Selection for growth, muscling and fatness alters the maternal performance and intermediary metabolism of Merino ewes

This thesis is presented for the degree of Doctor of Philosophy of Murdoch University

Mark Bradley Ferguson

B. Ag. Sci. (Hons) (University of Melbourne)

November 2012
I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

...........................................

Mark Bradley Ferguson
Abstract

There is growing interest in selectively breeding Merinos with higher growth and muscling and lower fatness. The effects of selection for these traits on ewe intermediary metabolism, body composition, reproduction and milk production and on lamb birthweight, survival and growth were studied in a series of experiments and analyses.

Ewes with higher genetic propensity for early growth had higher mature weight, reproductive rate, lamb birthweight, ewe milk production and lamb growth rate. Ewes with higher growth also had a higher circulating level of growth hormone during lactation.

Ewes with higher genetic propensity for muscling had a higher reproductive rate and produced lambs that were lighter at birth, but this did not result in lower lamb survival. Ewes with higher muscling maintained a higher condition score which may be at least partly attributed to a lower response to adrenaline at the level of the muscle in these higher muscled ewes. Similarly higher muscled ewes had lower growth hormone concentration in lactation which would result in lower mobilisation of tissues. In addition peripheral tissues were less responsive to insulin in high muscled ewes and blood glucose levels were also higher during the non-breeding state in high muscled ewes.

The genetic fatness of ewes was positively associated with lamb birthweight but only when nutrition was restricted suggesting that ewes with a higher genetic propensity for fatness can buffer lamb birthweight under periods of poor nutrition. Ewes with
higher genetic fatness had lower circulating growth hormone and a greater response to insulin providing potential mechanisms for the observed higher fatness. Furthermore, response to adrenaline at the level of liver was greater in ewes with higher fatness suggestive of a higher capacity for gluconeogenesis. The combined results of this work suggest that actively selecting Merino ewes to have higher growth, muscling and fatness is likely to have positive reproduction and therefore economic outcomes.
Table of Contents

Acknowledgements ........................................................................................................... ix
List of Figures ..................................................................................................................... xi
List of Tables ....................................................................................................................... xii
List of Abbreviations ......................................................................................................... xvi

Chapter 1 General Introduction ......................................................................................... 1

Chapter 2 Literature Review ............................................................................................... 5
  2.1 Ruminant energy metabolism ....................................................................................... 5
    2.1.1. Digestion of carbohydrate ................................................................................... 5
    2.1.2. Gluconeogenesis ................................................................................................. 6
    2.1.3. Storage of glucose as glycogen ........................................................................... 8
    2.1.4. Storage of energy as fat ..................................................................................... 10
    2.1.5. Fat depots ......................................................................................................... 11
    2.1.6. Fat mobilisation ................................................................................................. 12
    2.1.7. Muscle energy metabolism ............................................................................... 14
  2.2. Hormone regulation of growth and body composition .................................................. 16
    2.2.1. Growth Hormone .............................................................................................. 16
    2.2.2. Insulin-like growth factor I ............................................................................... 19
    2.2.3. Insulin ............................................................................................................... 21
    2.2.4. Adrenaline ......................................................................................................... 23
    2.2.5. Leptin .................................................................................................................. 25
  2.3. Adaptation to pregnancy and lactation ....................................................................... 26
    2.3.1. Accumulation of maternal tissues during early pregnancy .................................. 26
    2.3.2. Mobilisation of maternal tissues during late pregnancy ....................................... 27
    2.3.3. Mobilisation of maternal tissues during lactation .............................................. 28
    2.3.4. Re-building maternal tissues in late lactation and post weaning ......................... 30
  2.4. Measuring and breeding for changes to growth and body composition in sheep ......... 31
    2.4.1. Measuring body composition using dual-energy x-ray absorptiometry .............. 32
    2.4.2. Condition scoring .............................................................................................. 33
    2.4.3. GR tissue measurement for carcass grading ..................................................... 33
    2.4.4. C-site muscle and fat depth .............................................................................. 34
    2.4.5. Australian Sheep Breeding Values ................................................................... 34
  2.5. The impact of ewe nutrition and selection strategy on body composition and maternal traits ......................................................................................................................... 36
Chapter 7 Merino ewes selected for rapid lean growth have higher circulating growth hormone

