregnancy could also have major implications for the reproductive performance of the offspring in adult life. Maternal undernutrition has been reported to retard fetal ovarian development (Borwick *et al.*, 1997), ovulation rate (Rae *et al.*, 2002) and reproductive performance (Gunn *et al.*, 1995) in ewe progeny.

The size of the dam and her uterine capacity could have major effects on the postnatal life of her offspring. Uterine capacity can be defined as the physiological and biochemical limitations imposed on conceptus growth and development by the uterus. Several studies involving artificial insemination and embryo transfer have demonstrated the effects of uterine capacity, showing the restricting effects of maternal size on birth weight and postnatal growth (Walton and Hammond, 1938; Dickinson *et al.*, 1962; Allen *et al.*, 2002; Allen *et al.*, 2004).

The present study investigates the effects of ewe size and nutrition during pregnancy, on the growth and onset of puberty of female progeny.

Materials and methods

In 2005, 450 heavy (60.8 kg ± 0.18 kg) and 450 light (42.5 kg ± 0.17 kg) Romney dams were selected from a flock of over 2500 dams, synchronized using progesterone controlled-internal-drug-release devices (CIDR) and artificially inseminated with Suffolk semen. Approximately half of the ewes in each size group were provided with *ad libitum* feeding from day 21 of pregnancy until day 140 and the other half were restricted to maintenance feeding under pastoral grazing conditions. This resulted in four groups: heavy dams on maintenance feeding (HM), heavy dams on *ad libitum* feeding (HA), light dams on maintenance feeding (LM) and light dams on *ad libitum* feeding (LA). The aim of the maintenance-feeding regimen was to ensure throughout pregnancy that total dam liveweight gain was similar to that of the expected increase in conceptus mass. One week prior to lambing, all dams were provided with *ad libitum* feeding.

The average day of birth of the lambs was 28 August 2005. Lambs were weighed within 24 hours after birth. After weaning (6 December 2005) at the average age of 100 days, the female progeny were managed as one flock under commercial grazing conditions. The ewe lambs were weighed monthly until 1 year of age.

To investigate the onset of puberty, four crayon-harnessed teaser rams were joined with the ewe lambs from early March until mid June 2006. Ram-harness-crayon marks were recorded and the colour of the crayon changed every 14 days. Marks were recorded the day after each crayon change, to identify the period during which the lamb was in oestrus. Hence, a ewe lamb that was marked the day after the crayon change was recorded as being in oestrus during the previous two weeks. Onset of puberty was defined as being the time when a lamb had her first clear ram-harness-crayon mark.

The current study was conducted at Massey University, Keeble Sheep and Beef Cattle Farm, 5 km south of Palmerston North.

All animal manipulations were approved by the Massey University Animal Ethics Committee.

Statistical analysis

Data were analysed using SAS (2006). Repeated measures of weights and growth rates were analysed using the MIXED procedure with a linear model that included the fixed effects of dam size (heavy vs. light), nutrition (*ad libitum* vs. maintenance), birth rank (singleton vs. twin), time, the interactions of dam size by nutrition, dam size by time, nutrition by time and birth rank by time and the random effect of animal. Age of puberty and oestrus events were analysed using the MIXED procedure with a linear model that included the fixed effects of dam size, nutrition, birth rank, the interactions of dam size by nutrition and dam size by birth rank with the covariate of growth rate. Accumulated proportion of animals reaching puberty was analysed using the LIFETEST procedure including in the model the fixed effects of dam size, nutrition and birth rank. Total proportion of animals that reached puberty up to day 305 was analysed with the GENMOD procedure with a linear model that included the fixed effects of dam size, nutrition, birth rank and the covariate growth rate.

Results

At birth, lambs born to HA-dams were significantly heavier than lambs born to LM-dams (5.4 kg ± 0.10 vs. 5.0 kg ± 0.12, $P = 0.01$, respectively) (Table 5.1). The growth rate of lambs born to LM-dams was lower compared with that of the other groups over the first 22 days of life ($P < 0.05$) (Table 5.2). From 22 days of age until weaning (100 days of age) nutrition of the dam during pregnancy had an effect on live weight of the offspring, whereby lambs born to LM- and HM-dams were lighter than lambs born to LA- and HA-dams, respectively ($P < 0.05$).

Table 5.1: Effects of heavy or light dams fed either *ad libitum* or maintenance from days 21 - 140 of pregnancy on live weights (kg) from birth (day 0) until 396 days of age of female offspring. Table shows least square means ± S.E.

	Heavy		Light	
Age (days)	*Ad libitum*	Maintenance	*Ad libitum*	Maintenance
0	5.4^{b} ± 0.10	5.2^{ab} ± 0.10	5.3^{ab} ± 0.11	5.0^{a} ± 0.12
22	11.6^{b} ± 0.20	10.4^{a} ± 0.20	11.1^{b} ± 0.22	9.6^{a} ± 0.23
46	19.9^{c} ± 0.40	18.3^{ab} ± 0.39	19.2^{bc} ± 0.43	17.6^{a} ± 0.44
80	28.3^{c} ± 0.52	26.6^{ab} ± 0.51	27.2^{bc} ± 0.57	25.2^{a} ± 0.59
100	31.7^{b} ± 0.58	30.3^{ab} ± 0.57	31.0^{b} ± 0.64	28.9^{a} ± 0.67
187	37.3^{a} ± 0.51	37.0^{b} ± 0.51	36.7^{b} ± 0.56	35.1 ± 0.58^{a}
207	38.7^{b} ± 0.50	38.2^{b} ± 0.50	37.7^{ab} ± 0.55	36.2^{a} ± 0.57
235	35.7^{b} ± 0.46	35.4^{ab} ± 0.46	35.3^{ab} ± 0.51	34.1^{a} ± 0.53
263	41.1^{b} ± 0.47	40.9^{b} ± 0.47	40.3^{ab} ± 0.52	39.3^{a} ± 0.53
291	44.3^{b} ± 0.49	44.5^{b} ± 0.49	44.0^{b} ± 0.54	42.3 ± 0.56^{a}
319	46.5^{b} ± 0.54	47.1^{b} ± 0.55	45.8^{ab} ± 0.61	44.7^{a} ± 0.62
396	47.0^{ab} ± 0.59	47.5^{b} ± 0.58	46.3^{ab} ± 0.66	45.4^{a} ± 0.67

Means within row with different superscripts are significantly different ($P < 0.05$)

After weaning, this nutritional effect was no longer present. From 187 days until 396 days of age, lambs born to LM-dams were lighter and had lower growth rates compared with lambs born to HM-dams ($P < 0.05$). Except for lamb growth rate during the period 208 - 235 days of age and live weight at 235 days of age, when a tendency was observed ($P < 0.10$) between lambs born to LM- and HM-dams.

Table 5.2: Effects of heavy or light dams fed either *ad libitum* or maintenance from days 21 - 140 of pregnancy on growth rates (g/day) until 396 days of age of female offspring. Table shows least square means ± S.E.

	Heavy		Light	
Age (days)	*Ad libitum*	Maintenance	*Ad libitum*	Maintenance
0-22	275^{bc} ± 7.3	250^{bd} ± 7.9	261^{b} ±8.0	215^{a} ± 8.2
23-46	298 ± 10.0	286 ± 9.9	300 ± 10.9	280 ± 11.4
47-80	285^{b} ± 6.1	272^{ab} ± 6.1	273^{ab} ± 6.7	256^{a} ± 7.0
81-100	258 ± 6.1	254 ± 6.0	257 ± 6.8	242 ± 7.0
101-187	170^{b} ± 2.5	171^{b} ± 2.5	168^{ab} ± 2.8	162^{a} ± 2.9
188-207	160^{b} ± 2.3	160^{b} ± 2.3	156^{ab} ± 2.5	151^{a} ± 2.6
208-235	129 ± 1.9	129 ± 1.9	128 ± 2.0	124 ± 2.1
236-263	135^{ab} ± 2.2	136^{b} ± 2.2	129^{a} ± 2.4	131^{ab} ± 2.5
264-291	133^{b} ± 1.6	136^{b} ± 1.6	133^{ab} ± 1.8	129^{a} ± 1.8
292-319	129^{b} ± 1.6	132^{b} ± 1.7	127^{ab} ± 1.8	125^{a} ± 1.9
320-396	105^{ab} ± 1.4	107^{b} ± 1.4	103^{ab} ± 1.6	102^{a} ± 1.6

Means within row with different superscripts are significantly different ($P < 0.05$)

Table 5.3: Effects of heavy (H) or light (L) dams fed either *ad libitum* (A) or maintenance (M) from days 21 - 140 of pregnancy on the onset of puberty of female offspring. Table shows number of ewe lambs that reached puberty, percentage of ewe lambs that reached puberty (± 95% confidence interval), age at puberty, live weight at the average age of onset of puberty and the number of oestrus events (least square means ± S.E.).

	n	Reached puberty (*n*)	Reached puberty (%)	Age (days)	Live weight (kg)	Number of oestrus events
Dam size x nutrition						
HA	62	58	93.9 (84.0, 97.9)	260 ± 3.3	41.1^{b} ± 0.47	2.7 ± 0.20
HM	59	53	89.7 (78.2, 95.5)	262 ± 3.3	40.9^{b} ± 0.47	2.2 ± 0.19
LA	48	44	93.0 (81.5, 97.6)	264 ± 3.5	40.3^{ab} ± 0.52	2.4 ± 0.20
LM	44	43	98.1 (87.4, 99.7)	261 ± 3.8	39.3^{a} ± 0.53	2.8 ± 0.20
Birth rank						
Singletons	77	72	94.3 (85.8, 97.8)	259^{a} ± 2.9	41.7^{a} ± 0.41	2.6 ± 0.17
Twins	136	126	94.9 (89.1, 97.7)	264^{b} ± 3.0	39.0^{b} ± 0.31	2.5 ± 0.13

Different superscripts within a column are significantly different ($P < 0.05$)

Nutrition during pregnancy and dam size did not influence the number of animals reaching puberty, neither did it effect the age of the onset of puberty (Table 5.3). After adjusting for live weight, singletons reached puberty five days earlier than twins ($P = 0.011$).

Mean live weight at the average age of onset of puberty (day 263) was 41.1 kg ± 0.47; 40.9 kg ± 0.47; 40.3 kg ± 0.52 and 39.3 kg ± 0.53 kg for offspring born to HA-, HM-, LA- and LM-dams, respectively, with lambs born to LM-dams being significantly lighter than lambs born to HA- and HM-dams ($P < 0.05$).

Discussion

The present study shows that nutrition during pregnancy had an effect on the growth of the offspring until weaning. This effect was most likely caused by dams that were fed maintenance during pregnancy having lower milk yields than dams fed *ad libitum* (Wallace, 1938). This is reflected in the growth rates of the progeny, whereby lambs born to LM-dams had the lowest growth rates during the first 22 days of life compared with the other groups. This lower growth rate in lambs born to LM-dams is in agreement with work of Greenwood *et al.* (1998), who found that smaller newborns showed lower growth rates during the first two weeks of life (Greenwood *et al.*, 1998). The effect of dam size on the growth rate and live weight after weaning, found between the lambs born to LM- and HM-dams, is most likely due to genetic differences between heavy and light dams.

The live weights at the time of the onset of puberty in the groups are in agreement with work of Keane (1976). The threshold of live weight for reaching puberty was shown to be in the range 33 to 42 kg, for Suffolk crossbreds (Keane, 1976).

Mean oestrus events from this dataset are only indicative, as it was assumed that the data were normally distributed. The mean age of onset of puberty for each of the four groups was similar and this is in agreement with work of Da Silva *et al.* (2001), in which no differences were found in the time of onset of puberty or number of ovarian cycles between growth-restricted and normally grown lambs. However, when dam’s energy requirements were 50% restricted, ovarian development was significantly retarded in 47- and 62-day-old fetuses (Borwick *et al.*, 1997). Also ovulation rate was significantly reduced in lambs born to undernourished dams in contrast to those fed to requirements (Rae *et al.*, 2002).

Overall, singleton ewe lambs reached puberty five days earlier than did twin lambs. This is probably a result of singleton lambs having greater growth rates than twins throughout the study.

Although no differences were found in the onset of puberty among groups, this does not preclude the possibility of a longer-term or other impact on female reproductive performance, like lower volume-percentage of primordial follicles (de Bruin *et al.*, 1998) or reduced size of the uterus and ovarian volume (Ibanez *et al.*, 2000).

In conclusion, prior to weaning, maternal nutrition had an effect on live weight and growth rate of female progeny and after weaning dam size significantly affected live weight and growth rate. No differences in the age of onset of puberty were found among progeny born to either *ad libitum* or maintenance-fed heavy or light dams.

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CHAPTER 6

EFFECTS OF EWE SIZE AND NUTRITION DURING PREGNANCY ON GLUCOSE METABOLISM, FAT METABOLISM AND ADRENAL FUNCTION OF POST-PUBERTAL FEMALE TWIN OFFSPRING

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Abstract

Little is known about the long-term metabolic effects of maternal constraint on the offspring and whether a possible interaction between dam size and nutrition during gestation exists, affecting metabolic functions in the offspring. Heavy (H) (60.8 kg ± 0.18) and light (L) (42.5 kg ± 0.17) Romney dams were allocated to *ad libitum* (A) or maintenance (M) nutritional regimens under New Zealand pastoral grazing conditions, from days 21 - 140 post-insemination. One week prior to lambing, all dams and offspring were managed as one group and provided with *ad libitum* feeding. At 16 months of age, female twin-born offspring were catheterized and given intravenous insulin (0.15 IU/kg) (ITT), glucose (0.17 g/kg) (GTT) and epinephrine (1 μg/kg) (ETT) challenges to assess their glucose and fat metabolism and adrenal function. No effects of dam size or interactions between dam size and dam nutrition were found on glucose or fat metabolism or adrenal function. In response to the ETT, M-ewes showed greater ($P < 0.05$) peak glucose concentrations, increased ($P < 0.05$) glucose AUC and tended ($P < 0.10$) to have increased maximum change in glucose and NEFA concentrations compared to A-ewes. No effects of dam nutrition were found on glucose tolerance, insulin resistance or adrenal function in response to GTT and ITT. In conclusion, dam size had no effect on glucose metabolism, adrenal function or fat metabolism in 16-mo-old female twin offspring. Dam nutrition during pregnancy from day 21-140 had no major effect on glucose metabolism, adrenal function or lipolysis, however, it did affect gluconeogenesis and/or glycogenolysis, with increased glucose production in ewes born to maintenance-fed dams. These results indicate that M-ewes could have an advantage over A-ewes in physiological stressful situations in life (e.g., pregnancy, lactation) as their liver may be able to supply more glucose to support their growing conceptus and milk production to increase the chances of survival of their offspring.

Introduction

There is increasing support in the literature for the concept that adverse conditions *in utero* may result in developmental adaptations that permanently change structure, physiology, metabolism and postnatal growth of offspring (Wu *et al.*, 2006). Men and women exposed *in utero* to the Dutch famine developed glucose intolerance (Ravelli *et al.*, 1998; de Rooij *et al.*, 2006), an atherogenic lipid profile (Roseboom *et al.*, 2000) and abdominal obesity (Ravelli *et al.*, 1999) in adult life. Reduced maternal nutrient intake in sheep has resulted in glucose intolerance (Oliver *et al.*, 2002; Gardner *et al.*, 2005; Ford *et al.*, 2007), altered hypothalamus-pituitary-adrenal (HPA)-axis function (Hawkins *et al.*, 2000a; Bloomfield *et al.*, 2003; Gardner *et al.*, 2006), increased adiposity (Gardner *et al.*, 2005; Ford *et al.*, 2007) and altered postnatal growth (Greenwood *et al.*, 1998; Ford *et al.*, 2007) in their offspring. However, there have been inconsistent results observed in the offspring among ovine studies (Mellor, 1983; Robinson *et al.*, 1999b; Wu *et al.*, 2006; Symonds, 2007; Kenyon, 2008). These inconsistencies could be due to differences in dam live weight and body condition, the timing within pregnancy, duration and level of the nutritional manipulation, postnatal management of the young offspring, and the timing and nutritional levels prior to the period of investigating the offspring (Mellor, 1983; Robinson *et al.*, 1999b; Wu *et al.*, 2006; Symonds, 2007; Kenyon, 2008).

Dam size may also play an important role in the development of adult health and disease, as dam size could affect fetal growth through the size of the placenta which influences the nutrient supply to the developing fetus (Mellor, 1983). Embryo transfer and cross-breeding experiments in large and small breeds of sheep (Dickinson *et al.*, 1962; Gootwine *et al.*, 1993), horses (Walton and Hammond, 1938; Allen *et al.*, 2002) and pigs (Wilson *et al.*, 1998) have shown that fetal growth can be either increased or restricted from the normal genetic potential by varying dam size. However, little is known about the long-term metabolic effects of dam size on the offspring and there is no information about a possible interaction of dam size and nutrition during gestation, affecting metabolism in the offspring.

Our previous work in the same cohort of animals has shown that maintenance nutrition of the dam during pregnancy, under commercial grazing conditions, altered the bone mineral content/lean mass ratio of the fetal hindquarters when compared to *ad libitum* feeding, irrespective of dam size (Firth *et al.*, 2008). Furthermore, we have shown that dam nutrition affected birth weight in twin-born lambs (Kenyon *et al.*, 2009) and that dam nutrition and dam size during pregnancy affected postnatal growth (van der Linden *et al.*, 2007) of the offspring. Therefore, we examined the effects of dam size and nutrition during pregnancy under commercial grazing conditions on glucose and fat metabolism and adrenal gland function in post-pubertal female twin-born offspring. Further, the study-design allowed for testing of potential interactions between dam nutrition and size. We hypothesized that offspring born to light dams and maintenance-fed dams during pregnancy would have augmented glucose intolerance and insulin resistance, impaired adrenal function and reduced lipolysis compared to offspring born to *ad libitum*-fed and heavy dams. We also hypothesized that a possible interaction between dam nutrition and size would amplify these alterations in metabolic function of the offspring.

Materials and Methods

The study was conducted at the Massey University Keeble Sheep and Beef farm, 5 km south of Palmerston North, New Zealand. All experimental animal procedures were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Dams

Four hundred and fifty heavy (H) (60.8 kg ± 0.18) and 450 light (L) (42.5 kg ± 0.17) Romney dams were selected from the extremes in a commercial flock of 2900 ewes, on the basis of size, as determined by live weight, and bred using artificial insemination as previously described by Kenyon *et al.* (2009). From day 21 until day 140 post-insemination, the dams were randomly allocated, within size, to *ad libitum* (A) or maintenance (M) nutritional regimens under New Zealand pastoral grazing conditions (resulting in 78.4 kg ± 0.37 vs. 65.0 kg ± 0.35; $P < 0.05$; for A- and M-dams at d140) (Kenyon *et al.*, 2009). Pasture herbage was the only nutritional source and the average pre- and post-grazing pasture covers during the period days 21 – 140 were 1330 kg DM/ha ± 140.0 and 804.0 kg DM/ha ± 133.4 respectively, for the M-feeding regimen and 2304.0 kg DM/ha ± 156.8 and 1723.3 kg DM/ha ± 149.7 for the A-feeding regimen (Kenyon *et al.*, 2009).

From day 140 of pregnancy through to weaning, all dams and their lambs were provided with *ad libitum* feeding. Singleton- and twin-born offspring born to H-dams were heavier ($P < 0.05$) at birth (5.51 kg ± 0.05 and 5.37 kg ± 0.05; for offspring born to H- and L-dams, respectively) and weaning (32.7 kg ± 0.36 and 31.2 kg ± 0.33; for offspring born to H- and L-dams, respectively) than offspring born to L-dams. Twin-born offspring born to A-dams were heavier ($P < 0.05$) at birth (5.23 kg ± 0.06 and 4.52 kg ± 0.06; for offspring born to A- and M-dams, respectively) and weaning (30.6 kg ± 0.42 and 28.2 ± 0.41; for offspring born to A- and M-dams, respectively) than offspring born to M-dams (Kenyon *et al.*, 2009). After weaning, female progeny were managed and fed to nutritional requirements as one group under commercial New Zealand farming practice (van der Linden *et al.*, 2007). The study, therefore, utilized a two-by-two factorial design, two nutrition treatments (M vs. A) and two dam-size treatments (H vs. L).

Ewe offspring

At 16 months of age, 48 twin-born ewe offspring were housed indoors in two, random, consecutive batches of 24 ewes (n = 12 ewes born to the HA-, HM-, LA-, and LM-dam treatment groups, as described above (Kenyon *et al.*, 2009). Each group of 12 ewes contained eight ewes from female-female twin sets, born to four dams, and four ewes from female-male twin sets, born to four dams; birth weight differences within the twin pairs were < 25%. The ewe offspring born to the heavy or light dams fed either maintenance or *ad libitum* during pregnancy, will be referred to as H-, L-, A-, or M-ewes, respectively.

The randomly selected ewes were housed in a large shed, as one batch for one week, followed by housing in individual pens for two weeks prior to the metabolic challenges. Ewes had free access to water and were fed to achieve an average liveweight gain of 100 g/day (19 MJ ME/day (Geenty and Rattray, 1987)). The feed was a mixture of pelleted food (500 g of 12 MJ

ME/kg) and lucerne chaff (1500 g of 8.6 MJ ME/kg) (average ewe live weight prior to housing was 50 kg (± 4.4 S.D.). Ewes were fed daily between 1 and 2 pm; feed intake (offered less refusals) was recorded at 8 am each day.

Three days prior to the start of the metabolic challenges, both jugular veins were catheterized with indwelling polyvinyl catheters after administration of local anaesthetic (Lopaine, Lignocaine Hydrochloride U.S.P. 20 mg/mL, Ethical Agents LTD, Auckland, New Zealand); catheters were secured to the neck with tape and secured on the animal's back under a meshed stocking. This was followed by single prophylactic intramuscular (hind leg) administration of antibiotics (Duplocillin® LA, Intervet LTD, Newmarket, Auckland, 2 mL per 50 kg live weight). One catheter was used for hormone/metabolite administration and the other for blood collection.

After an overnight fast, ewes were submitted to an insulin tolerance test on day 1 (ITT; 0.15 IU/kg live weight, Humulin R, Eli Lilly, Indianapolis, IN), a glucose tolerance test on day 2 (GTT; 0.17 g/kg live weight, Dextrose 40%, Bomac Laboratories LTD, Auckland, New Zealand), and an epinephrine tolerance test on day 3 (ETT; 1 μg/kg live weight, Sigma-Aldrich Inc. St Louis, MO, USA), between 8 and 9 am. Blood samples (5 mL) were collected in vacutainers containing EDTA (BD Vacutainer Systems, UK) at -5, 0, 2, 10, 20, 30, 40, 50, 60 and 120 min from the insulin administration, at -5, 0, 2, 5, 10, 20, 30, 40, 50, 60, 120 minutes from the glucose administration and at -5, 0, 2, 5, 7, 10, 20, 45 and 60 min from the epinephrine administration. On all three days, ewes were re-fed after completion of the sampling. All blood samples were immediately placed on ice until centrifugation at 3000 rpm (1006 G) for 15 min. Triplicate plasma aliquots were stored at -20ºC until analysis.

Assays

Plasma metabolite concentrations were measured using a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan) using commercial kits for glucose and cholesterol (Roche, Mannheim, Germany) and non-esterified free fatty acids (NEFA) and triglyceride (Randox Laboratories Ltd, Ardmore, Crumlin, UK). Insulin was measured by radioimmunoassay (RIA) with ovine insulin as the standard (Sigma, Batch no. I9254) (Rumball *et al.*, 2008a). The minimal detectable concentration was 0.03 ng/mL; inter- and intra-assay coefficients of variation were 14.3% and 11.5%, respectively.

Plasma cortisone and cortisol concentrations were measured using mass spectrometry (Rumball *et al.*, 2008b). The internal standards were cortisol-d2 for cortisol; corticosterone-d8 for cortisone. A 100 μL volume of internal standard (20 ng/mL in water) was added to 200 μL plasma. Steroids were extracted using 1 mL ethyl acetate. After removal of the organic supernatant, samples were dried, re-suspended in 100 μL mobile phase (80% methanol and 20% water), and transferred to HPLC injector vials. A 25 μL volume was injected onto an HPLC mass spectrometer system consisting of a Surveyor MS pump and autosampler followed by an Ion Max APCI source on a Finnigan TSQ Quantum Ultra AM triple-quadrapole mass spectrometer all controlled by Finnigan Xcaliber software (Thermo Electron Corp., San Jose, CA). The mobile phase was isocratic, flowing at 600 μL/min through a Luna 3 μC18(2) 100A 250 X 4.6 column at 40° C (Phenomenex, Auckland, New Zealand). Retention times were as follows: cortisol, 5.9 min and cortisone, 5.5 min. Ionization was in positive mode, and Q2 had 1.2 mTorr of argon for both steroids. The mass transitions, for internal standard and steroid, respectively, were as follows: cortisol-d2, 365.3 – 122.2 at 28 V, and cortisol, 363.3 – 121.2 at 28 V; corticosterone-d8, 355.3 – 125.2 at 24 V, and cortisone, 361.1 – 163.0 at 28 V. Mean inter- and intra-assay coefficient of variation values were as follows: cortisol, 11.1 and 10.6%; cortisone, 12.0 and 6.7%.

Plasma variables

Glucose tolerance test (GTT)

Basal glucose and insulin concentration were calculated as the average of the concentrations in the blood samples taken at -5 and 0 min before commencement of the GTT. The maximum changes in glucose and insulin concentrations were calculated as the differences between the basal concentration and their respective maximum concentrations after administration of the glucose bolus. Glucose tolerance was measured as the area under the glucose curve ($GluAUC_{GTT}$) and absolute insulin secretion was measured as the area under the plasma insulin curve ($InsAUC_{GTT}$) in response to GTT.

Insulin tolerance test (ITT)

Basal glucose, insulin, cortisone and cortisol concentration were calculated as the average of the concentrations in the blood samples taken at -5 and 0 min before commencement of the ITT. The maximum changes in glucose, cortisone, and cortisol concentrations were calculated as the differences between the basal concentrations and their respective maximum (cortisone and cortisol) or minimum (glucose) concentrations after administration of the insulin bolus.

Insulin resistance was measured as the area under the glucose curve ($GluAUC_{ITT}$) in response to the ITT. Absolute cortisone ($CsoneAUC_{TT}$) and cortisol secretion ($CortAUC_{ITT}$) were measured as the area under the plasma cortisone and cortisol curve respectively in response to the ITT.

Epinephrine tolerance test (ETT)

Basal glucose, NEFA, insulin, triglyceride and cholesterol concentrations were calculated as the average of the concentrations in the blood samples taken at -5 and 0 min before commencement of the ETT. The maximum changes in glucose, NEFA and insulin concentrations were calculated as the differences between the basal concentrations and their respective maximum concentrations after administration of the epinephrine bolus.

Absolute glucose ($GluAUC_{ETT}$), insulin ($InsAUC_{ETT}$), NEFA ($NefaAUC_{ETT}$), triglycerides ($TrigAUC_{ETT}$) and cholesterol ($CholAUC_{ETT}$) secretion were measured as the area under the curves in response to the ETT.

Statistical analyses

All data were analysed using the MIXED procedure of SAS (2006). Plasma hormone and metabolites concentrations of all challenges were log transformed to normalize the data for statistical analyses over time and are presented as least square means with their 95% confidence interval. Repeated measures of plasma glucose, insulin, cortisol, cortisone, NEFA, triglycerides and cholesterol concentrations over time of all challenges were analysed with a mixed linear model that included fixed and random effects. The fixed effects considered were dam nutrition, dam size, and time of sampling, the interactions dam nutrition by dam size, dam nutrition by time of sampling and dam size by time of sampling. Batch was considered as a random effect. Birth weight and live weight at time of the challenge were fitted as covariables. Effect of animal was considered as random effect to account for repeated measures on the same individual through the time. The random residuals were modelled with a heterogeneous variance structure; based on the Akaike's information criterion, a compound symmetry error structure was determined as the most appropriate residual covariance structure for repeated measures over time within animals.

Metabolic variables were analysed using the same mixed linear model as stated above including the fixed effects of dam nutrition, dam size, and the interaction of dam nutrition by dam size and the random effect of batch. Metabolic variables are presented as least square means ± standard error (S.E.).