Chapter 8 Lamb energy metabolism at birth is altered by maternal genotype and lamb gestation length

Chapter 9 Ewes selected for high muscling mobilise less muscle glycogen and ewes selected for leanness release less glucose in response to adrenaline
Chapter 10 Breeding for increased muscling and reduced fatness decreases the response to insulin in reproducing Merino ewes .......................................................... 249
10.1 Introduction .................................................................................................. 249
10.2 Materials and Methods ............................................................................. 251
  10.2.1 Experimental Design ........................................................................... 251
  10.2.2 Animals ................................................................................................. 252
  10.2.3 Preparation of animals ......................................................................... 253
  10.2.4 Experimental Procedure ...................................................................... 253
  10.2.5 Body composition measurement .......................................................... 255
  10.2.6 Chemical Methods .............................................................................. 256
  10.2.7 Statistical Analysis .............................................................................. 256
10.3 Results ......................................................................................................... 257
  10.3.1 Liveweight, condition score and whole body fat percentage ................. 257
  10.3.2 Effect of physiological state, liveweight and condition score on basal glucose ........................................................................................................ 258
  10.3.3 The effect of muscling group on SSGIR ................................................. 259
  10.3.4 The effect of HFAT on SSGIR ............................................................... 260
  10.3.5 The effect of HWT on SSGIR ............................................................... 262
10.4 Discussion ................................................................................................... 264
Chapter 11 General Discussion ....................................................................... 273
11.1 Growth ......................................................................................................... 273
11.2 Muscling ...................................................................................................... 275
11.3 Fatness ......................................................................................................... 276
11.4 Associated changes in muscle metabolism .......................................................... 277
11.5 Future breeding direction in the Australian Merino ........................................... 278
11.6 Reflection on methodologies used ........................................................................ 279

12. Bibliography ........................................................................................................... 281
Acknowledgements

This thesis is an accomplishment not just for me but for all of those who have helped and guided me along the way. As with the decade before, my enchanting wife Nisha has been there to guide me through the last six years of study and I am eternally grateful for her unwavering support and belief. During the journey I lost a great mentor in Dr Norm Adams and I am deeply saddened that Norm was not here to see the completion of this work but I will be forever grateful that I had the opportunity to have worked with, and learnt from him. He was a scholar of unquestionable integrity and brilliance and it was the greatest of pleasures to have studied under his guidance. I am fortunate that during the time this thesis was completed, three wonderful children graced our world. My beautiful daughter, Sitara Devi and gorgeous sons, Jai Nikhil and Kiran Shah together you have changed my world.

The work contained within this thesis was made possible by the generous support of many people. I was extremely fortunate to have the assistance, guidance and friendship from Mrs Jan Briegel and it is inconceivable to imagine the last six years without her. I would also particularly like to thank my supervisors Dr Graham Gardner and Dr Dave Pethick for their support, guidance, friendship and encouragement throughout. Never were there two men more dedicated to doing great science with practical outcomes for industry.

To Mum and Dad I thank you for creating possibilities and for a lifetime of love and support, to my brothers, I owe thanks to Brett for ending up at university in the first place and to Tim for an eternal passion to breed the perfect sheep. To Ian and Ganga thank you for your enduring support.
My industry collaborators were a very important part of this work and they not only made the sheep available for these studies but also provided considerable insight and knowledge that has helped shaped this thesis. They also provided many days of assistance and many opportunities to laugh. To Ian and Debbie Robertson, to Bill and Kay and Geoff and Emma Sandilands and to the board of Merinotech (WA) Limited, my sincerest thanks. Thanks also to Paul Daly for his many hours of help.

The opportunity to spend many evenings with Gus Rose was undoubtedly the highlight of my many trips south. To Kel Pearce, Johan Greeff, Geoff Cox, Steve Bell, and all of those who helped the DXA days pass, my thanks. I also thank Matt Wilmot, Hayley Norman, Valérie Kromm and Harriet Pugh for providing endless assistance and opportunities to laugh at various times throughout this process.

The animal house experiment described in the last four chapters of this thesis involved most of the research community in Perth, in addition to those already mentioned above. I would like to thank Kristy Glover, Margaret Blackberry, Beth Paganoni, Carolina Vinoles Gil, Peter McGilchrist, Sarah Bonny, Jim McMahon, Jen Clulow, Elizabeth Hulm, Paul Young, Shimin Liu, Mike Carthew, Phil Bullock, Rob Kelly, Mal Boyce, Paul Kenyon, Megan Chadwick, Di Mayberry, Andrew Toovey, Allan Rintoul, Dean Thomas, Roberta Bencini, Nic Wryde, Anthony Wryde and Ken Chong for making it possible. There were others too, too numerous to mention that made this possible.

Finally a thank you to my friend, mentor and boss Dr Andrew Thompson for his continued support. I am fortunate to have had the financial support provided by an Australian Post-graduate Award, the Sheep CRC, CSIRO Livestock Industries and Murdoch University.
List of Figures

Figure 3.1 Predicted response (±s.e.) of lamb survival to the Australian sheep breeding value (ASBV) for percentage increase in ewe hogget-age clean fleece weight (HCFW) for single and twin born lambs. ................................................................. 74

Figure 4.1 Relationships between Dual-energy X-ray Absorptiometry (DXA) determined tissue masses and weighed and chemically determined values for a) total fat (carcass plus internal fat), b) carcass fat, c) carcass lean and d) carcass bone weight. Equations for lines of best fit are presented in Table 4.1. .................................................. 89

Figure 5.1 Live weight a), and condition score b), of ewes across the experiment in relation to day 0 (7 February 2006). ........................................................................................................ 110

Figure 5.2 The effect of eye muscle depth (EMD) Australian sheep breeding value (ASBV) on dual-energy X-ray absorptiometry (DXA) measured fat tissue (liveweight included in the model). ........................................................................ 112

Figure 5.3 The effect of eye muscle depth (EMD) Australian sheep breeding value (ASBV) on dual-energy X-ray absorptiometry (DXA) measured lean tissue (corrected to constant liveweight). ........................................ 113

Figure 6.1 Live weight a), and condition score b), of ewes fed high (black line filled in circles) or low (grey line open circles) nutrition across the experiment in relation to day 0 (13 February 2006). ...................................................................................... 136

Figure 6.2 The effect of Australian sheep breeding value (ASBV) for ewe fatness (HFAT) on DXA fat when measured at conception ( --- ), pre-lambing (---), mid-lactation (--- ) and weaning (--- ). Liveweight was included in the analysis. The relationship is significant (P<0.05) only at conception and post weaning measurements. ................................................................. 139

Figure 6.3 The effect of ewe fatness (HFAT) Australian sheep breeding value (ASBV) on lamb birthweight when ewes are managed on low or high nutrition during pregnancy ........................................................................................................ 143

Figure 7.1 Australian Sheep Breeding Values (ASBV) at hogget age for subcutaneous fat depth (HFAT) and eye muscle depth (HEMD) of single bearing ewes used in the experiment (n=55). ................................................................. 170

Figure 7.2 Liveweight profile and experimental time line from day 0 (7 March 2007), arrows under the line represent blood sampling time points. .................................................. 178

Figure 7.3 The relationship between plasma growth hormone concentration and the Australian sheep breeding value (ASBV) fat depth at hogget age (HFAT) at a hogget-weight ASBV (HWT) of 2, 5 or 8 when ewes were pregnant (a), lactating (b) and non-breeding (c). ................................................................. 183