Results

A detailed description of dam weight and dam weight gain during pregnancy is presented in a separate publication (Kenyon *et al.*, 2009). No dam nutrition or size effects ($P > 0.10$) were found for birth weight and current weight in the 48 animals studied in this experiment. No dam nutrition or size effects ($P > 0.10$) were found on food intake between groups, during the period of individual housing, including the acclimatization period (data not shown).

No interactions between dam nutrition treatment and dam size ($P > 0.10$) were found during pregnancy, therefore the main dam nutrition and dam size effects are presented.

Glucose metabolism

In response to the GTT, M-ewes tended ($P = 0.08$) to have a greater maximum change in insulin concentration than A-ewes (Table 6.1 and Figure 6.1). No dam nutrition or size effects ($P >$

0.10) were found for basal glucose and insulin concentrations, glucose and insulin peak concentrations, or maximum change in glucose and insulin concentrations in response to the GTT. No effects of dam nutrition or size ($P > 0.10$) were found in $GluAUC_{GTT}$, $InsAUC_{GTT}$ in response to the GTT (Figure. 6.1).

In response to the ITT, no dam nutrition or size effects ($P > 0.10$) were found for basal glucose concentrations, glucose nadir concentrations, or maximum change in glucose concentrations (Table 6.2). No effects of dam nutrition or size ($P > 0.10$) were found in $GluAUC_{ITT}$ between groups in response to the ITT (Figure 6.2).

Table 6.1. The effects of heavy (H) or light (L) dams fed *ad libitum* (A) or maintenance (M) during pregnancy on the female offspring's glucose and insulin concentrations in response to glucose tolerance test. Data are presented as least square means (± S.E.).

		Dam treatment[A]				*P* value	
Metabolite/ hormone	Parameter	*Ad libitum*	Maintenance	Heavy	Light	Nutrition effect	Size effect
Glucose	Baseline (mM L^{-1})	3.93 ± 0.10	4.07 ± 0.10	4.04 ± 0.10	3.96 ± 0.10	NS	NS
	Log peak conc. (mM L^{-1})	2.33 ± 0.01	2.34 ± 0.01	2.33 ± 0.01	2.34 ± 0.01	NS	NS
	Max change (mM L^{-1})	6.35 ± 0.17	6.30 ± 0.17	6.25 ± 0.17	6.39 ± 0.17	NS	NS
Insulin	Baseline (ng mL^{-1})	0.44 ± 0.04	0.37 ± 0.04	0.40 ± 0.04	0.41 ± 0.04	NS	NS
	Log peak conc. (ng mL^{-1})	0.71 ± 0.08	0.81 ± 0.08	0.79 ± 0.08	0.74 ± 0.08	NS	NS
	Max change (ng mL^{-1})	1.67 ± 0.16	2.07 ±0.16	1.97 ± 0.16	1.76 ± 0.16	0.08	NS

[A] No interactions between dam nutrition and dam size were detected (*P* > 0.10); therefore, only the main effects are reported (*n* = 24 per dam treatment group); NS: non-significant

Table 6.2. The effects of heavy (H) or light (L) dams fed *ad libitum* (A) or maintenance (M) during pregnancy on the female offspring's glucose, cortisol and cortisone concentrations in response to an insulin tolerance test. Data are presented as least square means (± S.E.).

		Dam treatment^{A}				*P* value	
Metabolite/ hormone	Parameter	*Ad libitum*	Maintenance	Heavy	Light	Nutrition effect	Size effect
Glucose	Baseline (mM L^{-1})	3.62 ± 0.04	3.60 ± 0.04	3.59 ± 0.04	3.64 ± 0.04	NS	NS
	Log nadir conc. (mM L^{-1})	0.38 ± 0.03	0.34 ± 0.03	0.36 ± 0.03	0.36 ± 0.03	NS	NS
	Max change (mM L^{-1})	2.15 ± 0.04	2.18 ± 0.04	2.15 ± 0.04	2.19 ± 0.04	NS	NS
Cortisol	Baseline (ng mL^{-1})	8.34 ± 0.88	9.15 ± 0.88	8.34 ± 0.88	9.14 ± 0.88	NS	NS
	Log peak conc. (ng mL^{-1})	3.77 ± 0.05	3.78 ± 0.05	3.75 ± 0.04	3.84 ± 0.04	NS	NS
	Max change (ng mL^{-1})	36.7 ± 2.18	35.3 ± 2.17	34.66 ± 2.05	38.79 ± 2.06	NS	NS
Cortisone	Baseline (ng mL^{-1})	1.73 ± 0.11	1.93 ± 0.11	1.80 ± 0.11	1.86 ± 0.11	NS	NS
	Log peak conc. (ng mL^{-1})	1.48 ± 0.04	1.47 ± 0.04	1.50 ± 0.04	1.46 ± 0.04	NS	NS
	Max change (ng mL^{-1})	2.75 ± 0.17	2.54 ± 0.17	2.78 ±0.17	2.51 ± 0.17	NS	NS

[A] No interactions between dam nutrition and dam size were detected (*P* > 0.10); therefore, only the main effects are reported (*n* = 24 per dam treatment group); NS: non-significant

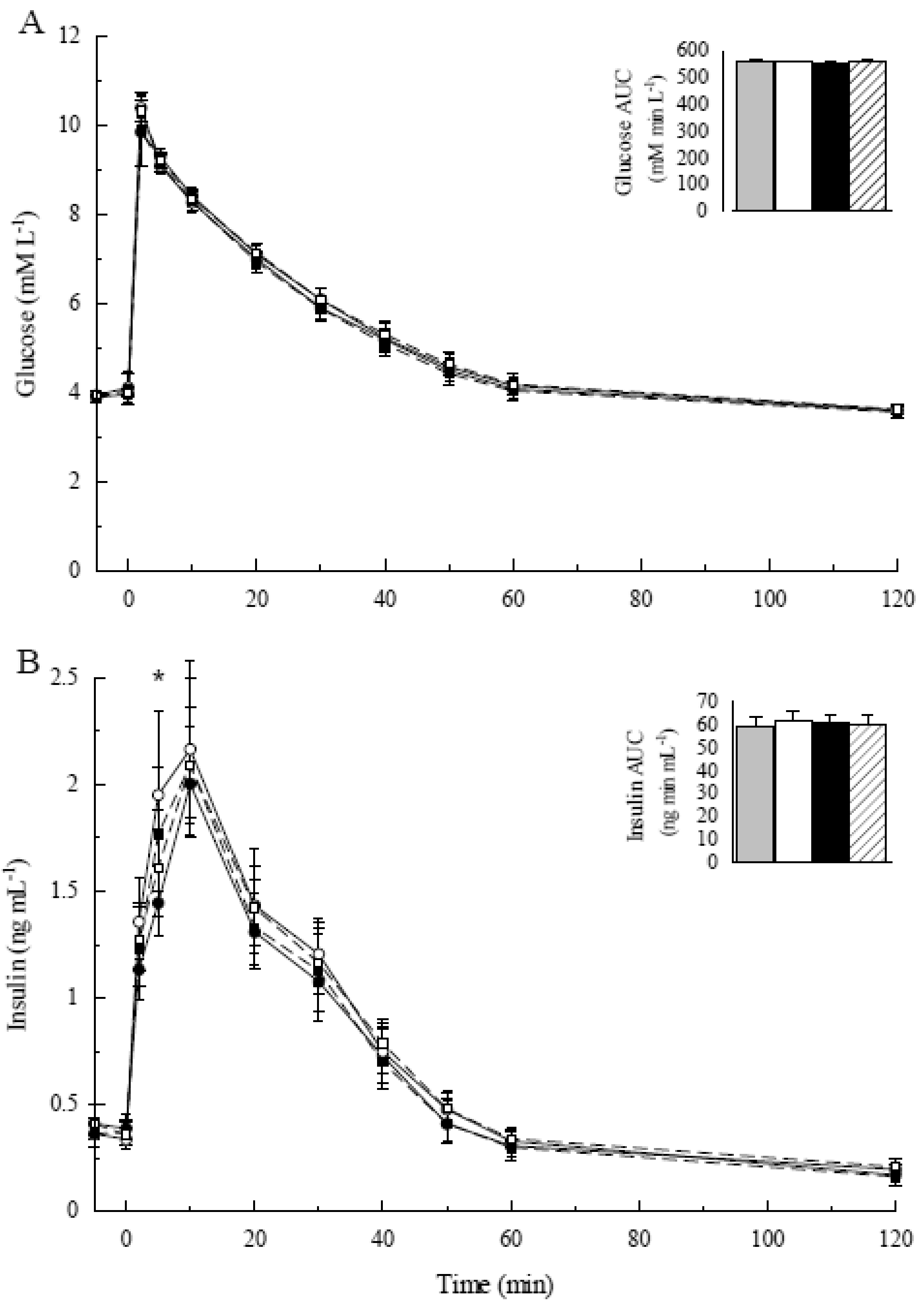

Figure. 6.1. Glucose (A) and insulin (B) responses to an intravenous glucose tolerance test (GTT) for ewes born to heavy (H) or light dams (L), fed *ad libitum* (A) or maintenance (M) during pregnancy. Data are presented as least square means (± 95% CI). Areas under the curve are shown as *inset* histograms and are presented as least square means (± S.E.). No interaction between dam nutrition and dam size was detected ($P > 0.10$); therefore, only the main effects are reported ($n = 24$ per dam treatment group). –●– and grey bars, *ad libitum*; –o–and open bars, maintenance; ---■--- and black bars, heavy; ---□--- and striped bars, light. * $P < 0.05$; dam nutrition effect.

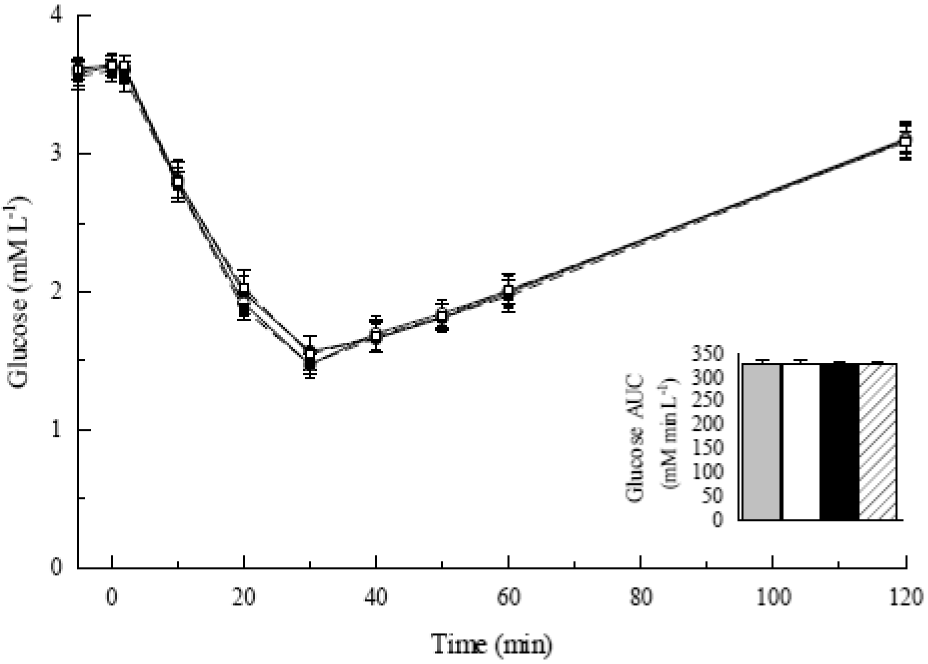

Figure 6.2. Glucose response to an intravenous insulin tolerance test (ITT) for ewes born to heavy (H) or light dams (L), fed *ad libitum* (A) or maintenance (M) during pregnancy. Data are presented as least square means (± 95% CI). Areas under the curve are shown as *inset* histograms and are presented as least square means (± S.E.). No interaction between dam nutrition and dam size was detected ($P > 0.10$); therefore, only the main effects are reported (n = 24 per dam treatment group). –●– and grey bars, *ad libitum*; –o–and open bars, maintenance; ---■--- and black bars, heavy; ---□--- and striped bars, light.

Adrenal function

There were no effects of dam nutrition or dam size found ($P > 0.10$) on basal concentrations, peak concentrations, or maximum change in concentrations of cortisol and cortisone (Table 6.2). No dam nutrition or size effects ($P > 0.10$) were found on CortAUC$_{ITT}$ (3203 ng min mL^{-1} ± 165.0 vs. 3134 ng min mL^{-1} ± 161.3; for M- and A-ewes, respectively; 3308 ng min mL^{-1} ± 165.0 vs. 3029 ng min mL^{-1} ± 161.3; for L- and H-ewes, respectively) or CsoneAUC$_{ITT}$ (282 ng min mL^{-1} ± 19.4 vs. 240 ng min mL^{-1} ± 19.0; for M- and A-ewes, respectively; 259 ng min mL^{-1} ± 18.1 vs.276 ng min mL^{-1} ± 17.4; for L- and H-ewes, respectively) in response to the ITT.

Fat metabolism

In response to the ETT, M-ewes showed greater (*P* = 0.01) peak glucose concentrations and tended to have greater maximum change in glucose (*P* = 0.098) and NEFA (*P* = 0.07) concentrations compared to A-ewes (Table 6.3). Basal insulin concentrations were greater (*P* = 0.046) in M-ewes compared to A-ewes. M-ewes tended (*P* = 0.096) to have lesser basal triglycerides concentrations than A-ewes (0.14 mM L^{-1} ± 0.01 vs. 0.16 mM L^{-1} ± 0.01; for M- and A-ewes, respectively). No dam nutrition (*P* > 0.10) effect was found on basal cholesterol concentrations (1.11 mM L^{-1} ± 0.03 vs. 1.08 mM L^{-1} ± 0.03; for A- and M-ewes, respectively) and no dam nutrition effects were found (*P* > 0.10) for basal glucose and NEFA concentrations and maximum change in insulin and NEFA concentrations in response to ETT (Table 6.3).

No effects of dam size (*P* > 0.10) were found for basal concentrations, peak concentration, and maximum change in concentration of glucose, NEFA and insulin concentrations in response to ETT. No dam-size effects (*P* > 0.10) were found for basal concentrations of triglycerides (0.15 mM L^{-1} ± 0.01 vs. 0.16 mM L^{-1} ± 0.01; for H- and L-ewes respectively) or cholesterol (1.09 mM L^{-1} ± 0.03 vs. 1.10 mM L^{-1} ± 0.03; for H- and L-ewes, respectively).

M-ewes showed greater (*P* < 0.05) $GluAUC_{ETT}$ during the first 20 min after the ETT (Figure 6.3). Area under the triglyceride curve was smaller (*P* = 0.001) in M-ewes compared to A-ewes over the 60-min period after epinephrine administration (8.3 mM min L^{-1} ± 0.40 vs. 9.9 mM min L^{-1} ± 0.41; M- and A-ewes, respectively), however, no differences were found for $TrigAUC_{ETT}$ during the first 20 min (Figure 6.4). No dam-nutrition effects (*P* > 0.10) were found for the area under the insulin (Figure 6.3), NEFA (Figure 6.4), and cholesterol curves (20.3 mM min L^{-1} ± 0.48 vs. 21.1 mM min L^{-1} ± 0.49; for M- and A-ewes, respectively). No dam-size effects (*P* > 0.10) were found for the area under the insulin (Figure. 6.3), NEFA, triglycerides (Figure 6.4) and cholesterol curves (20.6 mM min L^{-1} ± 0.49 vs. 20.8 mM min L^{-1} ± 0.48; for L- and H-ewes, respectively) in response to the ETT.

Table 6.3. The effects of heavy (H) or light (L) dams fed *ad libitum* (A) or maintenance (M) during pregnancy on the female offspring's glucose, NEFA and insulin concentrations in response to an epinephrine tolerance test. Data are presented as least square means (± S.E.).

Metabolite/ hormone	Parameter	Dam treatment[A]				*P* value	
		Ad libitum	Maintenance	Heavy	Light	Nutrition effect	Size effect
Glucose	Baseline (mM L^{-1})	3.84 ± 0.07	3.95 ± 0.07	3.85 ± 0.07	3.94 ± 0.07	NS	NS
	Log peak conc. (mM L^{-1})	1.48 ± 0.02	1.56 ± 0.02	1.51 ± 0.02	1.54 ± 0.02	0.01	NS
	Max change (mM L^{-1})	0.58 ± 0.12	0.86 ± 0.12	0.70 ± 0.12	0.74 ± 0.12	0.098	NS
NEFA	Baseline (mM L^{-1})	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	NS	NS
	Log peak conc. (mM L^{-1})	0.13 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.07	NS
	Max change (mM L^{-1})	0.13 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.07	NS
Insulin	Baseline (ng mL^{-1})	0.22 ± 0.02	0.29 ± 0.02	0.25 ± 0.02	0.27 ± 0.02	0.046	NS
	Log peak conc. (ng mL^{-1})	-0.81 ± 0.09	-0.70 ± 0.09	-0.77 ± 0.09	-0.75 ± 0.09	NS	NS
	Max change (ng mL^{-1})	0.26 ± 0.05	0.26 ± 0.04	0.27 ± 0.04	0.27 ± 0.04	NS	NS

[A] No interactions between dam nutrition and dam size were detected ($P > 0.10$); therefore, only the main effects are reported ($n = 24$ per dam treatment group)

NS: non-significant

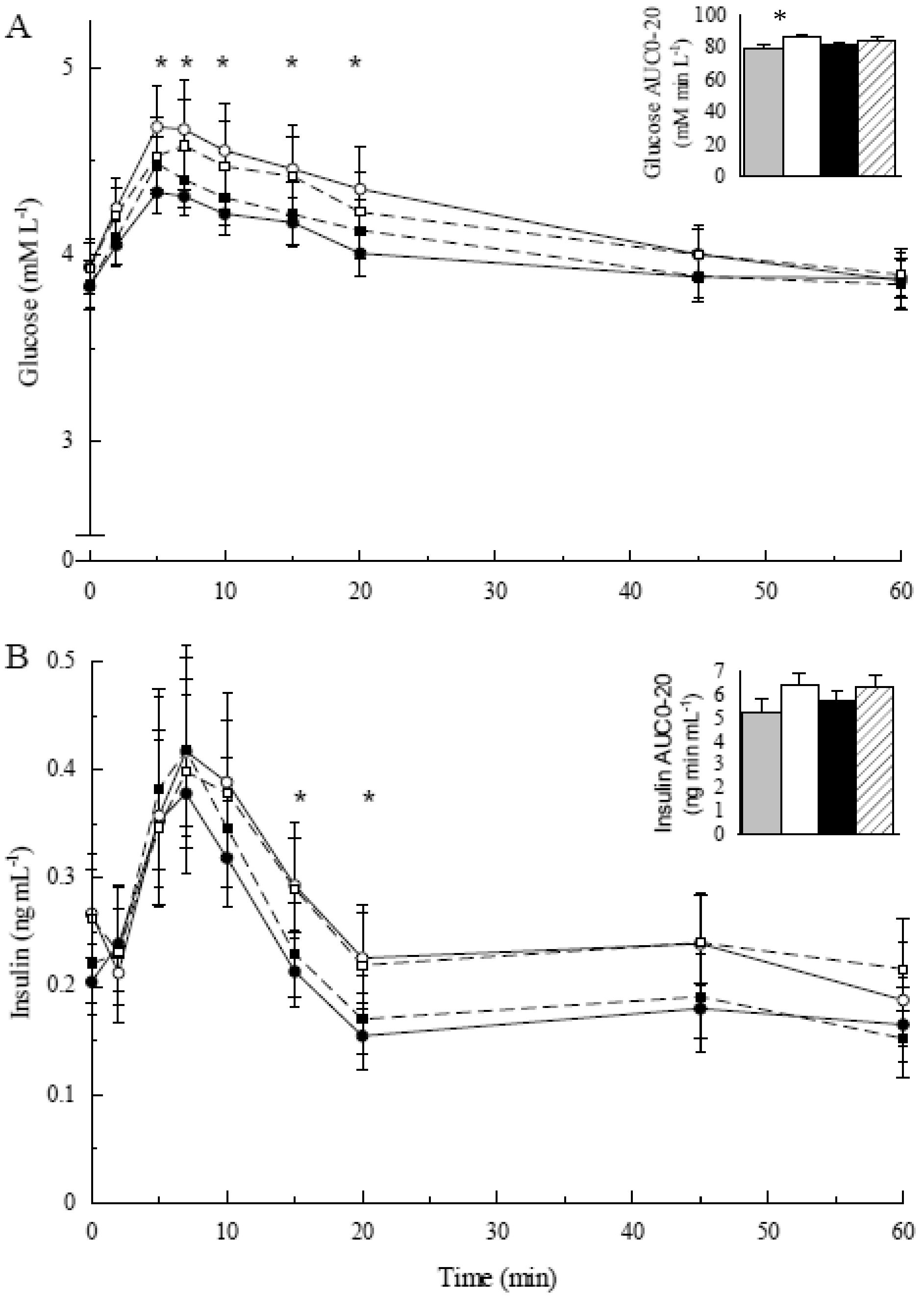

Figure 6.3. Glucose (A) and insulin (B) responses to an intravenous epinephrine tolerance test (ETT) for ewes born to heavy (H) or light dams (L), fed *ad libitum* (A) or maintenance (M) during pregnancy. Data are presented as least square means (± 95%CI). Areas under the curve (AUC 0-20 min) are shown as *inset* histograms and are presented as least square means (± S.E.). No interaction between dam nutrition and dam size was detected ($P > 0.10$); therefore, only the main effects are reported (n = 24 per dam treatment group). —●— and grey bars, *ad libitum*; —o— and open bars, maintenance; ---■--- and black bars, heavy; ---□--- and striped bars, light. * $P < 0.05$; dam nutrition effect.

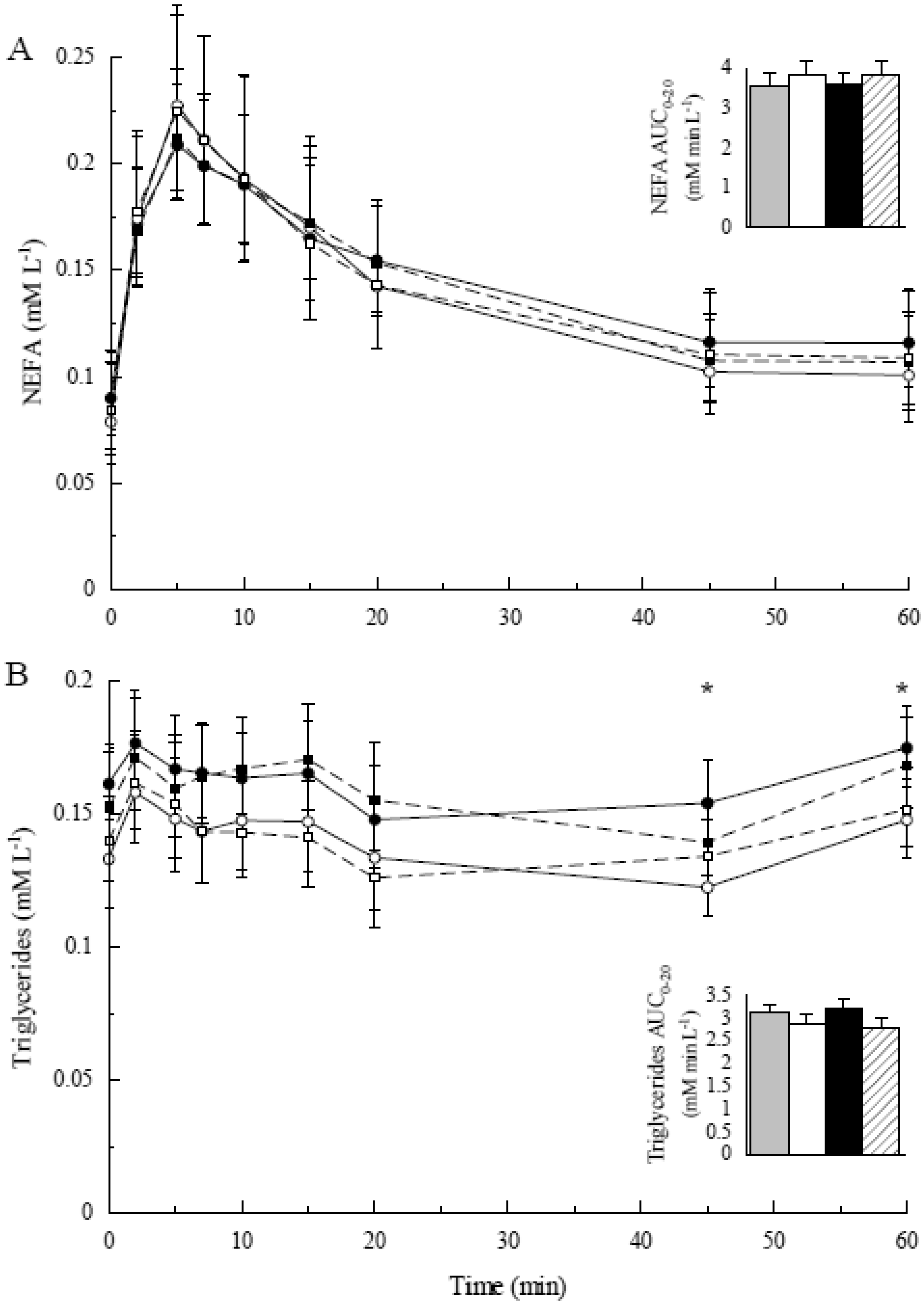

Figure 6.4. NEFA (A) and triglycerides (B) responses to an intravenous epinephrine tolerance test (ETT) for ewes born to heavy (H) or light dams (L), fed *ad libitum* (A) or maintenance (M) during pregnancy. Data are presented as least square means (± 95% CI). Areas under the curve (AUC 0-20 min) are shown as *inset* histograms and are presented as least square means (± S.E.). No interaction between dam nutrition and dam size was detected ($P > 0.10$); therefore, only the main effects are reported ($n = 24$ per dam treatment group). —●— and grey bars, *ad libitum*; —o— and open bars, maintenance; ---■--- and black bars, heavy; ---□--- and striped bars, light. * $P < 0.05$; dam nutrition effect.

Discussion

There was no interaction between dam nutrition and size during pregnancy on the metabolic function of 16-month-old offspring found in the present study. Dam size had no effect on glucose metabolism, fat metabolism and adrenal function in this study. Although the uterine capacity of small dams may be smaller compared to that of large dams and a reduction in fetal growth from its genetic potential may have occurred, without obligatorily altering metabolic function later in life. Dam nutrition showed a small effect on glucose metabolism in our study, with a slight insulin increment in M-ewes compared to A-ewes but no effect on adrenal function was found. Our results are in contrast to other studies that found a greater effect of dam nutrition during pregnancy on glucose metabolism (Oliver *et al.*, 2002; Gardner *et al.*, 2005; Husted *et al.*, 2008) and on HPA-axis function (Bloomfield *et al.*, 2003) in the offspring. However, in the previous studies, dam undernutrition regimens applied were more severe and for shorter periods during (mainly late) gestation (Oliver *et al.*, 2002; Gardner *et al.*, 2005; Husted *et al.*, 2008).

The dams in our study were fed maintenance from days 21 - 140 of gestation, not allowing them to build-up sufficient body reserves to maintain adequate milk production (Mellor, 1983). Therefore, the offspring born to the maintenance-fed dams were possibly also modestly nutrient deprived in early life which resulted in reduced growth rates until weaning (van der Linden *et al.*, 2007). We hypothesized that undernutrition during fetal life and during early postnatal life would result in glucose intolerance and insulin resistance at 16 months of age, however, no such effect was found. Perhaps the offspring born to maintenance-fed dams were programmed correctly *in utero* for their “poor” early postnatal environment (Gluckman *et al.*, 2005; Gardner *et al.*, 2007a) and consequently no impairment of the glucose metabolism at 16 months of age would be expected. However, insulin resistance is found to be related to adipose-tissue-specific reductions in GLUT-4 expression (Gardner *et al.*, 2005). We have not been able to investigate potential mechanisms at the molecular level, as these animals are being studied for lifetime effects, however, it could be that the sub-cellular insulin-signalling proteins downstream of the receptor may be programmed before development of increased adiposity and glucose intolerance commences (Fernandez-Twinn *et al.*, 2005).