Figure 9.1 Subcutaneous fat depth (HFAT) and eye-muscle depth (HEMD) Australian Sheep Breeding Value (ASBV), calculated 21 March 2008, for 8 ewes in each of the high, medium and low muscling groups. .................................................. 218
Figure 9.2 Glucose concentration area under curve between 0 and 10 minutes (AUC10) relative to adrenaline dose in: a) pregnancy; b) lactation; and c) non-breeding ewes with breeding values for subcutaneous fat over the loin at hogget age (HFAT) of -0.5, 0.5 and 1.5mm. ................................................................. 228

Figure 9.3 Lactate concentration area under curve between 0 and 10 minutes (AUC10) relative to an adrenaline dose in: a) pregnancy; b) lactation; and c) non-breeding ewes from high, medium and low muscling groups. ................................................................. 231

Figure 9.4 Area under curve (AUC) of lactate concentration between 0 and 10 minutes relative to adrenaline challenges in: a) pregnant; b) lactating; and c) non-breeding ewes from high, medium and low muscling groups and with a range in hogget-age subcutaneous fat depth (HFAT) Australian sheep breeding value (ASBV). Values presented are averages across all levels of adrenaline challenge. .............................................. 233

Figure 9.5 NEFA concentration area under curve between 0 and 10 minutes (AUC10) relative to adrenaline doses in pregnant, lactating and non-breeding ewes. ................. 236

Figure 10.1  The effect of ewe muscle group on steady state glucose infusion rate (SSGIR; 50% glucose solution) at insulin infusion rates of 0.6 and 6.0mU/kg.min. Values are predicted means ± s.e. and are averaged across pregnant, lactating and non-breeding states. Muscle groups are not significantly different (P>0.05) at the 0.6 infusion rate but are all significantly different (P<0.05) at the 6.0 rate. ........................................ 260

Figure 10.2 The effect of ewe fat breeding value (HFAT ASBV) on steady state glucose infusion rate (SSGIR; 50% glucose solution). Predicted means ± se at insulin infusion rates of 0.6 (grey line, open squares) and 6.0mU/kg.min (black line, closed squares) during (a) pregnant, (b) lactating, and (c) non-breeding. Symbols are adjusted raw data and each represent an experiment on a single sheep. .............................................. 261

Figure 10.3 The effect of ewe weight breeding value (HWT ASBV) on steady state glucose infusion rate (SSGIR; 50% glucose solution). Predicted means ± s.e. at insulin infusion rates of 0.6 (grey line, open squares) and 6.0mU/kg.min (black line, closed symbols) during (a) pregnancy, (b) lactation, and (c) non-breeding. Closed and open symbols are adjusted raw data for 0.6 and 6.0mU/kg.min insulin infusion rates, each represents an experiment on a single sheep. .............................................. 263

List of Tables

Table 3.1 Generalised linear regression model parameter estimates for the effect of birth type, ewe age, year, and ewe Australian sheep breeding value (ASBV) for hogget-age eye muscle depth (HEMD), weight (HWT) and clean fleec weight (HCFW) for the proportion of ewes having multiple lambs and the proportion of lambs surviving to weaning. Parameters for factors are differences compared with the reference level of ewe age = 2 years old, year = 2000 and in the case of lamb survival, birth type = single. ......................................................................................... 70
Table 3.2 $F$ values for the effect of lamb birth type or rear type, sex, sire, ewe age at birth, year of birth, and ewe Australian sheep breeding values (ASBVs) for hogget-age eye muscle depth (HEMD), weight (HWT), clean fleece weight (HCFW), coefficient of variation of fibre diameter (HFDCV) and significant interactions between terms on lamb weight at birth and weaning.

Table 4.1 Regression coefficients (± s.e.), model $F$ values and correlation coefficients for Dual-energy X-ray Absorptiometry (DXA) estimates of fat (DXA fat), lean tissue (DXA lean) and bone (DXA bone) compared with chemically determined and weighed measures of total fat, carcass fat, carcass lean and carcass bone. All terms are significant ($P<0.05$).