Lower birth weight is believed to be related to altered glucose and insulin metabolism in later life (Oliver *et al.*, 2002; Newsome *et al.*, 2003). If this is a true relationship, we might have underestimated the effects of dam nutrition during pregnancy on the development of impaired glucose metabolism in our study, as birth weights of all twins, both males and females, born to maintenance-fed dams were lighter than twins born to *ad libitum*-fed dams (Kenyon *et al.*, 2009), which was not observed in the sub-group studied in the present study.

Catecholamines consistently stimulate glycogenolysis, gluconeogenesis and lipolysis in sheep (Basset, 1970; Vernon, 1981; McDowell, 1983). The increase in plasma glucose and NEFA concentrations observed after epinephrine administration demonstrates that the dose of epinephrine used (1 µg/kg live weight) was sufficient to elicit a physiological response. This is in agreement with previous work in sheep (Carter *et al.*, 1989) and goats (Chapa *et al.*, 1996).

Dam nutrition during pregnancy affected glucose production in response to the epinephrine tolerance test. Ewes born to dams fed maintenance during pregnancy produced more glucose compared to *ad libitum*-fed dams. This could be due to either increased rate of glycogenolysis, greater glycogen stores or increased gluconeogenesis in response to epinephrine (McDowell, 1983) in the M-ewes compared to A-ewes. Offspring born to rats fed a low protein (LP) diet during pregnancy and lactation had altered hepatic key enzymes involved in glycolysis and gluconeogenesis (Desai *et al.*, 1997). Desai *et al.* (1997) found that glucokinase, a hepatic enzyme involved in glycolysis, was decreased and phosphoenolpyruvate carboxykinase (PEPCK), involved in gluconeogenesis, was increased in LP-offspring. Sheep exposed to betamethasone *in utero* showed increased glucose-6-phosphatase, involved in gluconeogenesis, activity at 3.5 years of age (Sloboda *et al.*, 2005). These results suggest that offspring exposed to betamethasone or born to malnourished dams shifted their metabolic control point to enhance the chances of survival under in postnatal nutritional circumstances (Sloboda *et al.*, 2005). The gluconeogenesis genes encoding phosphoenolpyruvate carboxykinase (PEPCK) and gluocose-6-phosphatase, which catalyze the first and last step in hepatic gluconeogenesis, respectively, are likely targets for fetal programming of hyperglycaemia and will possibly result in insulin resistance (McCurdy and Friedman, 2006) if pre- and postnatal nutrition do not match. In addition, liver sparing could have possibly occurred in the M-ewes (Haugen *et al.*, 2005; Baschat, 2006) which could have induced up-regulation of hepatic enzymes. Blair *et al.* (unpublished data) showed in a cohort of animals (‘siblings’ from the offspring studied in the current experiment) euthanized at day 140 of pregnancy, that those which were carried by dams fed maintenance had greater liver weights at day 140 ($P < 0.05$) compared to those carried by *ad libitum* fed dams (115.4 g ± 4.4 vs. 95.2 g ± 4.4; for M-fetuses and A-fetuses, respectively). Although we have not been able to investigate potential mechanisms at a molecular level, it is possible that an up-regulation and down-regulation of enzymes involved in gluconeogenesis and glycolysis, respectively (Desai *et al.*, 1997) and/or glycogenolysis have occurred in the ewes born to dams fed maintenance during pregnancy.

Although changes in NEFA concentrations are a tentative indicator of changes in the rate of lipolysis, the increase in plasma NEFA concentrations in response to catecholamines is most likely explained in terms of changes in the rate of lipolysis (Vernon, 1981). However, the rate of lipolysis in our study could have been underestimated by measuring NEFA, as fatty acids are rapidly re-esterified, or may be retained within the cell (Vernon, 1981). In addition, insulin can reduce the rate of fatty acid release from adipose tissue by stimulating glucose uptake and hence, fatty acid re-esterification (Vernon, 1981). In our study, we observed a two-fold increase in NEFA concentrations after epinephrine administration, and in contrast to our hypothesis of reduced lipolysis, M-ewes showed a slight increment in NEFA concentrations compared to A-ewes. However, no differences in area under the NEFA curve were found between the groups. Basal triglyceride concentrations tended to be lower in M-ewes compared to A-ewes. This is in contrast to a study conducted in rats using a LP diet during pregnancy, which found increased basal plasma NEFA and triglycerides concentrations in LP offspring after weaning (Burdge *et al.*, 2004). The lower basal triglyceride concentrations found in M-ewes could be explained by the greater basal insulin concentrations in M-ewes compared to A-ewes on the day of the epinephrine challenge, as greater insulin concentrations result in lower triglyceride secretion

(Durrington *et al.*, 1982). The basal insulin concentration at the day of the ETT could have been affected by the glucose or insulin challenges conducted on the previous two days, as no differences in basal insulin concentrations were found prior to the GTT and ITT between nutrition groups. Thus, to confirm triglycerides concentrations in M-ewes are lower than A-ewes, the ETT should be repeated with no challenges on days prior to the ETT.

In humans, it has been hypothesized that impairment of catecholamine-induced lipolysis may contribute to the development or maintenance of increased adipose tissue stores and obesity in humans (Jocken and Blaak, 2008). In our study, no impaired lipolysis in response to exogenous epinephrine administration was observed, however, our animals were not obese. Thus, it would be interesting to examine the effects of exogenous epinephrine in obese offspring that have experienced impairment *in utero*.

In conclusion, dam size had no effect on glucose metabolism, adrenal function or fat metabolism in 16-month-old female twin offspring. Dam nutrition during pregnancy from days 21 - 140 had no major effect on glucose metabolism, adrenal function or lipolysis, however, it did affect gluconeogenesis and/or glycogenolysis, with increased glucose production in ewes born to maintenance-fed dams. These results suggest that M-ewes could have an advantage over A-ewes in physiological stressful situations in life (e.g. pregnancy, lactation) as their liver may be able to supply more glucose to support their growing conceptus and milk production to increase the chances of survival of their offspring. This may have implications for the animal production industry as these results show that maternal maintenance nutrition during pregnancy could be beneficial for the productive performance of her offspring.

It would be of value to investigate if the animals born to dams fed maintenance show altered glucose homeostasis, lipolysis or gluconeogenesis and/or glycogenolysis when pregnant, lactating or later in life. In addition, more in-depth research at the molecular level in ruminants is necessary to understand how dam nutrition could alter gluconeogenesis and/or glycogenolysis in their offspring.

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CHAPTER 7

RELATIONSHIPS BETWEEN EARLY POSTNATAL GROWTH AND METABOLIC FUNCTION OF 16-MONTH-OLD FEMALE OFFSPRING BORN TO EWES EXPOSED TO DIFFERENT ENVIRONMENTS DURING PREGNANCY

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Abstract

It was hypothesized that exposure of the fetus to adverse conditions *in utero* due to either maternal constraint or nutrition may result in developmental adaptations altering metabolism and postnatal growth of the offspring. Heavy (H) and light (L) Romney dams were allocated to *ad libitum* (A) or maintenance (M) nutritional regimens, from days 21 - d140 of pregnancy. Female twin-born offspring born to the dams in the four treatment groups will be referred to as HA-ewes, LA-ewes, HM-ewes and LM-ewes. At 16 months of age, offspring were catheterized and given intravenous insulin (ITT), glucose (GTT) and epinephrine (ETT) challenges to assess their glucose and fat metabolism in relation to their birth weight and postnatal growth. In HA-ewes, the regression coefficients of growth rates prior to puberty on insulin and glucose curves in response to GTT ($InsAUC_{GTT}$) and ITT ($GluAUC_{ITT}$), respectively, were different from zero (560 ng min mL^{-1} ± 216.8 and 725 mmol min L^{-1} ± 324.8, respectively; $P < 0.05$) and were different from the regression coefficients of HM-ewes ($InsAUC_{GTT}$ -176 ng min mL^{-1} ± 198.6) and LM-ewes ($GluAUC_{ITT}$ -478 mmol min L^{-1} ± 334.6). This may indicate that HA-ewes may have showed puberty-related insulin resistance at 16 months of age with increasing growth rates prior to puberty compared to HM- or LM-ewes. In HM-ewes, the regression coefficients of growth rates after puberty on $InsAUC_{GTT}$ and $GluAUC_{ITT}$ were different from zero (880 ng min mL^{-1} ± 312.1 and 1626 mmol min l^{-1} ± 474.0, respectively; $P < 0.05$) and were different from those of HA-ewes (-400 ng min mL^{-1} ± 280.8 and -224 mmol min L^{-1} ± 440.9, respectively). These results may indicate that offspring born to heavy dams fed maintenance during pregnancy and with greater postnatal growth rates after puberty could develop glucose intolerance and insulin resistance in later life.

Introduction

There is increasing support in the literature for the concept that exposure of the fetus to adverse conditions *in utero* may result in developmental adaptations that permanently change the structure, physiology, metabolism and postnatal growth of the offspring (Wu *et al.*, 2006). Altered maternal nutrient intake in sheep resulted in offspring with glucose intolerance (Oliver *et al.*, 2002; Gardner *et al.*, 2005; Ford *et al.*, 2007), altered hypothalamus-pituitary-adrenal (HPA)-axis function (Hawkins *et al.*, 2000a; Bloomfield *et al.*, 2003; Gardner *et al.*, 2006), increased adiposity (Gardner *et al.*, 2005; Ford *et al.*, 2007) and altered postnatal growth (Greenwood *et al.*, 1998; Ford *et al.*, 2007). Dam size could affect fetal growth through the size of the placenta, which influences the nutrient supply to the developing fetus (Mellor, 1983). Embryo transfer and cross-breeding experiments in large and small breeds of sheep (Dickinson *et al.*, 1962; Gootwine *et al.*, 1993), horses (Walton and Hammond, 1938; Allen *et al.*, 2002) and pigs (Wilson *et al.*, 1998) have shown that fetal growth can be altered from the normal genetic potential by differing dam size.

In addition to the *in utero* environment affecting the offspring's metabolic function in later life, the postnatal growth trajectory has been found to play an important role in the development of metabolic dysfunction (Desai and Hales, 1997; Cottrell and Ozanne, 2008). For example, postnatal "catch-up" growth is associated with the development of glucose intolerance in adult life (Eriksson *et al.*, 2002; Bloomfield *et al.*, 2007), cardiovascular disease (Desai and Hales, 1997) and reduced longevity (Ozanne and Hales, 2005).

Our previous work has shown that maintenance nutrition of the ewe during pregnancy altered the bone mineral content/lean mass ratio of the fetal hindquarters when compared to *ad libitum* feeding, irrespective of dam size (Firth *et al.*, 2008). Furthermore, we have shown that dam nutrition affected birth weight in twin-born lambs (Kenyon *et al.*, 2009) and that dam nutrition and dam size during pregnancy affected postnatal growth of the female offspring (van der Linden *et al.*, 2007). Therefore, we examined, and report in this paper, the relationship between birth weight and early postnatal growth and metabolic function of 16-month-old female offspring born to dams differing in size and diet during pregnancy. We hypothesize that lower birth weight and greater postnatal growth rates until one year of age in female offspring born to light dams which were fed maintenance during pregnancy, would negatively affect their metabolic function at 16 months of age. In addition, we hypothesize that the pre-existing maternal body size (heavy vs. light) would exacerbate or reduce the effects of maternal nutrition during pregnancy on the metabolic function of 16-month-old offspring.

Materials and Methods

The study was conducted at the Massey University Keeble Sheep and Beef farm, 5 km south of Palmerston North, New Zealand. The study and all animal handling procedures were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Dams

Four hundred and fifty heavy (H) (60.8 kg ± 0.18) and 450 light (L) (42.5 kg ± 0.17) Romney dams were selected from the extremes in a commercial flock of 2900 ewes, on the basis of size, as determined by live weight, and bred using artificial insemination as previously described by Kenyon *et al.* (2009). From day 21 until day 140 post-insemination, the dams were randomly allocated, within size, to *ad libitum* (A) or maintenance (M) nutritional regimens under New Zealand pastoral grazing conditions. The aim of the M nutritional regimen was to ensure that total ewe live weight during pregnancy increased at a level similar to that of the expected conceptus mass. The aim of the A nutritional regimen was to provide dams with unrestricted food intake and, hence, no nutritional restriction to maternal or fetal growth and development (resulting in 78.4 kg ± 0.37 vs. 65.0 kg ± 0.35; $P < 0.05$; for H- and M-dams at day 140 (Kenyon *et al.*, 2009)). Pasture herbage was the only nutritional source and the average pre- and post-grazing pasture covers during the period days 21 - d140 were 1330 kg DM/ha ± 140.0 and 804.0 kg DM/ha ± 133.4, respectively, for the M-feeding regimen and 2304.0 kg DM/ha ± 156.8 and 1723.3 kg DM/ha ± 149.7 for the A-feeding regimen (Kenyon *et al.*, 2009). From day 140 of pregnancy through to weaning, all dams and their lambs were provided with *ad libitum* feeding. Singleton- and twin-born lambs (female and male lambs combined) born to H-dams (*n*

= 282) were heavier at birth (5.51 kg ± 0.05 vs. 5.37 kg ± 0.05; $P < 0.05$; for lambs born to H- and L-dams, respectively) and weaning (32.7 kg ± 0.36 vs. 31.2 kg ± 0.33; $P < 0.05$; for lambs born to H- and L-dams, respectively) than lambs born to L-dams ($n = 217$). Twin-born offspring (female and male lambs combined) born to A-dams ($n = 237$) were heavier at birth (5.23 kg ± 0.06 vs. 4.52 kg ± 0.06; $P < 0.05$; for twin-born lambs born to A- and M-dams) and weaning (30.6 kg ± 0.42 vs. 28.2 kg ± 0.41; $P < 0.05$; for twin-born lambs born to A- and M-dams) than lambs born to M-dams ($n = 262$) (Kenyon *et al.*, 2009). After weaning, the female offspring were managed and fed to nutritional requirements as one group under New Zealand commercial farming practice (van der Linden *et al.*, 2007) and the male offspring were slaughtered to obtain carcass information (to be reported elsewhere).The study, therefore, utilized a two by two factorial design: two dam-nutrition treatments (M vs. A) and two dam-size treatments (H vs. L). The term dam is used to refer to the G_0 generation of heavy and light ewes that underwent the nutritional treatment during pregnancy. The ewe offspring born to the heavy or light dams fed either maintenance or *ad libitum*, will be referred to as HA-, HM-, LA-, or LM-ewes, respectively.

Ewe offspring

At 16 months of age, 48 randomly selected twin-born ewe offspring were housed indoors in two, random, consecutive batches of 24 ewes ($n = 12$ ewes born to the HA-, HM-, LA-, and LM-dam treatment groups, as described above (Kenyon *et al.*, 2009)). Each group of 12 ewes contained eight ewes from female-female twin sets, born to four dams, and four ewes from female-male twin sets, born to four dams; birth weight differences within the twin pairs were < 25%.

The selected ewes were housed in a large shed, as one batch for one week, followed by housing in individual pens for two weeks prior to the metabolic challenges. Ewes had free access to water and were fed to achieve an average liveweight gain of 100 g/day (19 MJ ME/day (Geenty and Rattray, 1987)). The feed was a mixture of pelleted food (500 g of 12 MJ ME/kg) and lucerne chaff (1500 g of 8.6 MJ ME/kg) (average ewe live weight prior to housing was 50 kg (±

4.4 S.D.)). Ewes were fed daily between 1 and 2 pm; feed intake (offered less refusals) was recorded at 8 am each day.

Three days prior to the start of the metabolic challenges, both jugular veins were catheterized with indwelling polyvinyl catheters after administration of local anaesthetic (Lopaine, Lignocaine Hydrochloride U.S.P. 20 mg/mL, Ethical Agents LTD, Auckland, New Zealand); catheters were secured to the neck with tape and secured on the animal's back under a meshed stocking. This was followed by single prophylactic intramuscular (hind leg) administration of antibiotics (Duplocillin® LA, Intervet LTD, Newmarket, Auckland, 2 mL per 50 kg live weight). One catheter was used for hormone/metabolite administration and the other for blood collection.

After an overnight fast (food was removed between 6 and 7 pm the evening prior to the challenge), ewes were submitted to an insulin tolerance test on day 1 (ITT; 0.15 IU/kg live weight, Humulin R, Eli Lilly, Indianapolis, IN), a glucose tolerance test on day 2 (GTT; 0.17 g/kg live weight, Dextrose 40%, Bomac Laboratories LTD, Auckland, New Zealand), and an epinephrine tolerance test on day 3 (ETT; 1 μg/kg live weight, Sigma-Aldrich Inc. St Louis, MO, USA), between 8 and 9 am. Blood samples (5 mL) were collected in vacutainers containing EDTA (BD Vacutainer Systems, UK) at -5, 0, 2, 10, 20, 30, 40, 50, 60 and 120 min from the insulin administration, at -5, 0, 2, 5, 10, 20, 30, 40, 50, 60, 120 minutes from the glucose administration and at -5, 0, 2, 5, 7, 10, 20, 45 and 60 min from the epinephrine administration. On all three days, ewes were re-fed after completion of the sampling. All blood samples were immediately placed on ice until centrifugation at 3000 rpm (1006 G) for 15 min. Triplicate plasma aliquots were stored at -20ºC until analysis.

Assays

Plasma metabolite concentrations were measured using a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan) using commercial kits for glucose and cholesterol (Roche, Mannheim, Germany) and non-esterified free fatty acids (NEFA) and triglyceride (Randox Laboratories Ltd, Ardmore, Crumlin, UK). Insulin was measured by radioimmunoassay (RIA) with ovine insulin as the standard (Sigma, Batch no. I9254) (Rumball *et al.*, 2008a). The minimal detectable concentration was 0.03 ng/mL; inter- and intra-assay coefficients of variation were 14.3% and 11.5%, respectively.

Plasma cortisol concentrations were measured using mass spectrometry (Rumball *et al.*, 2008b). The internal standard was cortisol-d2. A 100 μL volume of internal standard (20 ng/mL in water) was added to 200 μL plasma. Steroids were extracted using 1 mL ethyl acetate. After removal of the organic supernatant, samples were dried, re-suspended in 100 μL mobile phase (80% methanol and 20% water), and transferred to HPLC injector vials. A 25 μL volume was injected onto an HPLC mass spectrometer system consisting of a Surveyor MS pump and autosampler followed by an Ion Max APCI source on a Finnigan TSQ Quantum Ultra AM triple-quadrapole mass spectrometer all controlled by Finnigan Xcaliber software (Thermo Electron Corp., San Jose, CA). The mobile phase was isocratic, flowing at 600 μL/min through a Luna 3 μ C18 (2) 100A 250 X 4.6 column at 40º C (Phenomenex, Auckland, New Zealand). Retention time was 5.9 min. Ionization was in positive mode, and Q2 had 1.2 mTorr of argon for the steroid. The mass transitions, for internal standard and steroid, respectively, were as follows: cortisol-d2, 365.3 – 122.2 at 28 V, and cortisol, 363.3 – 121.2 at 28 V. Mean inter- and intra-assay coefficient of variation values were 11.1 and 10.6%, respectively.

Metabolic variables of the offspring (G_1)

Area under the curves for all variables included the area under the baseline.

Glucose tolerance test

Glucose tolerance was measured as the area under the glucose curve ($GluAUC_{GTT}$) and absolute insulin secretion as the area under the plasma insulin curve ($InsAUC_{GTT}$) during the 120 minutes after the glucose administration.

Insulin tolerance test

Insulin resistance was measured as the area under the glucose curve ($\mathrm{GluAUC}_{\mathrm{ITT}}$) and absolute cortisol secretion was measured as the area under the plasma cortisol curve ($\mathrm{CortAUC}_{\mathrm{ITT}}$) during the 120 minutes after the insulin administration.

Epinephrine tolerance test

Absolute glucose ($\mathrm{GluAUC}_{\mathrm{ETT}}$), insulin ($\mathrm{InsAUC}_{\mathrm{ETT}}$), NEFA ($\mathrm{NefaAUC}_{\mathrm{ETT}}$), triglycerides ($\mathrm{TrigAUC}_{\mathrm{ETT}}$) and cholesterol ($\mathrm{CholAUC}_{\mathrm{ETT}}$) secretion were measured as the area under the curves during the first 20 minutes after epinephrine administration. Areas under the curves during the first 20 minutes were used as area under the curves during 60 minutes showed no relationship with birth weight or growth rates.

Birth weight and growth of the ewe offspring

The average day of birth of the lambs was 28 August 2005 and the lambs were weighed within 24 h after birth as previously described by Kenyon *et al.* (2009). After weaning at the average age of 100 days, the ewe lambs were weighed monthly until 1 year of age, as previously described by van der Linden *et al.* (van der Linden *et al.*, 2007). Growth rates of the ewe lambs (G_1) were calculated for four periods: $\mathrm{Growth}_{\mathrm{wean}}$: growth rates from birth to weaning (4 months of age); $\mathrm{Growth}_{\mathrm{postwean}}$: growth rates post weaning (4 to 7 months of age); $\mathrm{Growth}_{\mathrm{prepub}}$: growth rate prior to onset of puberty (7 to 9 months of age); $\mathrm{Growth}_{\mathrm{postpub}}$: growth rates post puberty (9 to 12 months of age).

Statistical analysis

Birth weight and growth rates of the ewe lambs were analysed using the MIXED procedure (SAS, 2006) with a linear model that included the fixed effects of dam nutrition, dam size, the interaction dam nutrition by dam size and the random effect of batch. Data are presented as least square means and their standard error (± S.E.). Area under the curves were analysed using the same mixed linear model as stated above including the fixed effects of dam nutrition, dam size, and the interaction of dam nutrition by dam size and the random effect of batch. Metabolic variables are presented as least square means and their standard error (± S.E.).

Birth weight and the four growth periods ($\mathrm{Growth}_{\mathrm{wean}}$, $\mathrm{Growth}_{\mathrm{postwean}}$, $\mathrm{Growth}_{\mathrm{prepub}}$ and $\mathrm{Growth}_{\mathrm{postpub}}$) of the ewe offspring were regressed on their metabolic variables at 16 months of age (glucose metabolism: $\mathrm{GluAUC}_{\mathrm{GTT}}$, $\mathrm{InsAUC}_{\mathrm{GTT}}$ and $\mathrm{GluAUC}_{\mathrm{GTT}}$; adrenal function: $\mathrm{CortAUC}_{\mathrm{ITT}}$; fat metabolism: $\mathrm{GluAUC}_{\mathrm{ETT}}$, $\mathrm{InsAUC}_{\mathrm{ETT}}$, $\mathrm{NefaAUC}_{\mathrm{ETT}}$, $\mathrm{TrigAUC}_{\mathrm{ETT}}$ and $\mathrm{CholAUC}_{\mathrm{ETT}}$) for each of the dam treatment interaction (dam nutrition by dam size; HM; HA; LM; LA) with the following linear regression model:

$$y_{klm} = \beta_{0k} + \beta_{1k} x_{kl} + \mathrm{M}_m + e_{klm}$$

where y_{klm} is the metabolic variable measured on ewe l from dam treatment interaction k in batch m, β_{0k} and β_{1k} are regression coefficients describing the regression line in dam treatment interaction k, M_m is the random effect of batch m and e_{klm} is the residual error corresponding to the observation y_{klm}.

Multiple comparisons were performed and therefore $\alpha(0.05)$ was corrected using the Bonferroni correction for multiple tests (Narum, 2006):

$$\alpha_{corr} = \alpha \Big/ \sum_{i=1}^{k} \left(1/i \right)$$

Associations were significant at $\alpha_{corr} = 0.02$ and considered a trend $\alpha_{corr} = 0.05$

Thus, if a relationship within a group is significant it is represented in a regression coefficient (β_1) that is significantly different from zero. If a relationship within a group is not significant it is represented in a regression coefficient (β_1) that is not significantly different from zero (regression line is horizontal).

Results

Birth weight, growth rates and metabolic variables of the ewe offspring

No dam-nutrition or dam-size effects ($P > 0.10$) were found on birth weight, $\text{Growth}_{\text{postwean}}$ or $\text{Growth}_{\text{postpub}}$ of the ewe offspring (Table 7.1). Growth rates from birth to weaning ($\text{Growth}_{\text{wean}}$) tended ($P < 0.10$) to be greater in HA-offspring compared to LM-offspring. However, growth rates prior to puberty ($\text{Growth}_{\text{prepub}}$) tended ($P < 0.10$) to be greater in LM-offspring than in HM- and LA-offspring.

No dam nutrition or dam size effects ($P > 0.10$) were found in area under the glucose ($\text{GluAUC}_{\text{GTT}}$) and insulin ($\text{InsAUC}_{\text{GTT}}$) curves in response to the glucose tolerance test, area under the glucose curve ($\text{GluAUC}_{\text{ITT}}$) in response to the insulin tolerance test or area under the NEFA curve ($\text{NefaAUC}_{\text{ETT}}$) in response to the epinephrine tolerance test at 16 months of age (Table 7.1). Offspring born to LM-dams had a greater ($P < 0.04$) area under the insulin ($\text{InsAUC}_{\text{ETT}}$) curve in response to epinephrine tolerance test than HA-offspring. In addition, HM-offspring tended ($P < 0.10$) to have greater $\text{InsAUC}_{\text{ETT}}$ than HA-offspring.

Table 7.1. The effects of heavy (H) or light (L) dams fed *ad libitum* (A) or maintenance (M) during pregnancy on birth weight (BW; kg), growth from birth to weaning (Growth$_{wean}$; g/day), growth from weaning-7 months of age (Growth$_{postwean}$; g/day), growth from 7-9 months of age (Growth$_{prepub}$; g/day) and growth from 9-12 months of age (Growth$_{postpub}$; g/day) and glucose-metabolism variables at 16 months of age (GluAUC$_{GTT}$ and InsAUC$_{GTT}$: glucose AUC and insulin AUC in response to GTT, respectively; GluAUC$_{ITT}$: glucose AUC in response to ITT) and fat-metabolism variables at 16 months of age (InsAUC$_{ETT\ 0\text{-}20}$ and NefaAUC$_{ETT\ 0\text{-}20}$: insulin AUC and NEFA AUC in response to ETT, respectively) of ewe offspring. Table shows least square means ± S.E.

	Variables*									
Dam treatment	BW (kg)	Growth$_{wean}$ (g/day)	Growth$_{postwean}$ (g/day)	Growth$_{prepub}$ (g/day)	Growth$_{postpub}$ (g/day)	GluAUC$_{GTT}$ (mmol min L^{-1})	InsAUC$_{GTT}$ (ng min mL^{-1})	GluAUC$_{ITT}$ (mmol min L^{-1})	InsAUC$_{ETT}$ (ng min mL^{-1})	NefaAUC$_{ETT}$ (mmol min L^{-1})
HA	4.8	250 ^{d}	74	53 ^{cd}	44	553.4	63.5	334.9	4.8 ^{a}	3.7
(*n* = 12)	± 0.19	± 10.0	± 5.9	± 9.2	± 6.5	± 9.46	± 5.64	± 7.81	± 0.70	± 0.47
LA	4.7	244^{cd}	74	45 ^{c}	51	568.4	55.2	325.5	5.9^{ab}	3.4
(*n* = 12)	± 0.19	± 10.0	± 5.9	± 9.2	± 6.5	± 9.34	± 5.89	± 7.74	± 0.69	± 0.49
HM	4.9	236^{cd}	82	43 ^{c}	50	548.9	57.7	322.8	6.7^{ab}	3.4
(*n* = 12)	± 0.19	± 10.0	± 5.9	± 9.2	± 6.5	± 9.30	± 6.18	± 7.71	± 0.68	± 0.47
LM	4.5	224 ^{c}	70	68 ^{d}	41	565.9	65.5	338.0	6.8 ^{b}	4.2
(*n* = 12)	± 0.19	± 10.0	± 5.9	± 9.2	± 6.5	± 9.73	± 6.18	± 8.32	± 0.69	± 0.47

^{ab} different superscripts are significantly different ($P < 0.05$) between dam treatments and within variables; ^{cd} different superscripts tend to be different ($P < 0.10$) between dam treatments and within variables. *Interaction of dam size by maternal nutrition was not significant ($P > 0.10$) for all variables.

Glucose metabolism of the ewe offspring

Birth weight, $\text{Growth}_{\text{wean}}$ and $\text{Growth}_{\text{postwean}}$ were not related to $\text{GluAUC}_{\text{GTT}}$, $\text{InsAUC}_{\text{GTT}}$ and $\text{GluAUC}_{\text{ITT}}$ in response to the GTT and ITT, respectively, of ewe offspring at 16 months of age (data not shown).

In the period before puberty, the regression coefficient (β_1) of $\text{Growth}_{\text{prepub}}$ on $\text{InsAUC}_{\text{GTT}}$ of HA-ewes was significantly (P = 0.01) different from zero, indicating that HA-ewes had increased $\text{InsAUC}_{\text{GTT}}$ with increasing growth rates prior to puberty (Figure 7.1 and Table 7.2). This regression coefficient of $\text{Growth}_{\text{prepub}}$ on $\text{InsAUC}_{\text{GTT}}$ of HA-ewes was significantly different (P = 0.02) from that of HM-ewes and tended (P = 0.05) to be different from that of LA-ewes. This indicates that HA-ewes had greater $\text{InsAUC}_{\text{GTT}}$ with increasing growth rates prior to puberty than did HM- or LA-ewes.

Thus, for example, if HA- and HM-ewes had growth rates of 0 kg/day prior to puberty, HA-ewes would have an predicted $\text{InsAUC}_{\text{GTT}}$ of 34 ng min mL^{-1} (34 + 560 × 0; Table 2) and HM-ewes would have an predicted $\text{InsAUC}_{\text{GTT}}$ of 66 ng min mL^{-1} (66 + -176 × 0; Table 2). However, if HA- and HM-ewes had growth rates of 0.1 kg/day prior to puberty, HA-ewes would have an predicted $\text{InsAUC}_{\text{GTT}}$ of 90.0 ng min mL^{-1} (34 + 560 × 0.1) and HM-ewes would have an predicted $\text{InsAUC}_{\text{GTT}}$ of 48.4 ng min mL^{-1} (66 + -176 × 0.1).

The regression coefficient of $\text{Growth}_{\text{prepub}}$ on $\text{GluAUC}_{\text{ITT}}$ of HA-ewes was significantly (P = 0.03) different from zero, indicating that HA-ewes had increased $\text{GluAUC}_{\text{ITT}}$ with increasing growth rates prior to puberty. This regression coefficient of $\text{Growth}_{\text{prepub}}$ on $\text{GluAUC}_{\text{ITT}}$ of HA-ewes was significantly different (P = 0.01) from that of LM-ewes, indicating that HA-ewes had greater $\text{GluAUC}_{\text{ITT}}$ with increasing growth rates prior to puberty than did LM-ewes.

Table 7.2. Linear regression equations* of pre-puberty growth rates ($\text{Growth}_{\text{prepub}}$; 7-9 months of age; kg/day) on glucose-metabolism variables at 16 months of age ($\text{GluAUC}_{\text{GTT}}$: glucose AUC and $\text{InsAUC}_{\text{GTT}}$: insulin AUC in response to GTT; $\text{GluAUC}_{\text{ITT}}$: glucose AUC in response to ITT) of ewes born to heavy (H) or light (L) dams fed either *ad libitum* (A) or maintenance (M) during pregnancy.

		Independent variable				
Dam treatment	Dependent variable	Intercept (β_0)	*P* value β_0	$\text{Growth}_{\text{prepub}}$ (β_1)	*P* value β_1	R^2
HA	$\text{GluAUC}_{\text{GTT}}$ (mmol min L^{-1})	539 (± 23.2)	0.0001	336 (± 383.4)	NS	0.43
LA		551 (± 15.4)	0.0001	202 (± 276.2)	NS	0.23
HM		533 (± 13.9)	0.0001	225 (± 239.5)	NS	0.16
LM		590 (± 21.2)	0.0001	- 496 (± 286.4)	0.09	0.19
HA	$\text{InsAUC}_{\text{GTT}}$ (ng min mL^{-1})	34 (± 12.6)	0.0001	560 (± 216.8) ^{b}	0.01	0.43
LA		57 (± 9.9)	0.0001	- 46 (± 202.0) ^{ab}	NS	0.03
HM		66 (± 11.5)	0.0001	- 176 (± 198.6) ^{a}	NS	0.08
LM		49 (± 16.3)	0.0001	282 (± 261.5) ^{ab}	NS	0.11
HA	$\text{GluAUC}_{\text{ITT}}$ (mmol min L^{-1})	295 (± 18.9)	0.0001	725 (± 324.8) ^{b}	0.03	0.51
LA		325 (± 14.2)	0.0001	9 (± 259.0) ^{ab}	NS	0.09
HM		318 (± 12.5)	0.0001	118 (± 222.5) ^{ab}	NS	0.01
LM		367 (± 25.8)	0.0001	- 478 (± 334.6) ^{a}	NS	0.38

*all regression equation models are significant ($P < 0.01$); NS: non significant. ^{ab} different superscripts are significantly different ($P < 0.05$; using Bonferroni correction) between dam treatments and within dependent metabolic variable.

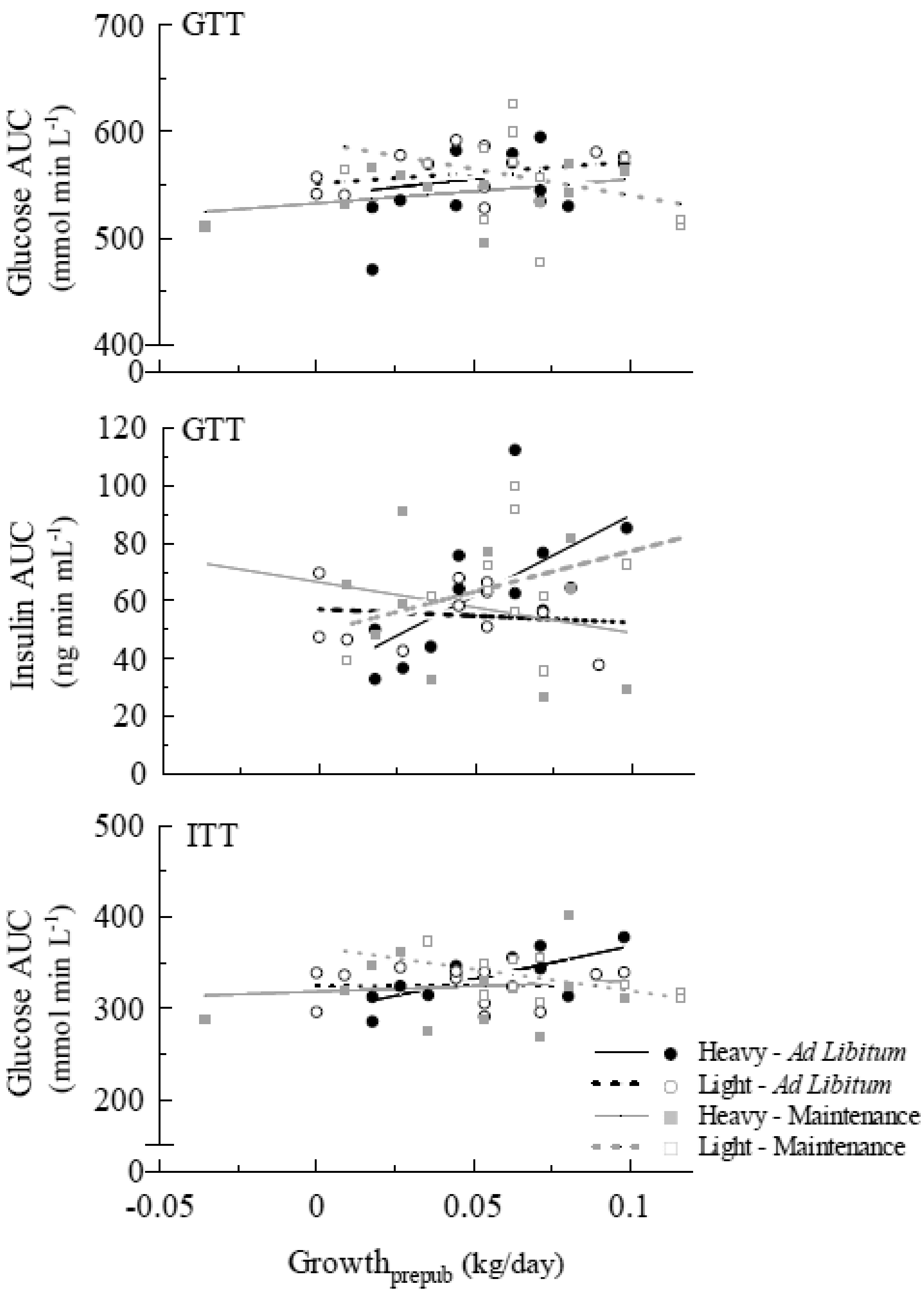

Figure 7.1. Linear regressions of pre-puberty growth rates (Growth$_{prepub}$; 7-9 months of age) on glucose-metabolism variables at 16 months of age (GluAUC$_{GTT}$: glucose AUC and InsAUC$_{GTT}$: insulin AUC in response to GTT; GluAUC$_{ITT}$: glucose AUC in response to ITT) of ewes born to heavy or light dams fed either maintenance or *ad libitum* during pregnancy. Black solid line and ● heavy – *ad libitum*; black dotted line and ○ light – *ad libitum*; grey solid line and ■ heavy – maintenance; grey dotted line and □ light – maintenance.

In the period after puberty, the regression coefficient of $\text{Growth}_{\text{postpub}}$ on $\text{GluAUC}_{\text{GTT}}$ of HA-ewes was significantly (*P* = 0.03) different from zero, indicating that HA-ewes had decreased $\text{GluAUC}_{\text{GTT}}$ with increasing growth rates after puberty (Figure 7.2 and Table 7.3). In addition, the regression coefficient of $\text{Growth}_{\text{postpub}}$ on $\text{GluAUC}_{\text{GTT}}$ of LM-ewes was significantly (*P* = 0.01) different from zero, indicating that LM-ewes had increased $\text{GluAUC}_{\text{GTT}}$ with increasing growth rates after puberty. The regression coefficient of $\text{Growth}_{\text{postpub}}$ on $\text{GluAUC}_{\text{GTT}}$ of LM-ewes was significantly different (*P* = 0.002) from that of HA-ewes, indicating that LM-ewes had greater $\text{GluAUC}_{\text{GTT}}$ with increasing growth rates after puberty than did HA-ewes. The regression coefficient of $\text{Growth}_{\text{postpub}}$ on $\text{GluAUC}_{\text{GTT}}$ of HM-ewes tended to be different (*P* = 0.04) from that of HA-ewes, indicating that HM-ewes tended to have greater $\text{GluAUC}_{\text{GTT}}$ with increasing growth rates after puberty than did HA-ewes.

The regression coefficient of $\text{Growth}_{\text{postpub}}$ on $\text{InsAUC}_{\text{GTT}}$ of HM-ewes was significantly (*P* = 0.02) different from zero, indicating that HM-ewes had increased $\text{InsAUC}_{\text{GTT}}$ with increasing growth rates after puberty. The regression coefficient of $\text{Growth}_{\text{postpub}}$ on $\text{InsAUC}_{\text{GTT}}$ of HM-ewes was significantly different (*P* = 0.005) from that of HA-ewes, indicating that HM-ewes had greater $\text{InsAUC}_{\text{GTT}}$ with greater growth rates after puberty than did HA-ewes. The regression coefficient of $\text{Growth}_{\text{postpub}}$ on $\text{InsAUC}_{\text{GTT}}$ of HM-ewes tended to be different (*P* = 0.05) from that of LA-ewes, indicating that HM-ewes tended to have greater $\text{InsAUC}_{\text{GTT}}$ with increasing growth rates after puberty than did LA-ewes.

The regression coefficient of $\text{Growth}_{\text{postpub}}$ on $\text{GluAUC}_{\text{ITT}}$ of HM-ewes was significantly (*P* = 0.001) different from zero, indicating that HM-ewes had increased $\text{GluAUC}_{\text{ITT}}$ with every kg of growth. The regression coefficient of $\text{Growth}_{\text{postpub}}$ on $\text{GluAUC}_{\text{ITT}}$ of HM-ewes was significantly different (*P* = 0.001) from that of HA-, LA- and LM-ewes, indicating that HM-ewes had greater $\text{InsAUC}_{\text{GTT}}$ with increasing growth rates after puberty than did HA-, LA- and LM-ewes.

Table 7.3. Linear regression equations[*] of post-puberty growth rates (Growth$_{prepub}$; 9-12 months of age; kg/day) on glucose-metabolism variables at 16 months of age (GluAUC$_{GTT}$: glucose AUC and InsAUC$_{GTT}$: insulin AUC in response to GTT; GluAUC$_{ITT}$: glucose AUC in response to ITT) of ewes born to heavy (H) or light (L) dams fed either *ad libitum* (A) or maintenance (M) during pregnancy.

		Independent variable				
Dam treatment	Dependent variable	Intercept (β_0)	*P* value β_0	Growth$_{postpub}$ (β_1)	*P* value β_1	R^2
HA	GluAUC$_{GTT}$ (mmol min L^{-1})	605 (± 25.2)	0.0001	- 1138 (± 515.9) ^{a}	0.03	0.56
LA		567 (± 20.8)	0.0001	68 (± 345.0) ^{ab}	NS	0.005
HM		520 (± 30.4)	0.0001	551 (± 576.5) ^{ab}	NS	0.28
LM		522 (± 17.1)	0.0001	926 (± 345.9) ^{b}	0.01	0.42
HA	InsAUC$_{GTT}$ (ng min mL^{-1})	76 (± 13.0)	0.0006	- 400 (± 280.8) ^{a}	NS	0.12
LA		49 (± 12.5)	0.002	117 (± 209.9) ^{ab}	NS	0.08
HM		13 (± 16.8)	NS	880 (± 312.1) ^{b}	0.02	0.49
LM		48 (± 11.1)	0.0001	371 (± 212.5) ^{ab}	NS	0.26
HA	GluAUC$_{ITT}$ (mmol min L^{-1})	344 (± 21.0)	0.0001	- 224 (± 440.9) ^{b}	NS	0.16
LA		323 (± 16.8)	0.0001	48 (± 295.3) ^{b}	NS	0.09
HM		243 (± 24.7)	0.0001	1626 (± 474.0) ^{a}	0.001	0.53
LM		327 (± 14.8)	0.0001	114 (± 296.7) ^{b}	NS	0.32

[*] all regression equation models are significant (*P* < 0.01); NS: non significant; ^{ab} different superscripts are significantly different (*P* < 0.05; using Bonferroni correction) between dam treatments and within dependent metabolic variable.

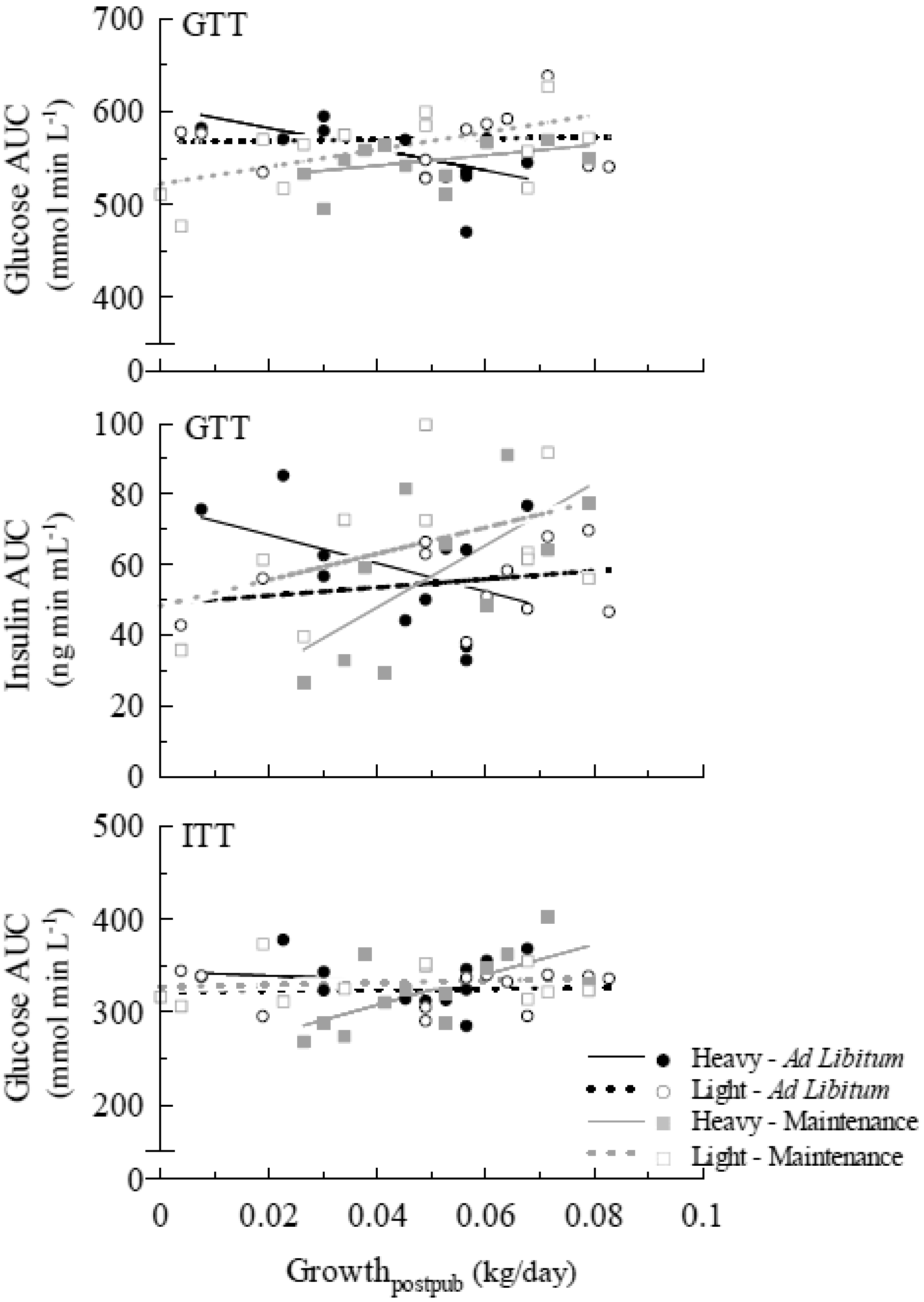

Figure 7.2. Linear regressions of post-puberty growth rates (Growth$_{\text{postpub}}$; 9-12 months of age) on glucose-metabolism variables at 16 months of age (GluAUC$_{\text{GTT}}$: glucose AUC and InsAUC$_{\text{GTT}}$: insulin AUC in response to GTT; GluAUC$_{\text{ITT}}$: glucose AUC in response to ITT) of ewes born to heavy or light dams fed either maintenance or *ad libitum* during pregnancy. Black solid line and ● heavy – *ad libitum*; black dotted line and ○ light – *ad libitum*; grey solid line and ■ heavy – maintenance; grey dotted line and □ light – maintenance.

Adrenal function of the ewe offspring (G_1)

Birth weight, $\text{Growth}_{\text{wean}}$, $\text{Growth}_{\text{postwean}}$, $\text{Growth}_{\text{prepub}}$ and $\text{Growth}_{\text{postpub}}$ were not related to $\text{CortAUC}_{\text{ITT}}$ in response to the ITT at 16 months of age (data not shown).

Fat metabolism of the ewe offspring (G_1)

Birth weight, $\text{Growth}_{\text{wean}}$, $\text{Growth}_{\text{postwean}}$, $\text{Growth}_{\text{prepub}}$ and $\text{Growth}_{\text{postpub}}$ were not related to $\text{GluAUC}_{\text{ETT}}$, $\text{TrigAUC}_{\text{ETT}}$ and $\text{CholAUC}_{\text{ETT}}$ in response to the ETT at 16 months of age.

$\text{Growth}_{\text{wean}}$, $\text{Growth}_{\text{postwean}}$, $\text{Growth}_{\text{prepub}}$ and $\text{Growth}_{\text{postpub}}$ were not related to $\text{InsAUC}_{\text{ETT}}$.

The regression coefficients of birth weight on $\text{InsAUC}_{\text{ETT}}$ of LA-ewes (*P* = 0.0001) and LM-ewes (*P* = 0.04) were significantly different from zero, indicating that LA- and LM- ewes had increased $\text{InsAUC}_{\text{ETT}}$ with every kg increase of birth weight (Fig. 7.3 and Table 7.4). The regression coefficient of birth weight on $\text{InsAUC}_{\text{ETT}}$ of LA-ewes was significantly different from that of HA-ewes (*P* = 0.001), and HM-ewes (*P* = 0.01), indicating that LA-ewes had greater $\text{InsAUC}_{\text{ETT}}$ with every kg increase of birth weight than did HA- and HM-ewes.

Birth weight, $\text{Growth}_{\text{postwean}}\text{Growth}_{\text{prepub}}$ and $\text{Growth}_{\text{postpub}}$ were not related to $\text{NefaAUC}_{\text{ETT}}$ at 16 months of age. The regression coefficient of $\text{Growth}_{\text{wean}}$ on $\text{NefaAUC}_{\text{ETT}}$ of LM-ewes was significantly (*P* = 0.03) different from zero, indicating that LM-ewes had decreased $\text{NefaAUC}_{\text{ETT}}$ with increasing growth rates prior to weaning (Figure 7.3 and Table 7.5). This regression coefficient of $\text{Growth}_{\text{wean}}$ on $\text{NefaAUC}_{\text{ETT}}$ of LM-ewes tended to be different (*P* = 0.03) from that of HM-ewes indicating that LM-ewes had smaller $\text{NefaAUC}_{\text{ETT}}$ with increasing growth rates prior to weaning than did HM-ewes.

Table 7.4. Linear regression equations* of birth weights (kg) on fat-metabolism variable $\text{InsAUC}_{\text{ETT}}$ at 16 months of age (insulin AUC in response to ETT) of ewes born to heavy (H) or light (L) dams fed either *ad libitum* (A) or maintenance (M) during pregnancy.

Dam treatment	Dependent variable	Independent variable				R^2
		Intercept (β_0)	*P* value β_0	Birth weight (β_1)	*P* value β_1	
HA	$\text{InsAUC}_{\text{ETT}}$ (ng min mL^{-1})	5.2 (± 3.3)	NS	- 0.4 (± 0.7) ^{b}	NS	0.11
LA		-10.9 (± 3.6)	0.004	3.3 (± 0.7) ^{a}	0.0001	0.76
HM		2.0 (± 3.7)	NS	0.5 (± 0.7) ^{b}	NS	0.33
LM		-5.6 (± 4.8)	NS	2.2 (± 1.0) ^{ab}	0.04	0.24

*all regression equation models are significant ($P < 0.01$); NS: non significant
^{ab} different superscripts are significantly different ($P < 0.05$; using Bonferroni correction) between dam treatments and within dependent metabolic variable.

Table 7.5. Linear regression equations* of growth rates (from birth until 4 months of age, $\text{Growth}_{\text{wean}}$: kg/day) on fat-metabolism variable $\text{NefaAUC}_{\text{ETT}}$ at 16 months of age (NEFA AUC in response to ETT) of ewes born to heavy (H) or light (L) dams fed either *ad libitum* (A) or maintenance (M) during pregnancy.

Dam treatment	Dependent variable	Independent variable				R^2
		Intercept (β_0)	*P* value β_0	$\text{Growth}_{\text{wean}}$ (β_1)	*P* value β_1	
HA	$\text{NefaAUC}_{\text{ETT}}$ (mmol min L^{-1})	7.0 (± 5.1)	NS	- 11.9 (± 20.3)	NS	0.11
LA		3.6 (± 4.8)	NS	0.4 (± 19.3)	NS	0.02
HM		2.1 (± 2.4)	NS	6.8 (± 9.6)	NS	0.07
LM		12.1 (± 3.5)	0.001	- 33.7 (± 15.1)	0.03	0.41

*all regression equation models are significant ($P < 0.01$); NS: non significant.

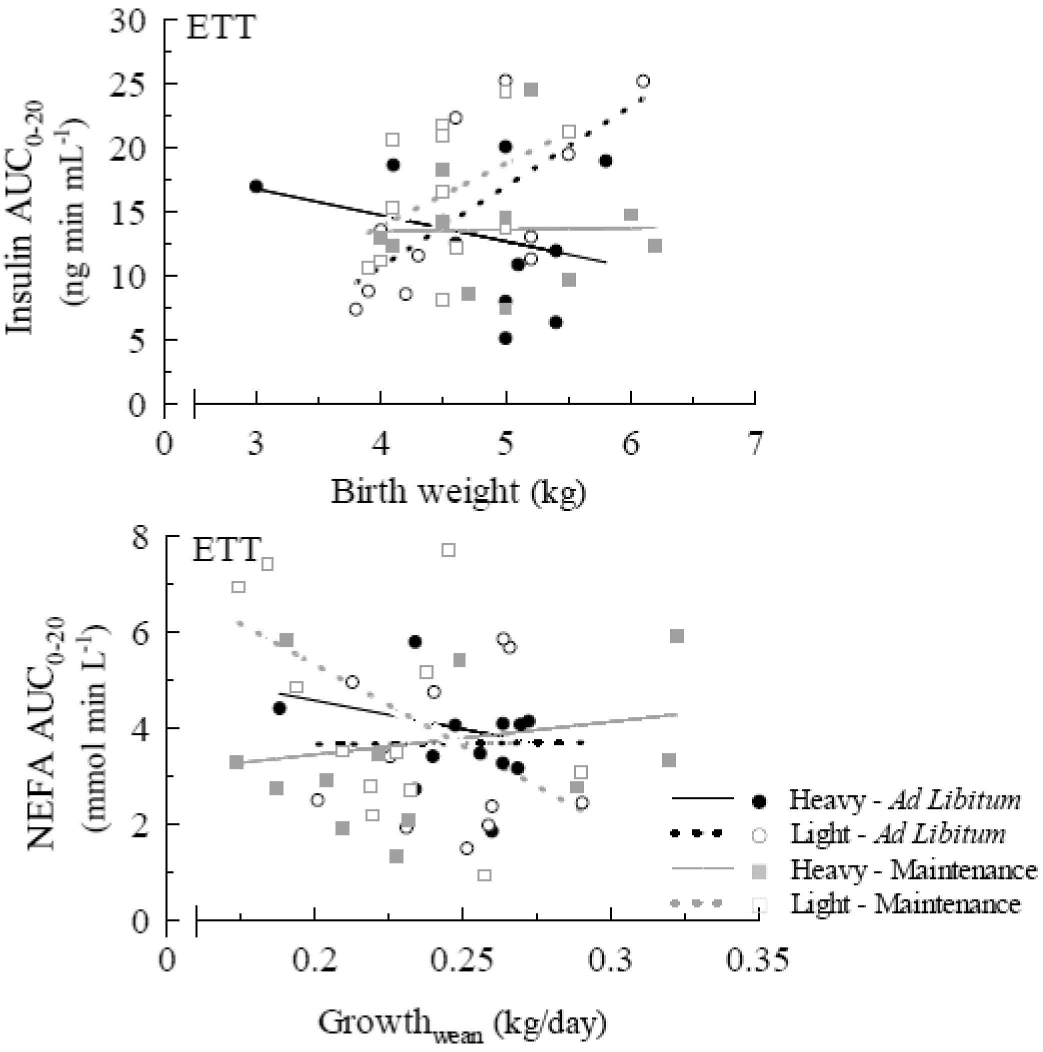

Figure 7.3. Linear regressions of birth weight and growth rates to weaning (Growth$_{wean}$; birth - 4 months of age) on fat-metabolism variables at 16 months of age (InsAUC$_{ETT}$: insulin AUC and NefaAUC$_{ETT}$: NEFA AUC in response to ETT) of ewes born to heavy or light dams fed either *ad libitum* or maintenance during pregnancy. Black solid line and ● heavy – *ad libitum*; black dotted line and ○ light – *ad libitum*; grey solid line and ■ heavy – maintenance; grey dotted line and □ light – maintenance.

Discussion

We hypothesized that low birth weight and greater postnatal growth rates until one year of age in female offspring born to light dams which were fed maintenance during pregnancy, would negatively affect their metabolic function at 16 months of age. In addition, we hypothesized that the pre-existing maternal body size (heavy vs. light) would exacerbate (light) or reduce (heavy) the effects of maternal nutrition during pregnancy.