Table 4.2 Predicted means and regression coefficients (± s.e.), model $F$ values and correlation coefficients for models of carcass traits predicted by 15 month old weight (WT EBV), depth of eye muscle (EMD EBV) and subcutaneous fat at the C site (FAT EBV) estimated breeding values. Coefficients are shown with and without carcass weight (CW) or liveweight (LW) included. Only significant terms ($P<0.05$) are included.

Table 4.3 Regression coefficients (± s.e.), model $F$ values and correlation coefficients for models of carcass traits predicted by GR depth, condition score and liveweight. All terms are significant ($P<0.05$) unless marked otherwise.

Table 5.1 $F$ values for the effect of time, the number of lambs born and reared (rear type) and breeding values for weight (HWT) and eye muscle depth (HEMD) at hogget age, on ewe liveweight and condition score.

Table 5.2 Liveweight, condition score, and total mass of fat, lean, and bone tissue measured by dual-energy x-ray absorptiometry (DXA) of ewes at conception, pre-lambing, lactation and post-weaning.

Table 5.3 $F$ values for the effect of physiological state, breeding values for weight (HWT), subcutaneous fat depth (HFAT), eye muscle depth (HEMD) at hogget age, and interactions of physiological state with HWT, HFAT and HEMD on total mass of fat, lean, and bone tissue measured by dual-energy x-ray absorptiometry (DXA).

Table 5.4 $F$ values for the effect of breeding values for weight (HWT), and eye muscle depth (HEMD) at hogget age, lamb birth type, lamb sex on lamb weights at birth and weaning and for the effect of breeding values for weight (HWT), and eye muscle depth (HEMD) at hogget age, time of milking and interaction between time of milking and HWT on ewe milk production.

Table 5.5 Plasma concentration of leptin, insulin-like growth hormone-1 (IGF-I), urea nitrogen and albumin in ewes during conception, mid-pregnancy, late-pregnancy, lactation, and post-weaning.

Table 5.6 $F$ values for the effect of physiological state, breeding values for weight (HWT), sub-cutaneous fat depth (HFAT), eye muscle depth (HEMD) at hogget age, and interactions of physiological state with HWT and HEMD on ewe plasma concentrations of leptin, insulin-like growth factor-I (IGF-I), urea nitrogen and albumin.
Table 6.1 F values for the effect of ewe nutrition treatment, time, the number of lambs born and reared (rear type), and breeding values for fat (HFAT), eye muscle depth (HMD) and weight (HWT) at hogget age and significant interactions between terms on ewe liveweight and condition score.................................................................137

Table 6.2 Total mass of fat, lean, and bone tissue measured by dual-energy x-ray absorptiometry (DXA) of ewes from low and high nutritional treatments at conception, pre-lambing, lactation and weaning.................................................................138

Table 6.3 F values for the effect of nutrition treatment, physiological state, the number of lambs carried (birth type), Australian sheep breeding values for subcutaneous fat depth (HFAT), eye muscle depth (HMD), and weight (HWT) at hogget age, and interactions of physiological state with nutrition, HWT, HFAT and HMD on total mass of fat, lean, and bone tissue measured by dual-energy x-ray absorptiometry (DXA)..........................................................................................................................140

Table 6.4 F values for the effect of ewe nutrition, lamb birth type, lamb sex, ewe breeding values for weight (HFAT), and eye muscle depth (HMD) at hogget age, and the significant interactions between terms on lamb weights at birth and weaning. ....144

Table 6.5 F values for the effect of nutrition treatment, milking occasion (time), the number of lambs carried (birth type), breeding values for subcutaneous fat depth (HFAT) and eye muscle depth (HMD) at hogget age, and significant interactions between terms on ewe milk production, and the percentage of fat, protein and lactose in the milk. ................................................................................................................................146

Table 6.6 Plasma concentration of leptin, insulin-like growth factor I (IGF-I), urea nitrogen and albumin from ewes in low and high nutrition treatments at conception, mid-pregnancy, pre-lambing, lactation and at lamb weaning.........................................................149