However, no relationship was found between altered glucose homeostasis at 16 months of age and birth weight or postnatal growth up to 7 months of age in the offspring, which is consistent with other studies (Oliver *et al.*, 2002; Eriksson *et al.*, 2006).

On the other hand, relationships were found between glucose homeostasis at 16 months of age and growth rates prior to puberty ($\text{Growth}_{\text{prepub}}$) and growth rates after puberty ($\text{Growth}_{\text{postpub}}$). A shift in metabolism seems to have occurred. Prior to puberty, HA-ewes produced more insulin at 16 months of age (increased predicted $\text{InsAUC}_{\text{GTT}}$ with increasing growth rates) and were more insulin resistant at 16 months of age (increased predicted $\text{GluAUC}_{\text{ITT}}$ with increasing growth rates) than the HM- and LM-ewes. However, no such relationship between glucose intolerance and insulin resistance at 16 months of age and growth rates after puberty was observed in HA-ewes. Interestingly, after puberty HM-ewes produced more insulin and were more insulin resistant at 16 months of age with increasing growth rates after puberty compared to the other groups. A possible explanation for the relationship observed prior to puberty in the HA-ewes, is puberty-related insulin resistance, as described in human children (Amiel *et al.*, 1991; Moran *et al.*, 1999).

Puberty-insulin resistance is related to increased growth hormone (GH) concentrations, which stimulate anabolic growth and lipolysis (Gower and Caprio, 2006) and secretion of insulin-like-growth factor I (IGF-I) (Moran *et al.*, 2002). Exogenous GH administration is associated with both an elevation in circulating free fatty acids (FFA) and a decrease in insulin sensitivity (Keller and Miles, 1991), as an elevation in FFA is associated with skeletal muscle resistance to insulin-stimulated glucose uptake. Therefore, pubertal metabolism appears to be optimized to permit or promote anabolic growth (Gower and Caprio, 2006). However, we cannot explain why the association was only observed in HA-ewes and not in the other groups. After puberty, the relationship between growth rate in early postnatal life and glucose homeostasis at 16 months of age observed in HM- and LM-ewes, is in agreement with the concept that postnatal growth and sub-optimal nutrition during pregnancy are predictors of later development of glucose intolerance and insulin resistance (Symonds, 2007). This could indicate that M-ewes, regardless of dam size, may develop glucose intolerance and insulin resistance later in life. However, the relationship between growth rate after puberty and insulin resistance (greater predicted $\mathrm{InsAUC}_{\mathrm{GTT}}$ and $\mathrm{GluAUC}_{\mathrm{ITT}}$) at 16 months was most profound in HM-ewes and significantly different from HA-ewes. This may suggest that the pre-existing dam size (heavy) exacerbate the effects of nutrition during pregnancy on glucose and insulin metabolism of the offspring, given that the offspring have increased growth rates after puberty. The absolute insulin secretion after the glucose administration was positively related with growth in the offspring studied in the current study, this may indicate that no dysfunction at pancreatic level had occurred (Davies *et al.*, 1994). Thus, it may be more likely that the sub-cellular insulin-signaling proteins downstream of the receptor could be affected (Fernandez-Twinn *et al.*, 2005) especially at adipose tissue level (Gardner *et al.*, 2005), as mature animals are more likely to accumulate adipose tissue than muscle tissue.

However, size of the dam did affect the area under the insulin curve in response to the ETT. Ewes born to light dams produced more insulin (increased predicted $\mathrm{InsAUC}_{\mathrm{ETT}}$) with every kg that they were heavier at birth in response to the ETT at 16 months of age. This was not observed in ewes born to heavy dams, which would produce the same amount of $\mathrm{InsAUC}_{\mathrm{ETT}}$ at 16 months of age irrespective of their birth weight. This may indicate that offspring born to light dams, with greater birth weights, were ‘protected’ from the lipolytic action of epinephrine as insulin has anti-lipolytic effects (Ozanne *et al.*, 1999), therefore, possibly being more ‘thrifty’ (Prentice, 2005). However, insulin is a secondary response to an epinephrine challenge, and the role of increased insulin production in response to ETT observed at 16 months of age with increasing birth weight is not fully understood.

An increase in plasma NEFA concentrations in response to catecholamines is most readily explained in terms of changes in the rate of lipolysis (mobilization of adipose tissue to NEFA and glycerol) (Vernon, 1981). LM-ewes produced less NEFA (decreased predicted $\text{NefaAUC}_{\text{ETT}}$ with increasing growth rates) at 16 months of age with increasing growth rates from birth to weaning ($\text{Growth}_{\text{wean}}$) than HM-ewes. This may indicate that within the maintenance-fed group, offspring born to light dams are more ‘thrifty’ (Prentice, 2005), as with increasing early postnatal growth rates, the rate of lipolysis at 16 months of age is less (smaller predicted $\text{NefaAUC}_{\text{ETT}}$), thus ‘sparing’ their energy reserves, which is in agreement with the relationship found between birth weight and insulin production in offspring born to light dams.

In summary, ewes born to heavy dams fed *ad libitum* during gestation may have showed puberty-related insulin resistance at 16 months of age with increasing growth rates prior to puberty. Post-puberty, ewes born to heavy dams fed maintenance during pregnancy, produced more insulin, and were increasingly insulin resistant at 16 months of age with increasing growth rates after puberty, in response to a glucose and insulin challenge, respectively compared to ewes born to heavy dams fed *ad libitum*. These results may indicate that offspring born to dams fed maintenance during pregnancy and with greater postnatal growth rates after puberty could develop glucose intolerance and insulin resistance in later life.

Ewes born to light dams were more ‘thrifty’ at 16 months of age with every kg increase of birth weight and increasing postnatal growth rates until weaning in response to an epinephrine challenge.

Altogether, the observed relationships both between birth weight and (early) postnatal growth and the metabolic response to glucose, insulin and adrenaline challenges at 16 months of age of offspring born to heavy or light dams fed maintenance or *ad libitum* during pregnancy are interesting, and further research will be needed to determine the exact meaning and mechanism(s) of the observed relationships.

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CHAPTER 8

EFFECTS OF EWE SIZE AND NUTRITION ON FETAL MAMMARY GLAND DEVELOPMENT AND LACTATIONAL PERFORMANCE OF OFFSPRING AT THEIR FIRST LACTATION

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Abstract

Many environmental factors applied postnatally are known to affect milk production of the dam but, to date, the effects of different fetal environments on subsequent first lactational performance of the offspring have not been reported. Four-hundred and fifty heavy (H; 60.8 kg ± 0.18) and 450 light (L; 42.5 kg ± 0.17) dams were randomly allocated to *ad libitum* (A) or maintenance (M) nutritional regimens from day 21 until day 140 of pregnancy, under pastoral grazing conditions (HA: n = 151; HM: n = 153; LA: n = 155; LM: n = 153). At day 100 of pregnancy, a sub-group of twin-bearing dams were euthanized and fetal mammary glands collected. From one week before lambing, all remaining dams were fed *ad libitum* until weaning. After weaning, female progeny were managed and fed under pastoral conditions as one group. At two years of age, 72 twin-rearing ewe offspring were milked once a week, for 7 weeks. Fetuses from M-dams had heavier mammary glands ($P = 0.03$) compared to A-fetuses. Fetuses from H-dams had greater ($P = 0.0008$) mammary duct area compared to L-fetuses. At two years of age, M-offspring had greater milk yields at day 7 ($P = 0.02$) and day 28 ($P = 0.09$) of lactation and tended to have greater accumulated milk yields ($P = 0.11$) compared to A-offspring. Ewes born to M-dams showed greater lactose percentage at day 14 ($P = 0.002$), day 21 ($P = 0.06$) and day 28 ($P = 0.07$) of lactation and greater ($P = 0.049$) accumulated lactose yields and crude protein ($P = 0.06$) yields compared to A-offspring. Ewes born to H-dams displayed greater milk yields at day 14 ($P = 0.08$) and day 21 ($P = 0.02$) and had greater accumulated milk yield ($P = 0.08$) and lactose yield ($P = 0.04$) compared to L-offspring. Lambs born to M-offspring were heavier at birth ($P = 0.02$) and grew faster until weaning ($P = 0.02$), matching the milk yield and composition data, compared to their *ad libitum* counterparts. Birth weight was not affected ($P > 0.10$) by 'grand'dam size; however, lambs born to H-offspring grew faster from birth until day 49 of age ($P = 0.03$). In conclusion, dam nutrition during pregnancy affected the resulting offspring's milk production and composition and their lamb's growth. In addition, dam size affected the offspring's milk production, lactose yield and their lamb's growth. These findings are important for furthering our understanding of how the

environment to which the female fetus is exposed can affect her subsequent development and her ability to nourish the next generation.

Introduction

Milk is the sole source of nutrients for the new-born mammal; thus, its survival and potential to reach reproductive maturity is directly dependent upon the success of its dam's lactation. Many environmental factors applied postnatally are known to affect milk production of the dam (Walker *et al.*, 2004; Pulina *et al.*, 2006) but, to date, little is known about the effects of *in utero* conditions on an animal's subsequent lactational performance. Studies have shown that nutrition during fetal life affects fetal ovarian development (Borwick *et al.*, 1997), postnatal growth (Greenwood *et al.*, 1998), reproductive performance (Rae *et al.*, 2002), and metabolism (Ravelli *et al.*, 1998; Gardner *et al.*, 2005). In addition, fetal mammary duct area has been shown to be decreased by poorer dam nutrition during pregnancy (Jenkinson, 2003). Fetal mammary duct area could affect the secretory tissue mass in the mature female (Knight and Sorensen, 2001) and, therefore, future milk production.

Dam size could also play an important role in fetal development, as it affects fetal growth through the size of the placenta, which influences the nutrient supply to the developing fetus (Mellor, 1983). Embryo transfer and cross-breeding experiments in large and small breeds of sheep (Dickinson *et al.*, 1962; Gootwine *et al.*, 1993), horses (Walton and Hammond, 1938; Allen *et al.*, 2002), and pigs (Wilson *et al.*, 1998) have shown that fetal growth can be altered from the normal genetic potential by varying dam size, resulting in altered birth weight and postnatal growth.

Restricted development of the mammary gland of the female offspring, due to dam nutrition and its effects on subsequent milk production or milk composition has not been demonstrated. Neither have the long-term effects of dam size on milk production or composition of the offspring been addressed. To examine these questions, we investigated the effects of dam nutrition and dam size during gestation on fetal mammary gland development and the lactational performance of female offspring at two years of age. We hypothesized that offspring born to light or dams fed maintenance during pregnancy would have impaired fetal mammary gland development and subsequently a decreased milk production and lighter lambs compared to offspring born to heavy dams or dams fed *ad libitum* during pregnancy.

Materials and methods

This study was conducted at the Massey University Keeble Sheep and Beef farm, 5 km south of Palmerston North, New Zealand. The study and all animal handling procedures were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Dams

Approximately 450 of the heaviest (H; 60.8 kg ± 0.18) and 450 of the lightest (L; 42.5 kg ± 0.17) mixed-aged Romney dams were selected from the extremes in a commercial flock of 2900 ewes, on the basis of size, as determined by live weight, and bred using artificial insemination as previously described by Kenyon *et al.* (2009). From day 21 until day 140 post-insemination, the dams were randomly allocated, within size, to *ad libitum* (A) or maintenance (M) nutritional regimens (HA: n = 151; HM: n = 153; LA: n = 155; LM: n = 153) under New Zealand pastoral grazing conditions. Live weights of the dams at day 140 of pregnancy were 78.4 kg ± 0.37 and 65.0 kg ± 0.35 ($P < 0.05$) for A- and M-dams, respectively (Kenyon *et al.*, 2009). Pasture herbage was the only nutritional source, and the average pre- and post-grazing pasture covers during the period days 21 to 140 were 1330 kg DM/ha ± 140.0 and 804.0 kg DM/ha ± 133.4, respectively, for the M-feeding regimen, and 2304.0 kg DM/ha ± 156.8 and 1723.3 kg DM/ha ± 149.7 for the A-feeding regimen (Kenyon *et al.*, 2009).

From day 140 of pregnancy until weaning, all dams and their lambs were fed *ad libitum*. The offspring born to H-dams were heavier at birth than offspring born to L-dams (5.51 kg ± 0.05 vs. 5.37 kg ± 0.05; $P < 0.05$) and at weaning (32.7 kg ± 0.36 vs. 31.2 kg ± 0.33; $P < 0.05$). The offspring born to A-dams were heavier at birth than offspring born to M-dams (5.66 kg ± 0.05 vs. 5.23 kg ± 0.05; $P < 0.05$) and at weaning (32.8 kg ± 0.37 vs. 31.0 kg ± 0.36; (Kenyon *et al.*, 2009)). After weaning, female progeny were managed and fed to nutritional requirements as one group under New Zealand commercial farming practice (van der Linden *et al.*, 2007). The study, therefore, utilized a two by two factorial design: two nutrition treatments (M vs. A) and two dam-size treatments (H vs. L).

Fetal mammary glands

Based on previous findings by Jenkinson (2003), 15 twin-bearing dams were euthanized at day 100 of pregnancy (HA: n = 5; HM: n = 3; LA: n = 5; LM: n = 2; low numbers are due to inconsistent male:female ratio). A wide range of fetal organs and tissues was collected and frozen for further analysis (to be reported elsewhere). Female fetal mammary glands (HA: n = 8; HM: n = 5; LA: n = 8; LM: n = 2) were dissected, separated into left and right glands, weighed and placed in Bouin’s fixative for 20 hours. After this time, excess fixative was washed out in two changes of 70% ethanol and the glands were stored in 70% ethanol before processing into paraffin wax (Leica Histoembedder, Leica Instruments GmbH, Nussloch, Germany). Sections of 6-µm thickness were cut, and once the ducts became visible, every tenth section of 3 µm was observed under the microscope until the “complete duct system” was located. Two sections of the complete duct system (3 µm thick) of each animal were mounted on individual pre-cleaned slides (Superfrost, Menzel-Glaser, Menzel GmbH & Co KG, Braunschweig). The mounted sections were oven dried for 2 hours at 57ºC and the slides were automatically stained with haematoxylin and eosin (Leica Auto Stainer XL). Cover slides were automatically mounted on the stained sections using xylene containing rapid mounting medium Entallan (Merck, kGaA, Darmstadt, Germany) and stored at room temperature until analysis of duct area and duct number. Digital images (Figure 8.1), including µm scale bars, were taken of the duplicate haematoxylin- and eosin-stained sections per animal for determination of total duct area (µm^2), using an automated image analysis assay (Dragunow, 2008). This image analysis assay automatically processed and measured duct area in images using the Metamorph v6.2.6 (Molecular Devices) image analysis package. Color images were converted to 16-bit gray scale images and then the "Detect Light Holes Morphological" filter followed by a "Morphological Erode" filter (circle, diameter = 3) were applied to segment the regions of interest (mammary ducts). The images were then copied and calibrated to micrometers. The calibrated images were "Autothresholded for Light Objects" and "Integrated Morphometry Analysis" (with classifiers for intensity and area) was performed on the thresholded images to measure the thresholded area of each image. Total duct areas were then recorded.

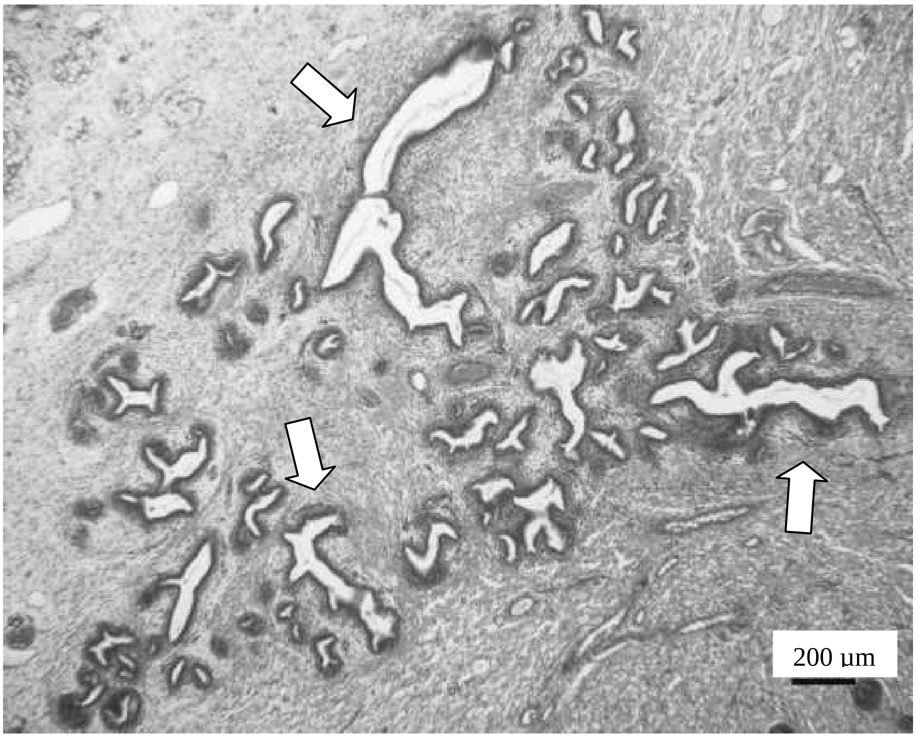

Figure 8.1. Digital image of a duplicate haematoxylin- and eosin-stained section of the fetal mammary gland at day 100 of pregnancy. Arrows indicate ducts

Ewes

At 18 months of age, oestrous cycles of the female progeny were synchronized using controlled-internal-drug-release devices (CIDR) which contained 0.3 g progesterone (Pharmacia & UpJohn, Auckland, New Zealand) and they were naturally mated with eight crayon-harnessed rams in two randomly allocated groups, 10 days apart. Ewes found to be carrying twin lambs by ultrasound at approximately day 70 of pregnancy were used in the current experiment. Twin-bearing ewes were used in this study as they are of greater economic importance for the New Zealand agricultural sector than ewes bearing singletons.

Seventy-two twin-rearing ewes (HA: *n* = 24; HM: *n* = 21; LA: *n* = 15; LM: *n* = 12) were milked once a week, for seven subsequent weeks, using the "oxytocin method" (Morgan *et al.*, 2006). The first milking commenced at day 7 (range of 5 to 9 days) after parturition. On each milking occasion, ewes were initially milked by machine followed by hand-stripping, after an intravenous injection of 1 IU synthetic oxytocin (Oxytocin V, 10 IU/mL, PhoenixPharm, Auckland, New Zealand). The time when the udder was empty was recorded. Animals were milked again (machine and hand-stripping) approximately 5 hours later, with the time and weight of the milk recorded. Lambs were separated from the ewes and bottle-fed as required during the intervening period. Daily milk yield was calculated using the formula:

$$\frac{24\ hours}{time\ between\ milkings} \times milk\ \ yield\ \ at\ \ 2^{nd}\ \ milking\ .$$

The same operators milked all ewes on each occasion in this study using a GSC 2000 milking machine (ThermaFlo, Palmerston North, New Zealand). Milk obtained by machine and hand-stripping was mixed and sub-sampled for analysis of milk composition (% fat, % crude protein, % lactose, and % casein). Milk samples were preserved with bronopol and refrigerated at 4ºC until analyses of composition using a FT120-FTIR, calibrated for sheep milk (DairyNZ, Hamilton, New Zealand). Two reference checks (fat and crude protein) were taken during lactation to account for changes in the matrix of the milk due to stage of lactation (DairyNZ). Before each milking, ewes were weighed and body condition scored (scale of 1 to 5; Jefferies,(1961)).

Ewes were drenched (Matrix Low Mineral, 1g/L Abamectin, 40g/L Levamisole, 22.7g/L Oxfendazole, Ancare New Zealand Ltd. Auckland, New Zealand) at the first two milkings followed by drenching every fortnight, to ensure milk production would not be affected by gastrointestinal nematode infections, which was confirmed by zero faecal egg counts.

Ewes were divided into four different milking groups, based on time of parturition and were grazed in rotation over five paddocks. Weekly herbage dry matter (DM) was estimated using a rising plate meter (Ashgrove Pastoral Products, New Zealand; 50 readings per paddock). The average grazing cover of the five paddocks was 1235.1 kg DM/ha ± 293.0.

Lambs

Lambs were ear tagged, identified to their mother and weighed within 24 h after birth (HA: *n* = 48; HM: *n* = 42; LA: *n* = 30; LM: *n* = 24). Lambs were weighed weekly for the first 7 weeks of life and at weaning (approximately 80 days of age). Lamb growth rates were calculated for the period from birth to day 49 of age and from birth to weaning.

Statistical analyses

Daily yields of milk and composition data were fitted using a third-degree orthogonal polynomial for each animal:

$$y_i = \alpha_0\phi_{0i} + \alpha_1\phi_{1i} + \alpha_2\phi_{2i} + \alpha_3\phi_{3i} + e_i$$

where y_i is the record of milk or composition taken at day *i*, α_n is the *n* regression, and ϕ_{ni} is rescaled value of day in milk i calculated as $\phi_{0i} = 1; \phi_{1i} = x;$ $\phi_{2i} = \frac{(3x^2 - 1)}{2}$; $\phi_{3i} = \frac{(5x^3 - 3x)}{2}$; $x = \frac{2(i - (50+1))}{(50-1)}$.

Accumulated yields of milk, lactose, crude protein, fat and milk net energy (Holmes *et al.*, 2002) were calculated over a 50-d lactation period for each animal using the estimates of the regression coefficients of the third-degree orthogonal polynomial. Concordance correlation coefficients were calculated for all variables and were 75% or higher, indicating a good fit of the models.

Fetal weight, fetal mammary gland weight and total duct area, and total number of ducts were analysed using the MIXED procedure of SAS (2006) with a linear model that included the fixed effects of dam nutrition, dam size, the interaction of dam nutrition by dam size, and the random effect of milking group. Significant covariates (*P* < 0.05; dam live weight, fetal weight, or fetal mammary gland weight) and their interaction with main effects stayed in the model.

Repeated measures analysis of milk yield, milk composition, ewe live weight, ewe body condition score, and lamb live weight were undertaken using the MIXED procedure of SAS (2006) with a linear model that included the fixed effects of dam nutrition, dam size, the interaction of dam nutrition and dam size, and the random effect of milking group. Accumulated milk, lactose, crude protein, fat yield and milk net energy were analyzed using the MIXED procedure with a linear model that included the fixed effects of dam nutrition, dam size, the interaction of dam nutrition by dam size, and the random effect of milking group. Lamb growth rates were analyzed using the MIXED procedure with a linear model that included the fixed effects of dam nutrition, dam size, the interaction of dam nutrition by dam size, and the random effect of milking group. Sex of the lamb was fitted in the model as a fixed effect where applicable.

Ewe efficiency was calculated as the regression (β_1) of accumulated milk, lactose, crude protein and fat yield (kg) and milk net energy (MJ) over 50-days lactation period, total lamb birth weight, weight at day 49 of lactation, and weaning weight on ewe metabolic live weight at mating ($LW^{0.75}$) of ewes for each of the dam treatment (dam nutrition or dam size) with the following model:

$$y_{klm} = \beta_{0k} + \beta_{1kl} x_{kl} + \mathrm{M}_m + e_{klm}$$

where y_{klm} is the dam parameter measured on ewe l from dam treatment k in milking group m, β_{0k} and β_{1k} are regression coefficients describing the regression line in dam treatment k, M_m is the random effect of milk group m and e_{klm} is the residual error corresponding to the observation y_{klm}.

The effect of sire was not included in the statistical model for the analyses of the data, as semen of the sires was evenly and randomly distributed over the dams among the treatment groups (Kenyon *et al.*, 2009). No interactions between dam nutrition and dam size were detected ($P > 0.10$); therefore, only the main effects are reported.

Results

Fetal mammary glands

Fetuses from M-dams had heavier ($P = 0.03$) mammary glands at day 100 of pregnancy compared to A-fetuses (Table 8.1). Dam nutrition had no effect ($P > 0.10$) on total duct area or total number of ducts. No dam-size effect was found on fetal mammary gland weight. However, H-fetuses had greater ($P = 0.0008$) mammary duct area compared to L-fetuses, without an effect of dam size on total number of ducts. Dam nutrition and size had no effect ($P > 0.10$) on fetus weight at day 100 of pregnancy.

Table 8.1. Fetal weight, fetal mammary gland weight (g), total duct area (μm^2), total number of ducts and total number of ducts containing lumen at day 100 of gestation of twin-fetuses carried by dams fed *ad libitum* (A) (n = 16) or maintenance (M) (n = 7) during gestation and fetuses carried by heavy (H) (n = 13) or light (L) (n = 10) dams. Table shows least square means (± S.E.)

	Treatment[1]				*P*-value	
Trait	A	M	H	L	Nutrition effect	Size effect
Fetal weight (g)	1228 ± 41.3	1129 ± 69.2	1213 ± 47.1	1144 ± 65.4	0.24	0.40
Fetal mammary gland weight (g)	3.98 ± 0.12	4.53 ± 0.20	4.16 ± 0.13	4.35 ± 0.19	0.03	0.43
Total duct area (μm^2 x 10^2)	3714 ± 320.2	3571 ± 517.6	4866 ± 352.7	2419 ± 496.0	0.82	0.0008
Number of ducts	49.7 ± 3.39	48.0 ± 5.48	51.9 ± 3.73	45.8 ± 5.25	0.79	0.36
Number of ducts with lumen	42.2 ± 3.09	42.3 ± 4.99	44.6 ± 3.40	40.0 ± 4.78	0.99	0.44

[1] No interactions between dam nutrition and dam size were detected ($P > 0.10$); therefore, only the main effects are reported

Ewe live weight and condition

Dam nutrition had no effect ($P > 0.10$) on ewe live weight during the first 49 days of lactation. Ewes born to H-dams were heavier ($P < 0.05$) throughout the first 49 days of lactation compared to L-ewes (Figure 8.2). No dam nutrition or size effects ($P > 0.10$) were found for ewe body condition score during the first 49 days of lactation (data not shown).

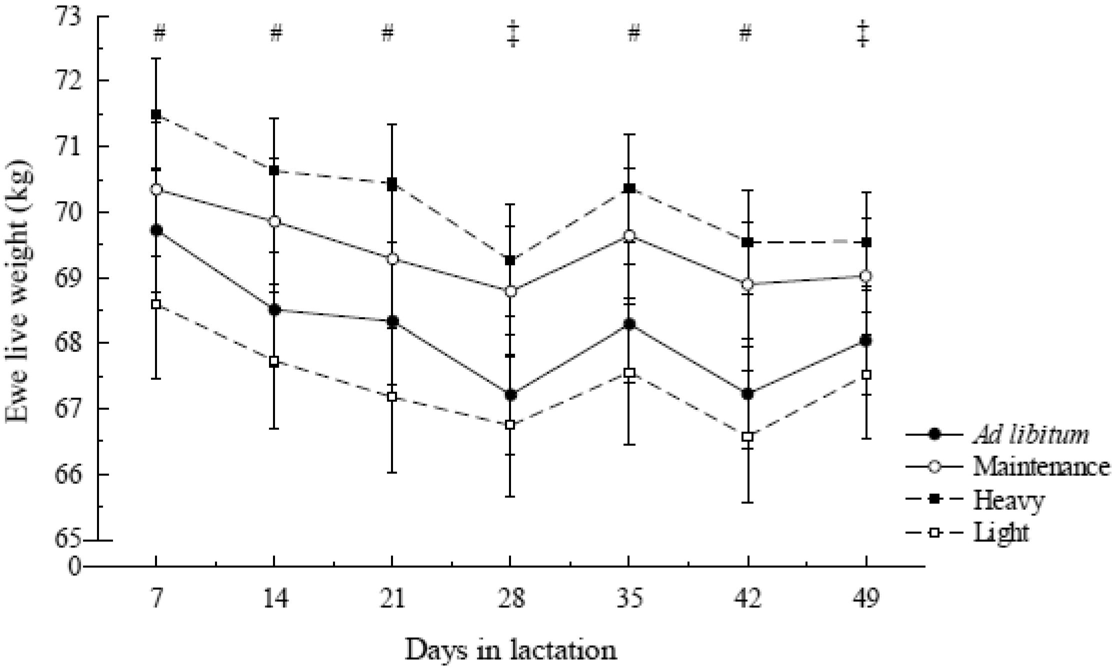

Figure 8.2. Ewe live weight during the first 49 days of lactation for ewes born to dams fed *ad libitum* (A) −●− (*n* = 39) or maintenance (M) −○− (*n* = 33) during pregnancy and ewes born to heavy (H) --■-- (*n* = 45) or light (L) --□-- (*n* = 27) dams. Data are presented as least square means (± S.E.). # $P < 0.05$; ‡ $P < 0.10$; dam size effect.

Lactation curve and milk composition

Ewes born to M-dams showed greater milk yields at day 7 ($P = 0.02$) and a trend on day 28 ($P = 0.08$) compared to A-ewes. Ewes born to M-dams showed greater lactose percentage at day 14 ($P = 0.002$), and a trend on day 21 ($P = 0.06$) and d 28 ($P = 0.07$) of lactation and showed greater ($P = 0.049$) accumulated lactose yields compared to A-ewes (Figure 8.3 and Table 8.2). Ewes born to M-dams tended ($P = 0.06$) to have greater accumulated crude protein yields compared to A-ewes. Ewes born to H-dams showed greater milk yields at day 14 (tendency, $P = 0.08$) and day 21 ($P = 0.02$) and tended ($P = 0.08$) to have greater accumulated milk yield compared to L-ewes. Ewes born to H-dams showed greater lactose percentage at day 21 ($P = 0.02$) of lactation and had greater ($P = 0.04$) accumulated lactose yields compared to L-ewes. Ewes born to L-dams showed greater fat percentage at day 7 (tendency, $P = 0.07$) at d 21 ($P = 0.03$) compared to H-ewes (Figure 8.4).

No dam nutrition or size effects were found ($P > 0.10$) for crude protein and casein percentage (data not shown), accumulated fat yield, or milk net energy over a 50-days lactation period.

Table 8.2. Accumulated milk, lactose, crude protein and fat yield (kg) and milk net energy (MJ) over 50-days lactation period of ewes born to dams fed *ad libitum* (A) ($n = 39$) or maintenance (M) ($n = 33$) during gestation and ewes born to heavy (H) ($n = 45$) or light (L) ($n = 27$) dams. Table shows least square means (± S.E.).

	Treatment[1]				*P* value	
Trait	A	M	H	L	Nutrition effect	Size effect
Milk yield (kg)	125.9 ± 2.58	132.2 ± 2.83	132.5 ± 2.34	125.7 ± 3.03	0.11	0.08
Lactose yield (kg)	6.59 ± 0.13	6.98 ± 0.14	6.99 ± 0.12	6.58 ± 0.15	0.049	0.04
Crude protein yield (kg)	5.87 ± 0.14	6.26 ± 0.15	6.19 ± 0.12	5.93 ± 0.16	0.06	0.20
Fat yield (kg)	10.18 ± 0.30	10. 35 ± 0.33	10.27 ± 0.27	10.26 ± 0.35	0.71	0.99
Milk net energy (MJ)	630.0 ± 14.93	649.2 ± 16.41	644.7 ± 13.55	634.5 ± 17.57	0.39	0.65

[1] No interactions between dam nutrition and dam size were detected ($P > 0.10$); therefore, only the main effects are reported.

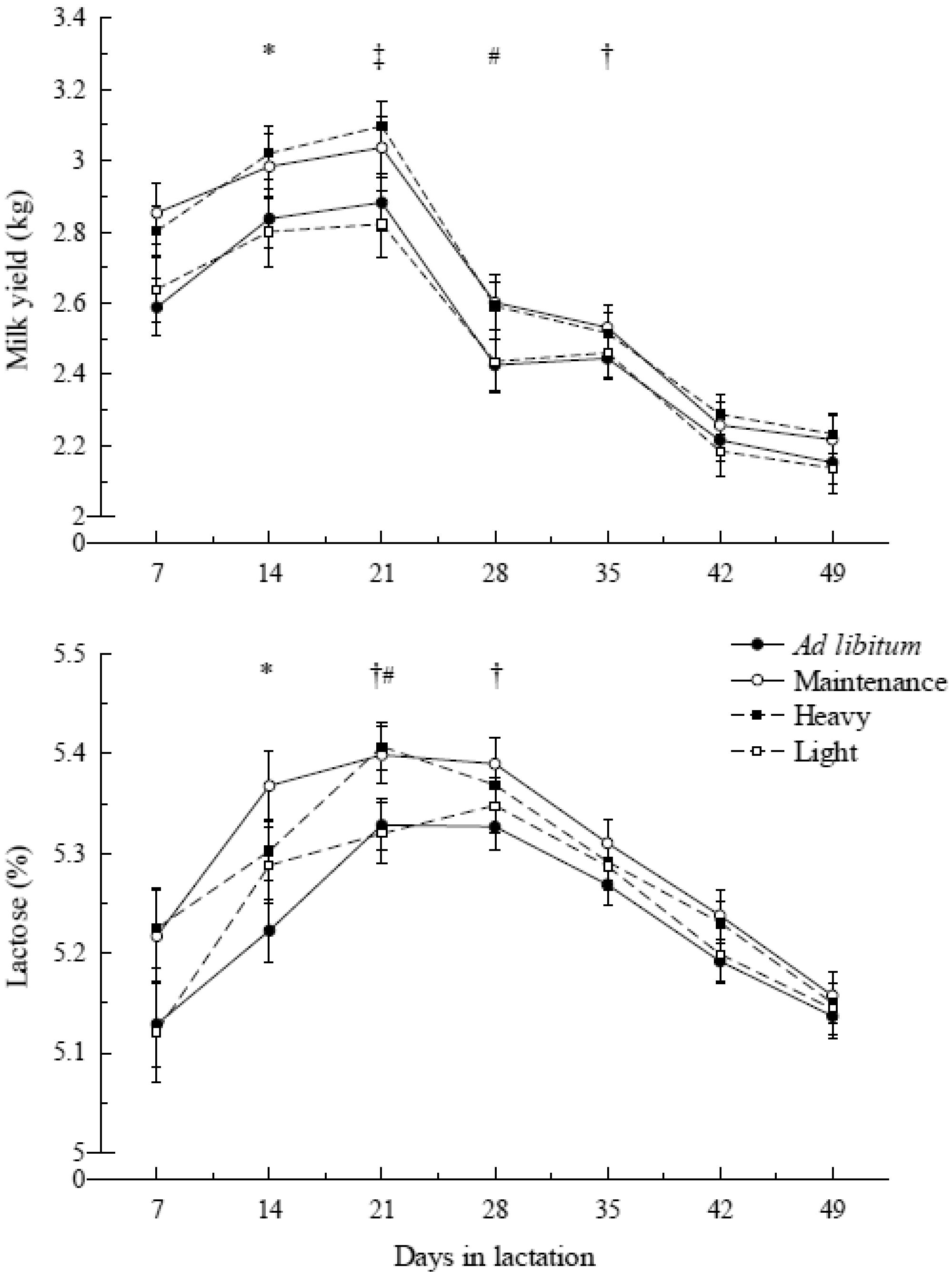

Figure 8.3. Milk yield (top) and lactose percentage (bottom) during the first 50 days of lactation for ewes born to dams fed *ad libitum* (A) −●− ($n = 39$) or maintenance (M) −○− ($n = 33$) during pregnancy and ewes born to heavy (H) --■-- ($n = 45$) or light (L)--□-- ($n = 27$) dams. Data are presented as least square means (± S.E.). * $P < 0.05$; † $P < 0.10$; dam nutrition effect, # $P < 0.05$; ‡ $P < 0.10$; dam size effect.

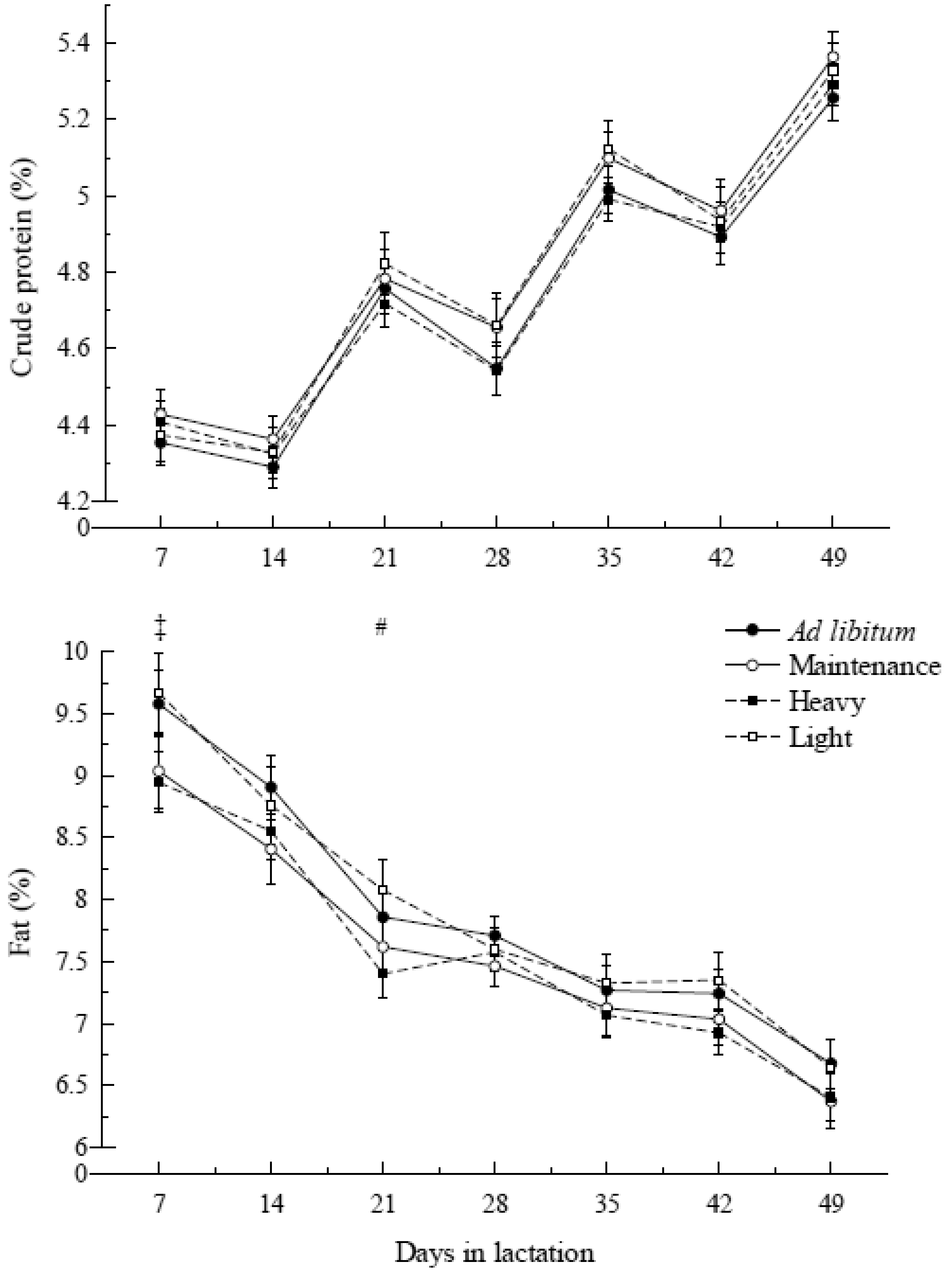

Figure 8.4. Crude protein percentage (top) and fat percentage (bottom) during the first 50 days of lactation for ewes born to dams fed *ad libitum* (A) −●− (*n* = 39) or maintenance (M) −○− (*n* = 33) during pregnancy and ewes born to heavy (H) --■-- (*n* = 45) or light (L)--□-- (*n* = 27) dams. Data are presented as least square means (± S.E.). # $P < 0.05$; ‡ $P < 0.10$; dam size effect.

Lamb birth and live weights

Lambs born to M-ewes were heavier ($P = 0.02$) at birth, remained heavier ($P < 0.05$) during the first 49 days after birth, and were heavier at weaning ($P = 0.05$) compared to lambs born to A-ewes (Table 8.3). In addition, lambs born to M-ewes had greater ($P = 0.02$) growth rates from birth to day 49 of age and from birth to weaning ($P = 0.02$). Birth weight was not affected ($P > 0.10$) by 'grand' dam size; however, lambs born to H-dams were heavier ($P < 0.05$) from 21 days until day 42 after birth, tended ($P = 0.09$) to be heavier at day 49, and were heavier ($P = 0.03$) at weaning. Lambs born to H-ewes had greater ($P = 0.03$) growth rates from birth to day 49 of age, however, no effect ($P > 0.10$) of 'grand' dam size on lamb growth rates were found from birth to weaning.

Table 8.3. Lamb birth weight (kg), lamb live weight (kg) at day 49 of age (d49) and weaning, and lamb growth rates (g/day) from birth to d49 ($\text{Growth}_{\text{birth-d49}}$) and from birth to weaning ($\text{Growth}_{\text{birth-wean}}$) for lambs born to 'grand' dams fed *ad libitum* (A) ($n = 78$) or maintenance (M) ($n = 66$) during pregnancy and lambs born to heavy (H) ($n = 90$) or light (L) ($n = 54$) 'grand' dams. Data are presented as least square means (± S.E.).

	Treatment[1]				*P* value	
Trait	A	M	H	L	Nutrition effect	Size effect
Birth weight (kg)	4.3 ± 0.09	4.7 ± 0.10	4.6 ± 0.09	4.4 ± 0.11	0.02	0.20
Weight at d 49 (kg)	18.2 ± 0.26	19.3 ± 0.28	19.0 ± 0.23	18.4 ± 0.30	0.002	0.09
Weaning weight (kg)	24.5 ± 0.45	25.7 ± 0.49	25.8 ± 0.42	24.4 ± 0.53	0.05	0.03
$\text{Growth}_{\text{birth-d49}}$ (g/day)	280 ± 4.10	295 ± 4.51	294 ± 3.73	281 ± 4.84	0.02	0.03
$\text{Growth}_{\text{birth-wean}}$ (g/day)	224 ± 7.69	237 ± 6.90	235 ± 6.98	226 ± 7.59	0.02	0.13

[1] No interactions between 'grand' dam nutrition and 'grand' dam size were detected ($P > 0.10$); therefore, only the main effects are reported.

Efficiency per kg metabolic ewe live weight at mating

The regression coefficients of ewe $LW^{0.75}$ on milk yield, lactose, crude protein and fat yield, milk net energy and lamb live weight at 49 days of age were significantly ($P < 0.05$) different from zero in A-ewes. The regression coefficient of ewe $LW^{0.75}$ on lamb weaning weight tended ($P < 0.10$) to be different from zero in A-ewes (Table 8.4). For M-ewes, the regression coefficients of ewe $LW^{0.75}$ on milk yield and crude protein yield tended ($P < 0.10$) to be different from zero. The regression coefficients of ewe $LW^{0.75}$ on lactose or fat yield, milk net energy, lamb birth weight, lamb live weight at 49 d of age, or lamb weaning weight were not significantly ($P > 0.10$) different from zero in M-ewes.

For H-ewes, the regression coefficients of ewe $LW^{0.75}$ on milk yield, lactose, crude protein and fat yield, milk net energy, and lamb weight at 49 days of age were significantly ($P < 0.05$) different from zero. The regression coefficients of ewe $LW^{0.75}$ on lamb birth and weaning weight tended ($P < 0.10$) to be different from zero in H-ewes. For L-ewes, the regression coefficients of ewe $LW^{0.75}$ on milk yield, lactose, crude protein and fat yield, milk net energy, lamb birth weight, lamb live weight at 49 days of age, or lamb weaning weight were not significantly ($P > 0.10$) different from zero.

Ewes born to H-dams tended to produce more kg of lamb at birth per kg increase in ewe $LW^{0.75}$ at mating compared to L-ewes (0.30 kg ± 0.15 vs. -0.19 kg ± 0.20; $P = 0.06$; for H- vs. L-ewes, respectively).

Table 8.4. Regression (β_1) of accumulated milk, lactose, crude protein and fat yield (kg) and milk net energy (MJ) over 50 days lactation period, total lamb birth weight, weight at day 49 of lactation and weaning weight on ewe metabolic live weight at mating ($LW^{0.75}$) of ewes born to dams fed *ad libitum* (A) (*n* = 39) or maintenance (M) (*n* = 33) during gestation and ewes born to heavy (H) (*n* = 45) or light (L) (*n* = 27) dams. Table shows estimate β_1 (± S.E.).

	Treatment[1]				*P* value	
Trait	A	M	H	L	Nutrition effect	Size effect
Milk yield (kg)	5.76 * ± 2.20	3.62 † ± 2.08	4.97 * ± 1.93	3.33 ± 2.57	0.48	0.61
Lactose yield (kg)	0.31 * ± 0.11	0.17 ± 0.11	0.23 * ± 0.10	0.20 ± 0.13	0.35	0.84
Crude protein yield (kg)	0.30 * ± 0.11	0.20 † ± 0.11	0.32 * ± 0.10	0.12 ± 0.13	0.52	0.24
Fat yield (kg)	0.52 * ± 0.20	0.30 ± 0.19	0.56 * ± 0.17	0.14 ± 0.23	0.43	0.16
Milk net energy (MJ)	35.8 * ± 12.44	18.9 ± 11.77	32.8 * ± 10.90	19.5 ± 14.58	0.32	0.47
Lamb birth weight (kg)	0.06 ± 0.18	0.22 ± 0.17	0.30 † ± 0.15	-0.19 ± 0.20	0.34	0.06
Lamb weight d49, kg	1.13 * ± 0.46	0.73 ± 0.44	1.20 * ± 0.41	0.30 ± 0.54	0.53	0.20
Lamb weaning weight, kg	1.15 † ± 0.67	0.30 ± 0.64	1.10 † ± 0.59	-0.23 ± 0.78	0.36	0.18

[1] No interactions between dam nutrition and dam size were detected ($P > 0.10$); therefore, only the main effects are reported. * β_1 significant different from zero ($P < 0.05$); † β_1 tend to be different from zero ($P < 0.10$).

Discussion

Dam nutrition effect

This study is the first to show that the plane of nutrition of the dam from days 21 to 140 of pregnancy alters fetal mammary gland weight at day 100 of pregnancy and milk yield, lactose and crude protein yields, and lactose percentage in offspring at two years of age.

Fetal mammary glands were heavier in fetuses carried by dams fed maintenance compared to their *ad libitum* counterparts; however, no differences in the number of ducts or ductal area were found. This is in contrast with results of Jenkinson (2003) who showed less ductal mammary gland area at day 101 in fetuses carried by dams fed maintenance compared to fetuses carried by dams fed 1.5 times maintenance from day 19 of pregnancy. Based on the results of Jenkinson (2003) and Berry *et al.* (2008) and the possibility that restricted development of the parenchymal tissue in early fetal life would reduce the secretory tissue mass in the mature female (Knight and Sorensen, 2001), it was hypothesized that M-ewes would have a reduced milk yield compared to A-ewes. However, milk production was greater in M-ewes and, in addition, the lambs of the M-ewes were heavier at days 49 of age and at weaning and grew faster compared to lambs born to A-ewes. This finding is in agreement with Koch (1972), who suggested that the environmental covariance correlation between offspring and dam is negative. Thus, offspring born to and reared by dams with low milk production would have high milk production when rearing their offspring, which would then result in greater weaning weights. The work of Koch (1972) would suggest a “critical window” during early postnatal life that affects the offspring’s milking and rearing ability; however, the current study showed that dam nutrition had an effect on the fetal mammary gland weight. Therefore, the combined results indicate it is not possible to identify whether the cyclical pattern of milk production is due to factors in the fetal period, the early postnatal period or a combination of both. A cross-fostering trial is necessary to untangle these possible contributions to milking performance.

No effect of plane of nutrition on duct area was found in this study, so the heavier mammary glands of M-fetuses compared to A-fetuses could be due to larger fat pads in the glands of the

M-fetuses. Fat pads mediate the action of growth hormone in mammary gland development (Walden *et al.*, 1998) which in turn could explain the greater milk yield by the M-ewes due to enhanced mammary gland differentiation in their first lactation. Mammary gland growth was reduced in the offspring of rats fed a low-protein diet during the dam's gestation and lactation (Fernandez-Twinn *et al.*, 2007). Subsequently, the mammary glands of offspring born to dams fed low-protein showed rapid compensatory development, specifically in epithelial density, for up to two weeks after weaning (Fernandez-Twinn *et al.*, 2007). Mammary gland size during lactation was not measured in this study, however, compensatory differentiation within the mammary gland could have occurred in M-ewes, given that they had greater liveweight gain compared to A-ewes after weaning until one year of age (56.9 g/day ± 1.5 vs. 52.0 g/day ± 1.5; $P = 0.02$; for M-ewes vs. A-ewes, respectively (van der Linden *et al.*, 2007)). Caution needs to be taken, however, when ruminants are compared with rodents, because rodents are less developed at birth compared to lambs.

The volume of milk produced is, in large part, a function of the amount of lactose produced (Shennan and Peaker, 2000); thus, the greater lactose synthesis in the M-ewes and, hence, a tendency to greater milk yields may be due either to increased metabolic activity of the mammary gland (Nielsen *et al.*, 2001; Xiao and Cant, 2005) or a greater amount of secretory tissue. The volume of milk produced is, to a much lesser extent, a function of the lactose concentration in the milk. A lower lactose concentration is associated with a greater volume of milk. However, M-ewes produced milk with a higher lactose percentage compared to A-ewes, indicating differences in the integrity of the glandular epithelium perhaps related to a difference in the regulation of the trans-epithelial ionic concentration gradient across the mammary epithelium. Thus, we suspect that the experience of the ewe as a fetus may affect not only the amount of secretory tissue present in the adult, but also its physiological activity.

Lamb birth weight is positively correlated with dam glucose production, without an increase in propionate availability in ewes, indicating that the precursors for glucose production are derived from glycerol and (non-essential) amino acids (Wilson *et al.*, 1983). In addition, Wilson et al. (1983) suggested that dam glucose production could be one of the factors influencing milk production and consequently have some control over lamb growth rates. Previous work, in the same cohort (van der Linden *et al*., (2010)), has shown that M-ewes had greater glucose production in response to epinephrine challenge compared to A-ewes at 16 months of age (86.1 mM min L^{-1} ± 1.89 vs. 79.1 mM min L^{-1} ± 1.93; $P = 0.01$; area under the glucose curve for M-ewes vs. A-ewes, respectively). These results indicate that M-ewes could have an advantage over A-ewes in physiological stressful situations in life (e.g. pregnancy or lactation), because their liver is able to supply more glucose to support their growing conceptus and milk production, which could explain heavier lambs at birth and greater milk yield and faster growing lambs in M-ewes compared to A-ewes as observed in the present study.

The lower concentration of lactose and lower milk yield in A-ewes is consistent with a low incidence of (sub)clinical mastitis (Auldist and Hubble, 1998). However, casein concentrations, which tend to be depressed in mastitis (Auldist and Hubble, 1998; Leitner *et al.*, 2004), did not differ between the groups. Somatic cell counts, which would have provided a more definitive indication of the mastitis status of the groups, were not measured. Thus, while not being able to counter the possibility that the differences in milk yield and composition between the groups reflected a difference in susceptibility to bacterial infection, this should not preclude consideration of more direct physiological explanations.

Dam nutrition had no effect on fat yield or energy content of the milk; however, M-ewes had heavier and faster growing lambs until weaning. Although, M-ewes had greater lactose and crude protein yields, these greater yields seem unlikely to totally explain the heavier lambs and greater growth rates. Therefore, it is suggested that dam nutrition *in utero* has affected either an unknown component in the milk or M-ewes were 'cued' *in utero* in such a way that these animals are more efficient at producing their offspring. This concept is supported by the observation that M-ewe $LW^{0.75}$ at mating did not drive lamb birth weight, lamb live weight at day 49 of age, or lamb live weight at weaning, whereas ewe $LW^{0.75}$ did drive these lamb variables in A-ewes. Thus, this may indicate that M-ewes are 'cued' by dam nutrition *in utero* to produce heavier offspring.

The current data indicate that ewes born to a dam fed maintenance during pregnancy may have an advantage over ewes born to A-dams. Thus, *ad libitum* feeding during pregnancy could be potentially more harmful than maintenance feeding as the lactational performance in the A-ewes was impaired compared to M-ewes, which is in agreement with work investigating the effects of *ad libitum* diet during pregnancy on subsequent lactational and reproductive performance in dairy cows (Lacasse *et al.*, 1993; Khan *et al.*, 2002; Beever, 2006). Further investigation is required to determine the mechanism(s) involved as it could have implications for growth and development of the next generation of offspring.

Dam size effect

No difference in fetal mammary gland weights was found between fetuses carried by heavy or light dams. However, H-fetuses showed greater duct area compared to L-fetuses, without a difference in duct number. The greater ductal area in the fetal mammary gland of H-fetuses may explain the increased milk yield in H-ewes compared to L-ewes, as secretory cells proliferate on alveoli branched off the ducts (Knight and Sorensen, 2001). In addition, H-ewes were heavier than L-ewes, and previous research has shown that larger/heavier animals within or between breeds can produce more milk (Gardner and Hogue, 1966; Hansen, 2000). A larger body size enables the animal to have greater energy intakes, which is reflected in a greater amount of precursors for milk synthesis reaching the mammary gland and subsequently greater milk yield (Armstrong and Prescott, 1970).

Larger animals have been found to be less efficient compared to their smaller counterparts (Gardner and Hogue, 1966; Hansen *et al.*, 1999), which is in agreement with the current results. The variation in lamb birth weight tended to be controlled by ewe $LW^{0.75}$ at mating for H-ewes, indicating that heavier H-ewes at mating produce heavier lambs at birth; no difference in lamb birth weight was found between dam size groups. In addition, lambs born to H-ewes did not grow faster until weaning than lambs born to L-ewes. However, lambs born to H-ewes were heavier at weaning than lambs born to L-ewes.

In conclusion, this is the first study to show that dam nutrition during pregnancy affects fetal mammary gland weight, milk production, lactose percentage, lactose and crude protein yields of the offspring, and growth to weaning of the ‘grand’ offspring. Dam size during pregnancy affects milk production of the offspring and growth to d 49 of age of the ‘grand’ offspring. These findings are important for furthering our understanding of how the environment to which the female fetus is exposed can affect her subsequent development and her ability to mother the next generation. However, the precise mechanism(s) involved requires further investigation.

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CHAPTER 9

EFFECTS OF EWE SIZE AND NUTRITION DURING PREGNANCY ON THE PERFORMANCE OF TWO-YEAR-OLD FEMALE OFFSPRING

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Abstract

This study investigated the effects of dam size and nutrition during gestation on the reproductive performance of female primiparous offspring at two-years of age. Four hundred and fifty heavy (H) (60.8 kg ± 0.18) and 450 light (L) (42.5 kg ± 0.17) dams were randomly allocated to *ad libitum* (A) or maintenance (M) nutritional regimens from day 21 until day 140 of pregnancy, under pastoral grazing conditions. One week prior to lambing, all dams and their lambs were provided with *ad libitum* feeding through to weaning. After weaning, female progeny were managed and fed to requirements as one group. At two years of age, the oestrous cycles of the female-offspring (*n* =207) were synchronized and the offspring were naturally mated with rams. Ewes were scanned for pregnancy by ultrasound at day 70 of pregnancy. Within 24 hours after birth, lambs were weighed and body dimensions were measured. Lambs were also weighed at day 24 (L24) and weaning. No dam nutrition or size effects ($P > 0.10$) were found on reproductive performance of the ewe offspring. Lambs of M-'grand'dams were heavier at birth ($P = 0.02$) and weaning ($P = 0.03$) than lambs of A-'grand'dams. Twin-lambs of H-'grand'dams were heavier at birth ($P = 0.01$) than twin-lambs of L-'grand'dams, however, 'grand'dam size had no effect ($P > 0.10$) on lamb weaning weight. In summary, dam size had no effect on reproductive performance of the female offspring, with only a minor effect on the weight of 'grand'offspring. Thus, being born to a larger dam has no advantages over being born to smaller dam, in terms of number of lambs born and weight of lambs at birth and weaning. 'Grand'dam maintenance nutrition had no effect on reproductive performance however, it increased lamb birth and weaning weight and lamb growth rates of the 'grand'offspring. Therefore, this indicates that ewes born to dams fed maintenance during pregnancy have an advantage over A-ewes in physiological stressful situations, like pregnancy or lactation.

Introduction

The productive performance of a ewe is dependent on her ability to be multiple ovulating, to conceive, and to rear heavy lambs at weaning. Any factor that limits any of these will reduce the ewe's productivity. It is possible that the *in utero* environment that a female fetus experiences, affects her productive performance as an adult. Undernutrition during early gestation (Rae *et al.*, 2002) or during both late gestation and early postnatal life (Gunn, 1977; Gunn *et al.*, 1995) are known to reduce the ovulation rate of the offspring. It is known that undernutrition during early and late gestation could affect fetal ovarian development (Borwick *et al.*, 1997; Rae *et al.*, 2001; Borwick *et al.*, 2003), which may explain the afore-mentioned reduced ovulation rates. In addition, mammary gland development of the ovine fetus has been shown to be affected by the dam's nutrition during pregnancy (Jenkinson, 2003). These effects on the development of the ovary and mammary gland may result in a reduced number of lambs at birth and their presence and weight at weaning.

Dam size could also play an important role in fetal development, as it affects fetal growth through the size of the placenta, which influences the nutrient supply to the developing fetus (Mellor, 1983). Embryo transfer and cross-breeding experiments in large and small breeds of sheep (Dickinson *et al.*, 1962; Gootwine *et al.*, 1993), horses (Walton and Hammond, 1938; Allen *et al.*, 2002) and pigs (Wilson *et al.*, 1998) have shown that fetal growth can be altered from the "normal" genetic potential by differing maternal size, resulting in altered birth weight and postnatal growth. However, these studies have not determined the long-term effects of dam size in terms of productive performance of the offspring.

Therefore, we investigated the effects of dam size and nutrition during gestation on the performance of female primiparous offspring at two-years of age. We hypothesized that small dam size and maintenance nutrition during pregnancy would have a negative effect on the performance of the ewe offspring, resulting in fewer ewes mated, lower pregnancy rates and lighter lambs born and weaned per ewe.

Materials and methods

The study was conducted at the Massey University Keeble Sheep and Beef farm, 5 km south of Palmerston North, New Zealand. The study and all animal handling procedures were approved by the Massey University Animal Ethics Committee, Palmerston North, New Zealand.

Dams

Four hundred and fifty heavy (H) (60.8 kg ± 0.18) and 450 light (L) (42.5 kg ± 0.17) Romney dams were selected from the extremes in a commercial flock of 2900 ewes, on the basis of size, as determined by live weight, and bred using artificial insemination as previously described by Kenyon *et al.* (2009). From day 21 until day 140 post-insemination, the dams were randomly allocated, within size, to *ad libitum* (A) or maintenance (M) nutritional regimens under New Zealand pastoral grazing conditions (Table 9.1) (Kenyon *et al.*, 2009).

Table 9.1. The effect of being a heavy or light dam fed *ad libitum* or maintenance during pregnancy on live weight (kg) at pregnancy day 1 (P1), 53 (P53) and 140 (P140). Table shows least square means (± S.E.).

	n	P1 (kg)	P53 (kg)	P140 (kg)
Dam nutrition				
Ad libitum	242	55.68 ± 0.26	66.45 ± 0.27	78.36 ± 0.37
Maintenance	268	55.21 ± 0.25	56.11 ± 0.26	65.01 ± 0.35
P value		NS	< 0.05	< 0.05
Dam size				
Heavy	255	64.08 ± 0.26	69.43 ± 0.27	78.88 ± 0.36
Light	255	46.80 ± 0.26	53.12 ± 0.26	64.49 ± 0.37
P value		< 0.05	< 0.05	< 0.05

Data adapted from Kenyon *et al.*, (2009). NS: non significant.

Pasture herbage was the only nutritional source and the average pre- and post-grazing pasture covers during the period days 21 - 140 were 1330 kg DM/ha ± 140.0 and 804.0 kg DM/ha ± 133.4,respectively, for the M-feeding regimen and 2304.0 kg DM/ha ± 156.8 and 1723.3 kg DM/ha ± 149.7 for the A-feeding regimen (Kenyon *et al.*, 2009).

From day 140 of pregnancy through to weaning, all dams and their lambs were provided with *ad libitum* feeding. The offspring born to H-dams were heavier than offspring born to L-dams at birth (5.51 kg ± 0.05 vs. 5.37 kg ± 0.05; for offspring born to H-and L-dams, respectively) and at weaning (32.7 kg ± 0.36 vs. 31.2 kg ± 0.33; for offspring born to H-and L-dams, respectively) (Kenyon *et al.*, 2009). The offspring born to A-dams were heavier than offspring born to M-dams at birth (5.66 kg ± 0.05 vs. 5.23 kg ± 0.05; for offspring born to A- and M-dams, respectively) and at weaning (32.8 kg ± 0.37 vs. 31.0 kg ± 0.36; for offspring born to A- and M-dams, respectively) (Kenyon *et al.*, 2009). In addition, offspring born to A-dams had greater body dimensions than offspring born to M-dams at birth (Kenyon *et al.*, 2009). After weaning, female progeny were managed and fed to nutritional requirements as one group under commercial New Zealand farming practice (van der Linden *et al.*, 2007). The study, therefore, utilized a two by two factorial design, two dam nutritional treatments (M vs. A) and two dam-size treatments (H vs. L).

Ewes

The young ewes utilized in the present study were born to the dams in the above described study (van der Linden *et al.*, 2007; Kenyon *et al.*, 2009). Ewes born to dams fed maintenance or *ad libitum* during pregnancy will be referred to as M- and A-ewes, respectively. Ewes born to heavy or light dams will be referred to as H- and L-ewes, respectively.

At 18 months of age, oestrous cycles of the young ewes (n = 207) were synchronized using controlled-internal-drug-release devices (CIDRs) which contained 0.3 g progesterone (Pharmacia & UpJohn, Auckland, New Zealand) and they were naturally mated with eight crayon-harnessed rams in two randomly allocated groups, ten days apart. Ram-harness-crayon marks on the rumps of the ewes were recorded daily for five days, as an indicator of breeding activity. Ram-harness-crayon colours were changed 20 days after the first introduction of the ram, to allow for identification of animals mated in the second cycle and marks were recorded weekly for three weeks. Ewes were scanned for pregnancy by ultrasound at approximately day 70 of pregnancy (P70) and diagnosed as being either singleton-, twin- or triplet-bearing. Ewes were weighed and body-condition scored (Jefferies, 1961) at P0 (breeding), P70 (pregnancy scanning) and P135 of pregnancy and at weaning of their lambs.

Lambs

Lambs were ear-tagged and identified to the young ewes, weighed, and thoracic girth, crown-rump length, length of the right front-leg (from elbow (cubital) joint to toe of the hoof) and right hind-leg (from hip (coxofemoral) joint to toe of the hoof) were measured within 24 h after birth. Lambs were weighed at day 24 ± 2.2 (S.D.) of age (L24) and at weaning (L77 ± 12.6 (S.D.) of age). Lamb growth rates were calculated for the periods from birth until L24 ($LWG_{birth\text{-}L24}$), L24 until weaning ($LWG_{L24\text{-}wean}$) and from birth until weaning ($LWG_{birth\text{-}wean}$).

Statistical analysis

Ewe live weight, ewe body condition score, lamb body dimensions at birth, lamb live weight, lamb growth rates and total weight of lambs weaned per ewe mated were analysed using the MIXED procedure (SAS, 2006) with a mixed linear model. The model included the fixed effects of dam nutrition, dam size, sex of the lamb, the interaction between dam nutrition by dam size, birth rank or rearing rank, and birth rank of the ewe, the two- and three-way interactions between these effects and the random effect of mating group. Non-significant two-way interactions were removed from final analyses. All three-way interactions were non-significant and were removed from final analyses. The fixed effect of birth rank of the lamb was used in the analyses of lamb birth weight, lamb dimensions at birth, ewe live weight and body condition score at P0, P70 and P135. The fixed effect of rearing rank of the lamb was used in the analysis of lamb weight at L24, at weaning (L77) and growth rates, ewe live weight and body condition score at weaning (L77) and total weight of lambs weaned per ewe mated. Birth rank of the ewe was found to be non-significant (*P* > 0.10) for all the analyses and data are not shown.

Number of lambs at pregnancy scanning, birth and weaning was analysed using the MIXED procedure (SAS, 2006) with a linear model that included the fixed effects of dam nutrition, dam size, the interaction dam nutrition by dam size and the random effect of mating group.

Ewe efficiency was calculated as the regression (β_1) of total lamb weaning weight on ewe metabolic live weight ($LW^{0.75}$) at P0 for each of the dam treatments (dam nutrition or size) with the following model:

$$y_{klm} = \beta_{0k} + \beta_{1k} x_{kl} + \mathrm{R}_{klm} + e_{klm}$$

where y_{klm} is the total lamb weaning weight measured on ewe *l* from dam treatment *k*, β_{0k} and β_{1k} are regression coefficients describing the regression line in dam treatment *k*, R_{kl} is the effect of rearing rank *m* of ewe *l* from dam treatment *k* and e_{klm} is the residual error corresponding to the observation y_{klm}. The statistical analyses of ewe efficiency and total weight of lambs weaned per ewe mated was conducted on all ewes presented for breeding. Least square means and standard errors (S.E.) were obtained and used for multiple comparisons.

Ewe breeding performance was analysed as a binomial trait after logit transformation using the GENMOD procedure (SAS, 2006) with a linear model that included the fixed effects of dam nutrition, dam size and the interaction dam nutrition by dam size. Similarly, lamb survival (lambs present at weaning (L77) were coded 1, whereas lambs not present were coded 0) was analysed as a binomial trait after logit transformation using the GENMOD procedure (SAS, 2006) with a linear model that included the fixed effects of dam nutrition, dam size and the interaction dam nutrition by dam size. Data were back transformed and presented as least square means with 95% confidence interval (C.I.).

At the start of the experiment 207 (HA: n = 60; HM: n = 59; LA: n = 44; LM: n = 44) young ewes were presented for breeding. Non-pregnant ewes (HA: n = 8; HM: n = 8; LA: n = 5; LM: n = 9) or ewes carrying triplets (HA: n = 0; HM: n = 1; LA: n = 2; LM: n = 2) at pregnancy scanning were excluded from any analyses from P70 onwards. Thirty-two singleton- or twin-bearing ewes (HA: *n* = 10; HM: *n* = 8; LA: *n* = 7; LM: *n* = 7) lost one or more of their lamb(s) prior to weaning and were excluded for analyses of weights at L24 and weaning (L77).

No interactions between dam nutrition and dam size were detected (*P* > 0.10); therefore, only the main effects are reported.

Results

Ewe live weights and body condition scores

Dam nutrition had no effect (*P* > 0.10) on ewe live weight at P0, P70 or P135 (Table 9.2). At weaning, there was an interaction between dam nutrition and ewe pregnancy / rearing rank (*P* = 0.02), such that singleton-bearing and rearing (S-rearing) M-ewes tended to be heavier (*P* = 0.07) compared to S-rearing A-ewes (65.5 kg ± 1.05 vs. 62.9 kg ± 1.16; for S-rearing M- and A-ewes, respectively). Twin-bearing but singleton-rearing (TS-rearing) M-ewes were lighter (*P* = 0.03) than TS-rearing A-ewes (62.1 kg ± 1.77 vs. 67.8 kg ± 1.82; for TS-rearing M- and A-ewes, respectively). However, in the twin-bearing and -rearing (T-rearing) ewes no effect of dam nutrition was found (63.3 kg ± 1.01 vs. 63.0 kg ± 0.93; *P* > 0.10; for T-rearing M- and A-ewes, respectively).

Dam size had no effect (*P* > 0.10) on ewe live weight at P0. H-ewes tended to be heavier at P70 (*P* = 0.07) and were heavier at P135 (*P* = 0.0006) and at weaning (*P* = 0.02) than L-ewes. Twin-bearing ewes were heavier at P0 (*P* = 0.05), P70 (*P* = 0.001) and P135 (*P* = 0.002) than singleton-bearing ewes.

Neither dam nutrition, nor dam size, nor ewe pregnancy rank affected body condition score at P0, P70 or P135 (Table 9.3). At weaning, there was an interaction (*P* = 0.007) between dam nutrition and pregnancy / rearing rank, such that S-rearing M-ewes had greater (*P* = 0.0005) body condition scores than S-rearing A-ewes (1.9 ± 0.08 vs. 1.5 ± 0.09; for S-rearing M- and A-ewes, respectively). No dam-nutrition effect (*P* > 0.10) on body condition score was found in the T-rearing ewes (1.4 ± 0.08 vs. 1.4 ± 0.08; for T-rearing M- and A-ewes, respectively) or the TS-rearing ewes (1.6 ± 0.13 vs. 1.8 ± 0.14; for TS-rearing M- and H-ewes, respectively).

Table 9.2. The effects of heavy (H) or light (L) dams fed *ad libitum* (A) or maintenance (M) during pregnancy on the live weights (kg) of the ewe offspring at pregnancy day 0 (P0), 70 (P70) and 135 (P135) and at weaning (L77) of their lambs. Ewes were either bearing and rearing singleton (S) or twin (T) lambs or bearing twins but rearing singleton lambs (TS) to weaning (L77). Table shows least square means (± S.E.).

	n^{*}	P0 (kg)	n^{**}	P70 (kg)	P135 (kg)	n^{***}	L77 (kg)
Dam nutrition[‡]							
A	104	58.8 ± 0.81	89	65.8 ± 0.62	74.1 ± 0.92	69	64.6 ± 0.79
M	103	58.2 ± 0.80	84	66.0 ± 0.60	73.3 ± 0.88	72	63.8 ± 0.82
P value		NS		NS	NS		NS
Dam size[‡]							
H	119	59.0 ± 0.81	103	66.7 ± 0.60	75.9 ± 0.88	57	65.4 ± 0.75
L	88	58.0 ± 0.80	74	65.1 ± 0.63	71.5 ± 0.93	84	63.0 ± 0.81
P value		NS		0.07	0.0006		0.02
Pregnancy / rearing rank[†]							
S	72	57.3 ± 0.63	72	64.5 ± 0.66	71.7 ± 0.96	51	64.4 ± 0.79
T }	101	58.8 ± 0.54	101	67.2 ± 0.56	75.6 ± 0.82	70	63.2 ± 0.69
TS						20	65.0 ± 1.29
P value		0.05		0.001	0.002		NS

[*] Ewes presented for breeding; [**] Non-pregnant (*n* = 30) or ewes carrying triplets (*n* = 5) were excluded from further analysis; [***] Singleton- or twin-bearing ewes that weren't able to raise at least one lamb through to weaning (*n* = 32) were excluded from further analysis; [†] Pregnancy rank is used for live weight at P0, P70 and P135, rearing rank is used for live weight at weaning, based on if the ewes were either bearing and rearing singleton (S) or twin (T) lambs or bearing twins but rearing singleton lambs (TS) to weaning (L77); [‡] No interactions between dam nutrition and dam size were detected ($P > 0.10$); therefore, only the main effects are reported. NS: non significant.

Table 9.3. The effects of heavy (H) or light (L) dams fed *ad libitum* (A) or maintenance (M) during pregnancy on the body condition score (scale 1-5) of the ewe offspring at pregnancy day 0 (P0), 70 (P70) and 135 (P135) and at weaning (L77) of their lambs. Ewes were either bearing and rearing singleton (S) or twin (T) lambs or bearing twins but rearing singleton lambs (TS) to weaning (L77). Table shows least square means (± S.E.).

	n[*]	P0	*n*[**]	P70	P135	*n*[***]	L77
Dam nutrition[‡]							
A	104	2.4 ± 0.04	89	2.5 ± 0.03	2.0 ± 0.05	69	1.6 ± 0.08
M	103	2.4 ± 0.04	84	2.5 ± 0.03	2.1 ± 0.04	72	1.6 ± 0.07
P value		NS		NS	NS		NS
Dam size[‡]							
H	119	2.4 ± 0.04	103	2.5 ± 0.03	2.0 ± 0.04	57	1.6 ± 0.07
L	88	2.5 ± 0.04	74	2.5 ± 0.03	2.1 ± 0.04	84	1.6 ± 0.07
P value		NS		NS	NS		NS
Pregnancy / rearing rank[†]							
S	72	2.4 ± 0.03	72	2.5 ± 0.03	2.0 ± 0.05	51	1.7 ± 0.07
T }	101	2.4 ± 0.03	101	2.5 ± 0.03	2.1 ± 0.04	70	1.4 ± 0.06
TS						20	1.7 ± 0.10
P value		NS		NS	NS		0.005

[*] Ewes presented for breeding; [**] Non-pregnant (*n* = 30) or ewes carrying triplets (*n* = 5) were excluded from further analysis; [***] Singleton- or twin-bearing ewes that weren't able to raise at least one lamb through to weaning (*n* = 32) were excluded from further analysis; [†] Pregnancy rank is used for body condition score P0, P70 and P135, rearing rank is used for body condition score at weaning, based on if the ewes were either bearing and rearing singleton (S) or twin (T) lambs or bearing twins but rearing singleton lambs (TS) to weaning (L77); [‡] No interactions between dam nutrition and dam size were detected (*P* > 0.10); therefore, only the main effects are reported. NS: non significant.

Reproductive performance

Neither dam nutrition nor dam size affected (*P* > 0.10) ewe breeding performance (100% (100-100) vs. 100% (100-100) and 97.6% (92.4-99.3) vs. 100% (100-100); breeding percentage during the first 34 days of oestrus for A- and M-ewes and H- and L-ewes, respectively). In addition, neither dam nutrition nor dam size affected (*P* > 0.10) number of fetuses scanned per ewe mated, nor number of lambs born or weaned per ewe mated (Table 9.4). No effects of dam nutrition or dam size were found on lamb survival until weaning (81.6% (73.6-87.3 C.I.) vs. 81.4% (73.6-87.3) and 80.1% (73.2-85.5) vs. 82.8% (74.6-88.8); lamb survival percentage from birth until weaning for lambs born to A- and M-ewes and H- and L-ewes, respectively). M-ewes weaned significantly (*P* = 0.01) greater total weight of lambs per ewe mated than did A-ewes. No effect of dam size was found on the total weight of lambs weaned per ewe mated. Although, the relationship of ewe metabolic live weight on lamb weaning weight was significantly ($P < 0.05$) different from zero for M- and H-ewes, the regression slopes (β_1) between dam-nutrition or dam-size groups were found not to be different ($P > 0.10$).

Table 9.4. The effects of heavy (H) or light (L) dams fed *ad libitum* (A) or maintenance (M) during pregnancy on pregnancy scanning, lambing and weaning percentages of the ewe offspring and total weight (kg) of lambs weaned per ewe mated and ewe efficiency (kg). Table shows least square means (± S.E.).

	n	% of fetuses scanned*	% of lambs born**	% of lambs weaned***	Weight of lambs weaned (kg) / ewe****	Ewe efficiency β_1†
Dam nutrition‡						
A	104	144 ± 8	144 ± 7	132 ± 8	33.7 ± 1.55	1.32 ± 1.06
M	103	134 ± 8	130 ± 7	122 ± 8	36.2 ± 1.62	2.12 ± 1.08
P value		NS	NS	NS	0.01	NS
Dam size‡						
H	119	142 ± 7	142 ± 7	129 ± 7	35.7 ± 1.59	1.89 ± 0.97
L	88	135 ± 8	132 ± 8	125 ± 9	34.2 ± 1.59	1.44 ± 1.21
P value		NS	NS	NS	NS	NS

* Defined as the number of fetuses identified at pregnancy diagnosis per ewe presented for breeding; ** Defined as the number of lambs born alive per ewe presented for breeding; *** Defined as the number of lambs weaned alive per ewe presented for breeding; **** Defined as the total weight of lambs at weaning per ewe presented for breeding; † Total lamb weaning weight regressed on ewe metabolic live weight at mating of ewes presented for breeding; ‡ No interactions between dam nutrition and dam size were detected ($P > 0.10$); therefore, only the main effects are reported. NS: non significant.

Lamb live weights

Lambs whose ‘grand’dams were fed maintenance were heavier at birth (*P* = 0.02) and weaning (*P* = 0.03) than lambs whose ‘grand’dams were fed *ad libitum* (Table 9.5). There was a significant interaction (*P* = 0.004) between ‘grand’dam nutrition and birth / rearing rank for lamb live weight at L24, such that twin-born and reared (T-reared) lambs whose ‘grand’dams were fed maintenance were heavier (*P* = 0.001) than T-reared lambs whose ‘grand’dams were fed *ad libitum* (13.0 kg ± 0.18 vs. 12.4 kg ± 0.18; for T-reared lambs of M- and A-‘grand’dams, respectively). No such effect (*P* > 0.10) for live weight at L24 was found in singleton-born and reared (S-reared) lambs (13.5 kg ± 0.28 vs. 13.9 kg ± 0.32; for S-reared lambs of M- and A-‘grand’dams, respectively) or twin-born and singleton-reared (TS-reared) lambs (13.3 kg ± 0.41 vs. 13.1 kg ± 0.46; for TS-reared lambs of M- and A-’grand’dams, respectively).

After adjusting for birth weight, ‘grand’dam-nutrition effects on lamb weaning weight remained significant (*P* < 0.05), however, the interaction between ‘grand’dam nutrition and rearing rank for lamb live weight at L24 was no longer significant (*P* > 0.10).

There tended to be an interaction (*P* = 0.09) between ‘grand’dam size and lamb birth rank for birth weight, such that twin-born (T-born) lambs of H-‘grand’dams were heavier (*P* = 0.01) than T-born lambs of L-‘grand’dams (4.5 kg ± 0.10 vs. 4.2 kg ± 0.12; for T-born lambs of H- and L-‘grand’dams, respectively). No such relationship (*P* > 0.10) was found for birth weight in singleton-born (S-born) lambs (5.3 kg ± 0.16 vs. 5.4 kg ± 0.16; for S-born lambs of H- and L-‘grand’dams, respectively). Lambs with H-‘grand’dam were heavier (*P* = 0.01) at L24 than lambs from a L-’grand’dam. Dam size had no effect (*P* > 0.10) on lamb weaning weight.

After adjusting for birth weight, lambs of H-‘grand’dams tended (*P* = 0.09) to be heavier than lambs of L-’grand’dams at L24.

S-reared lambs were heavier at weaning (*P* = 0.0001) than T-reared lambs and TS-reared lambs (*P* = 0.0001).

After adjusting for birth weight, the birth / rearing-rank difference between S- and T-reared lambs at weaning remained significant, however, the birth / rearing-rank difference between S-reared lambs and TS-reared lambs at L24 became non-significant (*P* > 0.10).

Table 9.5. The effects of heavy (H) or light (L) 'grand'dams fed *ad libitum* (A) or maintenance (M) during pregnancy on weight (kg) at birth, L24 and weaning (L77) of 'grand'offspring. Lambs were born and reared either as a singleton (S) or twin (T), or born as a twin but reared as a singleton (TS) to weaning (L77). Table shows least square means (± S.E.).

	n^*	Birth weight (kg)	n^{**}	L24 weight (kg)	L77 weight (kg)
Dam nutrition[‡]					
A	139	4.7 ± 0.10	111	13.1 ± 0.20	26.6 ± 0.52
M	124	5.0 ± 0.10	102	13.3 ± 0.18	27.9 ± 0.48
P value		0.02		NS	0.03
Dam size[‡]					
H	161	4.9 ± 0.10	128	13.4 ± 0.16	27.7 ± 0.47
L	102	4.8 ± 0.10	85	13.0 ± 0.18	26.8 ± 0.53
P value		NS		0.01	NS
Birth rank and rearing rank[†]					
S	68	5.4 ± 0.12	51	13.7 ± 0.22	30.7 ± 0.59
T	} 195	4.3 ± 0.08	140	12.7 ± 0.13	25.5 ± 0.37
TS			22	13.2 ± 0.31	25.6 ± 0.92
P value		0.0001		0.0001	0.0001

[*] Number of lambs born; [**] Number of lambs born and raised as a singleton (S), born and raised as a twin (T) and born as twin but raised as a singleton (TS) to weaning (L77) ; [†] Birth rank was used in the birth weight analysis and rearing rank was used in the analysis for weight at L24 and at weaning (L77); [‡] No interactions between dam nutrition and dam size were detected ($P > 0.10$); therefore, only the main effects are reported. NS: non significant.

Lamb body dimensions at birth

'Grand'dam nutrition had no effect ($P > 0.10$) on thoracic girth, or length of the front and hind legs of lambs (Table 9.6). Lambs whose 'grand'dams were fed maintenance had greater ($P = 0.02$) crown-rump lengths than lambs whose 'grand'dams were fed *ad libitum*.

No 'grand'dam size effect ($P > 0.10$) was found on crown-rump length of the lambs. The hind legs of lambs of H-'grand'dams were longer ($P = 0.003$) than those of lambs of L-'grand'dams. There was a significant interaction ($P = 0.01$) between 'grand'dam size and birth rank for thoracic girth, such that S-born lambs of L-‘grand’dams had greater (*P* = 0.049) thoracic girths than lambs of H-‘grand’dams (42.8 cm ± 0.94 vs. 40.4 cm ± 0.81; for S-born lambs of L- and H-‘grand’dams, respectively). No such relationship (*P* > 0.10) for thoracic girths was found for T-born lambs (37.8 cm ± 0.60 vs. 38.9 cm ± 0.48; for T-born lambs of L- and H-’grand’dams, respectively).

Table 9.6. The effect of heavy (H) or light (L) ‘grand’dams fed *ad libitum* (A) or maintenance (M) during pregnancy on the thoracic girth (cm), crown-rump length (cm), length of right front and hind legs (cm) of ‘grand’ offspring. Lambs were born either as a singleton (S) or twin (T). Table shows least square means (± S.E.).

	n	Thoracic girth (cm)	Crown-rump length (cm)	Front leg (cm)	Hind leg (cm)
Dam nutrition[*]					
A	139	39.7 ± 0.36	53.6 ± 0.65	31.5 ± 0.24	37.5 ± 0.27
M	124	40.0 ± 0.33	55.0 ± 0.59	31.8 ± 0.22	37.4 ± 0.29
P value		NS	0.03	NS	NS
Dam size[*]					
H	161	40.0 ± 0.34	54.4 ± 0.63	32.0 ± 0.23	38.0 ± 0.28
L	102	39.7 ± 0.35	54.2 ± 0.62	31.2 ± 0.24	37.0 ± 0.28
P value		NS	NS	0.02	0.003
Birth rank					
S	68	41.4 ± 0.41	55.6 ± 0.68	32.1 ± 0.28	38.3 ± 0.32
T	195	38.3 ± 0.26	53.0 ± 0.56	31.1 ± 0.17	36.7 ± 0.24
P value		0.0001	0.0001	0.002	0.0001

[*] No interactions between dam nutrition and dam size were detected (*P* > 0.10); therefore, only the main effects are reported. NS: non significant.

There was a significant interaction (*P* = 0.04) between ‘grand’dam size and birth rank for the length of the front leg, such that twin-born lambs of H-‘grand’dams had longer (*P* = 0.0001) front legs than T-born lambs of L-‘grand’dams (31.9 cm ± 0.21 vs. 30.4 cm ± 0.27; for T-born lambs of H- and L-‘grand’dams, respectively). No such relationship (P > 0.10) for the length of the front leg was found for S-born lambs (32.1 cm ± 0.39 vs. 32.1 cm ± 0.39; for S-born lambs of H- and L-‘grand’dams, respectively).

S-born lambs had greater crown-rump lengths (*P* = 0.0001) and longer hind legs (*P* = 0.001) compared to T-born lambs.

Lamb growth rates

‘Grand’dam nutrition had no effect (*P* > 0.10) on $\text{LWG}_{\text{L24-weaning}}$ (Table 9.7).

There was a significant interaction (*P* = 0.005) between ‘grand’dam nutrition and birth / rearing rank for $\text{LWG}_{\text{birth-L24}}$ such that S-reared lambs of A-‘grand’dams had greater (*P* = 0.04) growth rates than S-reared lambs of M-‘grand’dams (398 g/day ± 14 vs. 364 g/day ± 13; for S-reared lambs of A- and M-’grand’dams, respectively). In addition, T-reared lambs whose ‘grand’dams were fed maintenance had greater (*P* = 0.0001) growth rates than T-reared lambs of A-‘grand’dams for $\text{LWG}_{\text{birth-L24}}$ (330 g/day ± 10 vs. 300 g/day ± 11; for T-reared lambs of M- and A- ‘grand’dams, respectively). No such relationship (*P* > 0.10) for $\text{LWG}_{\text{birth-L24}}$ was found for TS-reared lambs (350 g/day ± 20 vs. 322 g/day ± 23; for TS-reared lambs of M- and A-‘grand’dams, respectively).

There was a significant interaction (*P* = 0.03) between ‘grand’dam nutrition and birth / rearing rank for $\text{LWG}_{\text{birth-weaning}}$, such that S- reared lambs of A-‘grand’dams tended to have greater (*P* = 0.06) growth rates than S-reared lambs of M-‘grand’dams (316 g/day ± 10 vs. 295 g/day ± 9; for S-reared lambs of A- and M- ‘grand’dams, respectively). No such relationship (*P* > 0.10) for $\text{LWG}_{\text{birth-weaning}}$ was found for T-reared lambs (259 g/day ± 7 vs. 267 g/day ± 7; for T-reared lambs of A- and M- ‘grand’dams, respectively) and TS-reared lambs (306 g/day ± 14 vs. 279 g/day ± 13; for TS-reared lambs of A- and M- ‘grand’dams, respectively).

After adjusting for birth weight, the ‘grand’dam nutrition effects found on lamb growth rates, remained significant (*P* < 0.05), however, the interaction between ‘grand’dam nutrition and rearing rank for $\text{LWG}_{\text{birth-weaning}}$ became non significant (*P* > 0.10).

‘Grand’dam size had no effect (*P* > 0.10) on $\text{LWG}_{\text{L24-weaning}}$. Lambs of H-‘grand’dams tended (*P* = 0.08) to have greater growth rates than lambs of L-‘grand’dams for $\text{LWG}_{\text{birth-weaning}}$. There tended to be an interaction (P = 0.08) between ‘grand’dam size and birth / rearing rank for $\text{LWG}_{\text{birth-L24}}$, such that S-reared lambs of H-‘grand’dams had greater (P = 0.04) growth rates than S-reared lambs of L-‘grand’dams (402 g/day ± 14 vs. 361 g/day ± 13; for S-reared lambs of H- and L-‘grand’dams, respectively). No such relationship (P > 0.10) for $\text{LWG}_{\text{birth-L24}}$ was found for T-reared lambs (315 g/day ± 10 vs. 315 g/day ± 10; for T-reared lambs of H- and L-‘grand’dams, respectively) and TS-reared lambs (358 g/day ± 17 vs. 315 g/day ± 28; for TS-reared lambs of H- and L-‘grand’dams, respectively).

Table 9.7. The effects of heavy (H) or light (L) ‘grand’dams fed *ad libitum* (A) or maintenance (M) during pregnancy on growth rates (g/day) from birth to L24, from L24 to weaning (L77) and from birth to weaning (L77) of ‘grand’lambs, which were either born and reared as singleton or twin or born as a twin but reared as a singleton (TS) to weaning (L77). Table shows least square means (± S.E.).

*

	n	$\text{LWG}_{\text{birth-L24}}$ (g/day)	$\text{LWG}_{\text{L24-weaning}}$ (g/day)	$\text{LWG}_{\text{birth-weaning}}$ (g/day)
Dam nutrition†				
A	111	340 ± 12	268 ± 9	294 ± 8
M	102	348 ± 10	258 ± 8	281 ± 7
P value		NS	NS	NS
Dam size†				
H	128	359 ± 10	266 ± 8	292 ± 7
L	85	330 ± 12	260 ± 8	282 ± 7
P value		0.02	NS	NS
Rearing rank*				
S	51	381 ± 11	275 ± 9	306 ± 7
T	140	315 ± 9	244 ± 7	263 ± 6
TS	22	336 ± 17	269 ± 12	293 ± 10
P value		0.01	0.03	0.002

Number of lambs born and raised as a singleton (S), born and raised as a twin (T) and born as twin but raised as a singleton (TS) to weaning (L77);† No interactions between dam nutrition and dam size were detected (P > 0.10); therefore, only the main effects are reported. NS: non significant.

S-born lambs had greater growth rates than T-born lambs for $LWG_{L24\text{-weaning}}$ (*P* = 0.0002). TS-reared lambs had greater growth rates for $LWG_{L24\text{-weaning}}$ (*P* = 0.04) than T-reared lambs.

After adjusting for lamb birth weight, the significant ‘grand’dam size effects found on lamb growth rates remained significant (*P* < 0.05).

Discussion

Dam and ‘grand’dam nutrition

The number of fetuses per ewe presented for breeding was not affected by dam nutrition. Previous work has found reduced ovulation rate in ewes at 20 months of age born to dams fed 50% of the ME for pregnancy from mating to day 95 of pregnancy (Rae *et al.*, 2002). In contrast to the study by Rae *et al*. (2002) the oestrus of the ewes was synchronized for practical reasons in the present study. This treatment could have overridden any potential endocrine differences, masking potential differences in reproductive performance. Although, ovulation rate was not measured in the present study, it is possible that the maintenance feeding regimen during gestation of the dams was not severe enough to alter reproductive function in the female offspring. In addition, no effect of dam nutrition during pregnancy was found on the onset of puberty in the female offspring (van der Linden *et al.*, 2007).

Interestingly, M-ewes gave birth to heavier lambs, somewhat explained by the greater crown-rump lengths of these lambs, and weaned heavier lambs which contributed to greater total weight of lambs weaned per ewe mated compared to A-ewes. In addition, within the twin-born lambs, lambs whose ‘grand’dams were M-fed were heavier at L24 and grew faster from birth to L24 than lambs of A-’grand’dams. The current study is the first to show that maintenance nutrition of the ‘grand’dam during pregnancy positively affects the weight of the second generation offspring. This has implications for the design of optimal feeding regimen for ewes during pregnancy, in terms of the performance of future generations.

During the first three to four weeks of life milk is the sole source of nutrients for the new-born lamb (Degen and Benjamin, 2005), thus, its survival and potential to reach maturity is directly dependent upon the success of its dam's lactation. Therefore, the greater lamb live weights at weaning and greater early growth rates in lambs within the twin-born group whose 'grand'dams were M-fed could partly be driven by the greater milk production (Snowder and Glimp, 1991). A sub-set of ewes in the present study were milked (van der Linden *et al.*, 2009) and it was observed that twin-bearing and rearing M-ewes had greater milk yield (132.2 kg ± 2.83 vs. 125.9 kg ± 2.58; for M- and A-ewes, respectively), greater lactose (6.98 kg ± 0.14 vs. 6.59 kg ± 0.13; for M- and A-ewes, respectively) and crude protein yields (6.26 kg ± 0.15 vs. and 5.87 kg ± 0.14; for M- and A-ewes, respectively) compared to A-ewes. This indicates that at least some of the response in lamb live weight was driven by the milk production of the ewe. Thus, a maintenance diet of a dam during pregnancy positively affects its offspring's milk production. Thus, based on these finding, it would be beneficial to farmers to feed their pregnant ewes a maintenance diet to increase the productivity of the next generation. However, the present results are potentially in contrast with studies conducted in rodents where poorer growth of the 'grand'offspring is associated with poor nutritional regimen of the 'grand'dam (Zambrano *et al.*, 2005; Pinheiro *et al.*, 2008; Corson *et al.*, 2009), but the maintenance regimen in the present study is not as severe as the protein-restriction regimen used in the rodent studies.

No differences in ewe efficiency, defined as the relationship of ewe metabolic live weight at breeding on total lamb weaning weight, were found between nutrition groups. However, the regression slope of M-ewes was significantly different from zero, indicating that an increase in metabolic live weight of the M-ewes at breeding, resulted in an increase in the weight of their lambs at weaning. This relationship was not observed in A-ewes; indicating that, metabolic live weight of A-ewes had no effect on lamb weaning weight. Thus, from a practical point of view, it would be of benefit to farmers if the offspring born to maintenance fed dams were themselves heavier at their breeding, in terms of weaning weight of the lambs of the second generation, but there is no benefit if offspring born to *ad libitum* fed dams were themselves heavier at their breeding.

Wilson *et al.* (1983) found that lamb birth weight was positively correlated with dam glucose production and also suggested that dam glucose production could be one of the factors influencing milk production and consequently have some control over lamb growth rates. Previous work (van der Linden *et al*. (2010)) has shown that M-ewes had greater glucose production in response to an epinephrine challenge compared to A-ewes at 16 months of age, suggesting that M-ewes may have an advantage over A-ewes in their ability to positively affect fetal growth and their lactational performance, which may explain heavier lambs at birth and faster growing lambs born to M-ewes compared to lambs born to A-ewes.

No differences in lamb survival were observed between treatment groups. However, caution must be taken when interpreting this data due to relative low number of animals within each treatment group.

Dam and 'grand'dam size

Dam size had no effect on reproductive performance of the offspring, or lamb weaning weight of the 'grand' offspring. Although, twin-bearing H-ewes gave birth to heavier lambs compared to twin-bearing L-ewes. This could be explained by the observation that H-ewes were heavier at P70, at P135 and at weaning than L-ewes. In addition, no 'grand'dam size effect was found for lamb birth weight in singleton-bearing ewes. The heritability of adult weight of an animal is ~0.41 (Safari *et al.*, 2005), therefore, ewes born to H-dams are more likely to be larger as an adult than ewes born to L-dams. Thus, H-ewes probably had more space for the fetuses *in utero* (uteroplacental complex) (Gootwine *et al.*, 2007) and possibly a bigger digestive tract to be able to supply more nutrients to the fetuses. In addition, lambs born to H-ewes had longer front and hind legs at birth compared to those born to L-ewes. This is in agreement with the longer legs found in H-ewes at birth compared to L-ewes (Kenyon *et al.*, 2009) and this is therefore presumably due to genetic effects. However, no effect of 'grand'dam size was found on lamb weaning weight or lambing or weaning percentage, or total weight of lambs weaned per ewe indicating that heavier or larger animals might not be as efficient compared to their smaller counterparts, which is in agreement with previous work.

In conclusion, dam size had no effect on reproductive performance of the female offspring, with only a minor effect on the weight of 'grand'offspring. Thus, being born to heavy (larger) dam has no advantages over being born to light (smaller) dam, in terms of reproductive performance, number of lambs born and weight of lambs at birth and weaning. Maintenance nutrition of the ‘grand’dam had no effect on reproductive performance, however, it increased lamb birth and weaning weight and lamb growth rates of the ‘grand’offspring. Therefore, this indicates that ewes born to dams fed maintenance during pregnancy have an advantage over A-ewes in physiological stressful situations (e.g., pregnancy or lactation) and is likely to be driven by the ability of M-ewes to produce a greater amount of glucose in physiologically demanding situations. Therefore, it would be of high interest to investigate if the increase in lamb birth and weaning weight persists in the second parity and determine what mechanism(s) are driving this nutritional effect.

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CHAPTER 10

GENERAL DISCUSSION

Adverse conditions *in utero* and/or in early postnatal life have been shown to programme the postnatal physiology and metabolic function of the offspring (Fowden *et al.*, 2006). However, there are few data on the long-term effects of dam size and nutrition during pregnancy on the performance of the offspring under commercial grazing conditions. Therefore, the objectives of this thesis were to examine the effects of dam size (heavy vs. light) and dam nutrition (maintenance vs. *ad libitum*) during pregnancy on growth, metabolic function, lactational and reproductive performance of female offspring under New Zealand commercial pastoral grazing conditions.

The results of this thesis indicated that it is possible to alter the performance of the offspring by feeding dams differently during pregnancy (fetal programme effect). For example, offspring born to dams fed maintenance levels during pregnancy showed increased gluconeogenesis and/or glycogenolysis, greater lactational performance and gave birth to, and weaned, heavier lambs compared to offspring born to dams fed *ad libitum*. This is an exciting finding as it reinforces the concept that life-time production potential may be programmed during early life. In addition, with increased knowledge and a better understanding of how offspring can be programmed through altering the maternal environment through changes in nutritional regimen, it may be possible to significantly increase the production potential of the New Zealand ewe population. This could significantly improve financial returns to the New Zealand sheep industry and could potentially have implications for other animal industries also.

Dam nutrition effects

Female offspring born to dams fed maintenance during pregnancy were lighter at birth and had reduced growth rates up to weaning compared to offspring born to *ad libitum* fed dams (Chapter 5). However, after weaning, this maternal nutrition effect was no longer significant. The initial difference in weight is probably due to the lactational performance of the dams, as it has been shown that nutrition during pregnancy can affect the milk production of the dam (Wallace, 1938; Mellor and Murray, 1985). Therefore, the offspring born to dams fed maintenance were most likely exposed to suboptimal nutrition during early postnatal life as well as during pregnancy compared to offspring born to dams fed *ad libitum*. Although the female offspring were lighter at birth and weaning and grew slower, no differences in the onset of puberty were found compared to offspring born to *ad libitum* fed dams. One possibility is that the maintenance nutritional regimen of the dam during pregnancy, which aimed for an increase in ewe live weight similar to that of the expected conceptus mass, was not severe enough to induce differences in the reproductive axis of the offspring. However, it also is possible that the method used in the present study (vasectomized crayon harnessed rams) was not effective enough to detect more subtle (hormonal) changes between groups (Da Silva *et al.*, 2001). Further, all the offspring obtained the threshold of live weight for reaching puberty (range 33 to 42 kg, for Suffolk crossbreds) (Keane, 1976). It would be of interest for future studies to regularly collect blood samples to examine if maternal nutrition during pregnancy affects reproductive hormone concentrations (e.g. LH, FSH, oestrogen, leptin) prior to puberty and the onset of oestrous later in life. In addition, determination of ovulation rates between groups would have given more insight into the reproductive potential of these animals.

At 16 months of age, dam nutrition showed a small effect on glucose metabolism in the female twin offspring (Chapter 6). Twin-born M-ewes showed a small insulin increment compared to their A-counterparts. These results are in contrast to other studies that found a greater effect of dam nutrition during pregnancy on glucose metabolism (Oliver *et al.*, 2002; Gardner *et al.*, 2005; Ford *et al.*, 2007; Husted *et al.*, 2008) in offspring. However, in the previous studies, dam undernutrition regimens applied were more severe and for shorter periods during (mainly late) gestation (Oliver *et al.*, 2002; Gardner *et al.*, 2005; Ford *et al.*, 2007; Husted *et al.*, 2008) than in the present study. The random sub-group of female twin offspring used in the metabolic challenge trial did not differ in birth weight between maternal nutrition groups, however, birth weights of all twins, both males and females, born to maintenance-fed dams were lighter than twins born to *ad libitum*-fed dams (Kenyon *et al.*, 2009). Therefore, it is possible that our study could have underestimated the effects of maternal nutrition on glucose metabolism in later life as birth weight may be an important indicator for glucose impairment in later life (Oliver *et al.*, 2002).

Another explanation for the small effect observed on glucose metabolism could be that the offspring in our study were farmed under New Zealand pastoral grazing conditions and the control of food intake is less than in an individual housing situation. After weaning (at approximately seven months of age) the offspring were exposed to a summer drought, resulting in decreased growth rates and low live weights (van der Linden *et al.*, 2007). Perhaps the offspring born to maintenance-fed dams were programmed correctly *in utero* for their “suboptimal” postnatal environment (Gluckman *et al.*, 2005; Gardner *et al.*, 2007a) and consequently no impairment of the glucose metabolism at 16 months of age would be expected.

Age and physiological state of the female offspring at the time of the metabolic challenge trial might have contributed to the lack of alteration in metabolic function. Previous work has shown that age of the offspring negatively affects the development of impaired glucose metabolism and insulin resistance (Gatford *et al.*, 2004). Therefore, it would also be of value to investigate if dam size and nutrition during pregnancy may have altered the metabolic function of the offspring at a more mature age (e.g. 4 or 5 years of age) and /or in more physiological demanding circumstances (i.e. pregnancy, lactation). Research conducted by Husted *et al.* (2008) showed that offspring born to undernourished dams responded differently to a glucose tolerance test during pregnancy and lactation than did offspring born to control-fed dams. It would be of interest to explore different methods (e.g. proteomics, gene expression) in addition to metabolic challenges to assess metabolic functioning in animals, as there is much variation among studies making conclusive statements difficult.

On the other hand, when the growth rates of offspring were regressed on the area under the glucose and insulin curves in response to the GTT and ITT at 16 months of age, a relationship was found between impaired glucose metabolism and insulin resistance and growth rates prior to puberty in HA-ewes (Chapter 7). This relationship was not observed after puberty. Therefore, it was suggested that HA-ewes showed puberty-related insulin resistance, as described in human children (Amiel *et al.*, 1991; Moran *et al.*, 1999). Growth rates after puberty showed a positive relationship with glucose impairment and insulin resistance in HM-ewes. This finding is in agreement with the suggestion that postnatal growth is a predictor of later development of glucose intolerance (Symonds, 2007). This indicates that the HM-ewes may develop glucose intolerance later in life. The absolute insulin secretion after the glucose administration was positively related with growth of the offspring in the current study, this may indicate that no dysfunction at pancreatic level had occurred (Davies *et al.*, 1994). Thus, it may be more likely that the sub-cellular insulin-signalling proteins downstream of the receptor could be affected (Fernandez-Twinn *et al.*, 2005) especially at adipose tissue level (Gardner *et al.*, 2005), as mature animals are more likely to accumulate adipose tissue than muscle tissue. To confirm these hypotheses it would be of interest to examine insulin signalling pathways in (adipose) tissues of the offspring used in this work.

Maternal nutrition during pregnancy had no effect on the HPA-axis function at the adrenal level in response to the insulin tolerance test. However, it would have been of interest to examine ACTH concentrations as previous work has shown that a 10-day period of undernutrition during late pregnancy elevated ACTH concentrations compared to control-fed animals in response to an insulin tolerance test and CRH/AVP challenge, without finding differences in cortisol concentrations (Bloomfield *et al.*, 2003).

In response to an epinephrine challenge, ewes born to maintenance fed dams during pregnancy showed increased concentrations of glucose compared to those born to dams fed *ad libitum*. These results may indicate that under stressful situations M-ewes increased gluconeogenesis or glycogenolysis within the liver (Desai *et al.*, 1997; Sloboda *et al.*, 2005; McCurdy and Friedman, 2006). To confirm the hypothesis that maternal nutrition had indeed affected gluconeogenesis and/or glycogenolysis, future work should collect liver samples to confirm the possible up- or down- regulation of key hepatic enzymes (phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase for gluconeogenesis; glucokinase for glycogenolysis) involved in these processes.

In contrast to the hypothesis based on the findings of Jenkinson (2003) ewes born to M-dams had greater lactose percentage, lactose and crude protein yields and a trend towards greater accumulated milk yield during the first 49 days of lactation compared to those born to A-dams (Chapter 8). The measurement of blood metabolites (e.g. glucose, NEFA, triglycerides) and blood hormones (e.g. insulin, prolactin) during the lactation of the ewe offspring may have given a better insight into why differences in lactational performances were observed between treatment groups. In agreement with the lactation data, lambs born to M-ewes had greater growth rates compared to those born to A-ewes (Chapter 9). These results could be explained by the observation of the greater glucose production in response to the ETT. Pregnancy and lactation are physiologically stressful and if the rate of gluconeogenesis or glycogenolysis is indeed up-regulated in M-ewes, this would give them an advantage over A-ewes, by being able to give birth to heavier lambs, having greater lactational performances, and therefore weaning heavier lambs per ewe. However, to examine if this lactational effect persists into the second lactation, the ewe offspring should be milked again during their second lactation. In addition to determine if this maternal nutrition effect on lactation is an intergenerational effect, the lactational performance of the ‘grand’offspring (G_2) should be examined.

Another possibility to consider, to explain the heavier lambs born to M-ewes is the direct effect of ‘grand’dam nutrition on the ‘grand’offspring. In other words, the development of the female germline (‘grand’offspring) occurs while the female (offspring) is *in utero* (dam), thus, the ‘grand’offspring could have been directly affected by the nutritional regimen of the dam (Youngson and Whitelaw, 2008). The offspring of M-‘grand’dams could have been ‘cued’ to be thrifty, resulting in greater birth weight and weaning weight.

In conclusion, maintenance nutrition of the dam, in which the liveweight gain of the dam is similar to the expected increase in conceptus weight, from days 21 - 140 of pregnancy is beneficial to the female offspring, as offspring born to maintenance-fed dams may have increased gluconeogenesis and/or glycogenolysis, which enables them to produce heavier lambs at birth, have greater lactational performance, which results in heavier lambs at weaning during their first parity compared to offspring born to dams fed *ad libitum*.

The dam-nutrition effects observed could have implications for the animal-production industry, as the primiparous offspring born to dams exposed to maintenance nutrition during pregnancy (e.g. shortage of grass), have an advantage over offspring born to *ad libitum*-fed dams by being able to produce heavier lambs at birth and weaning. Therefore, it would be of interest to investigate if the increase in lamb birth and weaning weights persists in their second parity and what mechanism(s) are driving this effect. This could be achieved by regular collection of blood samples during pregnancy to determine if concentrations of metabolites (e.g. glucose, NEFA) are elevated in ewes born to maintenance-fed dams compared to A-ewes. In addition, tissue could be collected for molecular analyses (e.g. gene expression of glucokinase, PEPCK, glucose-6-phosphatase in the liver; GLUT-4 receptors in adipose and muscle tissue; β-cell differentiation in pancreas; ACTH receptors in adrenal gland) which might give more insight into the mechanisms of the observed results in the present study, however, this was not possible, as the female offspring were studied for life-time effects of maternal nutrition during pregnancy

Dam size effects

The results of this work have shown that dam size had no effect on growth of the offspring prior to weaning, however, after weaning, within the maintenance group, offspring born to heavy dams grew faster than those born to light dams (Chapter 5). Thus, lambs born to heavy dams may have an advantage (greater growth rates after weaning) over lambs born to light dams in situations where food supply is suboptimal for the dam during pregnancy. However, from a productive perspective, a heavier dam will consume more grass per year and could therefore be less productive on a per kilogram dry matter consumed, per kilogram of lamb weaned (Morel and Kenyon, 2006). Even though lambs born to heavy offspring had greater growth rates after weaning, no effect of dam size was found on the onset of puberty in female offspring. This is probably due to the fact that all the offspring obtained the threshold of live weight for reaching puberty at a similar age (range 33 to 42 kg, for Suffolk crossbreds) (Keane, 1976).

Dam size had no effect on glucose metabolism, fat metabolism and adrenal function of the female twin offspring (Chapter 6). Although the uterine capacity of small dams may be smaller compared to that of large dams (573 g ± 18.6 vs. 624 g ± 18.3; $P = 0.056$; for placenta weight of small and large ewes at day 140 of pregnancy, respectively; Blair *et al.*, unpublished data), there could have been a reduction in fetal growth from its genetic potential, without obligatorily altering metabolic function later in life. This is in itself an interesting finding and the first study to show that maternal size has no effect on metabolic function of the female offspring.

Offspring born to heavy dams had greater accumulated milk and lactose yields over the first 49 days of lactation (Chapter 8). No differences in fetal mammary gland weights were found between fetuses carried by heavy or light dams, however, H-fetuses showed greater duct area compared to L-fetuses without a difference in duct number. The greater ductal area in the fetal mammary gland of H-fetuses may explain the increased milk yield in H-ewes compared to L-ewes, as secretory cells proliferate on the ducts (Knight and Sorensen, 2001). In addition, H-ewes were significantly heavier than L-ewes and previous research has shown that larger/heavier animals within or between breeds can produce more milk (Gardner and Hogue, 1966; Hansen, 2000). A larger body size enables the animal to have greater energy intakes, which is reflected in a greater amount of precursors for milk synthesis reaching the mammary gland and, subsequently, greater milk yield (Armstrong and Prescott, 1970). Even though offspring born to heavy dams had an advantage over offspring born to light dams in lactational performance, overall, ‘grand’dam size had no effect on number of ‘grand’lambs born and no effect of dam size was found on birth and weaning weights of the ‘grand’offspring (Chapter 9).

Based on the findings in the present study, it can be concluded that female offspring born to heavy dams have no advantage over offspring born to light dams in terms of productive performance. In fact, from an agricultural point of view, grazing heavy ewes is less efficient compared to grazing light ewes as the offspring born to heavier dams do not increase total weight of lambs weaned per ewe. Based on these findings, farmers might benefit from selecting for smaller/lighter dams as they consume less grass and, therefore, more stock units per hectare can be grazed (Morel and Kenyon, 2006). However, this needs to be confirmed with a more in depth economic analyses.

Recommendations for further research

The study was conducted under commercial New Zealand farming practice and therefore the nutritional regimens of the dams during pregnancy were impossible to manage at an individual animal level. Rather than this being a limitation, the results indicate that offering different feeding regimens under commercial farming practice can indeed programme the offspring. If any nutritional treatment is to be effective at flock level, and therefore have implications for commercial farmers, nutritional level itself was most important to control the feed intake of the dams.

Due to the comparison of only two nutrition levels, it is unknown if the maintenance nutrition treatment increased performance or if the *ad libitum* nutrition treatment decreased performance. Further studies should consider a third nutritional treatment either slightly above or below maintenance.

The ‘critical window’ studied in the present study (days 21 - 140 of pregnancy) was relatively long and this makes it difficult to define which period during pregnancy (or early life) resulted in the observed effects. In addition, days 21 - 140 is probably too long in an agricultural situation due to variation in feed availability during winter. To narrow down the ‘critical windows’ *in utero* it would be of interest to examine when during pregnancy, for example, mammary gland development and subsequent lactational performance, is affected. It is known that mammary gland development commences during early pregnancy in sheep (Anderson, 1975). Therefore, in an ideal (funding) situation, the nutritional regimen of the dams during pregnancy may be split into three groups; from breeding (day 0) to day 21, from day 22 to day 40 and from day 41 through to mid-pregnancy (day 70) where dams are fed sub-maintenance, maintenance or *ad libitum* nutritional levels (Table 10.1) to determine during which period mammary gland development is affected. To cancel out the potential carry-over effects of maternal nutrition during pregnancy on her subsequent milk production, the lambs should be reared artificially. To determine if the lactational performance of the offspring in later life is due to the confounding effects of the intrauterine environment of the fetus and nutrition of the lamb during early postnatal life, half of the female offspring, born to each maternal treatment group, should be fed above maintenance for growth and the other half should be fed sub-maintenance until weaning.

Table 10.1. Research design to examine the effects of nutrition during pregnancy on the mammary gland development and subsequent lactational performance of the offspring.

		Duration during pregnancy		
Group	*n*	d0- d21	d22-d40	d41-d70
SSS	200	Sub-Maintenance	Sub-Maintenance	Sub-Maintenance
SAA	200	Sub-Maintenance	*Ad libitum*	*Ad libitum*
SAS	200	*Ad libitum*	Sub-Maintenance	*Ad libitum*
AAS	200	*Ad libitum*	*Ad libitum*	Sub-Maintenance
MMM	200	Maintenance	Maintenance	Maintenance
MAA	200	Maintenance	*Ad libitum*	*Ad libitum*
AMA	200	*Ad libitum*	Maintenance	*Ad libitum*
AAM	200	*Ad libitum*	*Ad libitum*	Maintenance
AAA	200	*Ad libitum*	*Ad libitum*	*Ad libitum*

From an agricultural perspective, commercial grazing conditions are beneficial as these results could lead to guidelines for farmers of how to feed their dams during early pregnancy to potentially increase the lactational performance of the ewe offspring and subsequently increase the total weight of lambs weaned born to these ewe offspring. In addition, to examine potential molecular changes induced by the nutritional regimen of the dam, sub-groups of dams (*n* = 8-10) need to be either sacrificed for tissue collection of the fetuses and dam during pregnancy and postnatal life of the ewe offspring (e.g. puberty, pregnancy, lactation) or other techniques should be used (e.g. biopsies, scanning of the animal) to examine tissues on molecular level. The study could focus on twin-bearing dams only, as those are of greater economical value to farmers compared to singleton-bearing dams.

Concluding statement

In summary, this PhD study showed that dam size and nutrition under commercial grazing conditions can affect the performance of the offspring. This PhD study gives valuable information of what could be investigated in the future to increase our knowledge to improve animal production.

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