Table 6.7 F values for the effect of nutrition treatment, physiological state, the number of lambs carried (birth type), breeding values for subcutaneous fat depth (HFAT) and eye muscle depth (HMD) at hogget age, and significant interactions between terms on plasma concentrations of leptin, insulin-like growth factor I (IGF-I), albumin and urea nitrogen..................................................................................................................................151

Table 7.1 F values for the effect of day of experiment, breeding values for weight (HWT), subcutaneous fat depth (HFAT), eye muscle depth (HMD) at hogget age and condition score on ewe liveweight (LW), condition score (CS) and fat amount measured by Dual-energy x-ray absorptiometry (DXA fat)...................................................................................179

Table 7.2 Ewe liveweight (LW), condition score (CS), and plasma concentrations of albumin, urea nitrogen (Urea N), glucose, non-esterified fatty acids (NEFA) and lactate across the breeding cycle.................................................................180

Table 7.3 F values for the effect of physiological state, its interaction with time of sample, breeding values for weight (HWT), subcutaneous fat depth (HFAT) and eye muscle depth (HMD) at hogget age on ewe plasma concentrations of albumin, urea nitrogen (urea N), glucose, non-esterified fatty acids (NEFA) and lactate. ..............180

Table 7.4 Mean ± s.e. of plasma concentrations of growth hormone, insulin like growth-factor-I (IGF-I), insulin and leptin across a physiological states.................................185
Table 7.5 F values for the effect of physiological state, its interaction with time of sample, breeding values for weight (HWT), subcutaneous fat depth (HFAT), eye muscle depth (HEMD) at hogget age, on ewe plasma concentrations of growth hormone, insulin-like growth factor-1 (IGF-I), insulin, and leptin. 

Table 8.1 Concentrations of glucose, albumin, urea nitrogen, non-esterified fatty acids (NEFA) and lactate in lamb plasma collected 1 hour and 24 hours post birth.

Table 9.1 F values for the effect of physiological state, muscle group, ewe Australian sheep breeding values (ASBVs) for sub-cutaneous fat depth (HFAT) and weight (HWT), adrenaline challenge and condition score and significant interactions on basal glucose concentration, maximum glucose concentration, time to maximum glucose concentration and area under curve of glucose response between 0 and 10 minutes relative to administering adrenaline (AUC10).

Table 9.2 F values for the effect of physiological state, muscle group, ewe Australian sheep breeding values (ASBVs) for subcutaneous fat depth (HFAT) and weight (HWT), adrenaline challenge and condition score and significant interactions on basal lactate concentration, maximum lactate concentration, time to maximum lactate concentration and area under curve of lactate response between 0 and 10 minutes relative to administering adrenaline (AUC10).

Table 9.3 Time to reach maximum NEFA concentration (minutes) following an adrenaline challenge in Merino ewes of high, medium and low muscling during pregnancy, lactation or non-breeding.

Table 9.4 F values for the effect of physiological state, muscle group, ewe Australian sheep breeding values (ASBVs) for subcutaneous fat depth (HFAT) and weight (HWT), adrenaline challenge and condition score and significant interactions on basal non-esterified fatty acid (NEFA) concentration, maximum NEFA concentration, time to maximum NEFA concentration and area under curve of NEFA response between 0 and 10 minutes relative to administering adrenaline (AUC10).

Table 10.1 Predicted means of steady state glucose infusion rate (SSGIR) at insulin infusion rates (IIR) of 0.6 and 6.0mU/kg.min, basal blood glucose, liveweight, adjusted liveweight (adjusted for conceptus and wool weights where appropriate) and condition score across three physiological states. Average standard error of means across states are presented.

Table 10.2 F values and regression coefficients (±s.e.) for the effect of physiological state, muscle group, insulin infusion rate, hogget weight (HWT) and fat depth (HFAT), Australian sheep breeding values (ASBV) and condition score (CS) and liveweight (LW) on steady state glucose infusion rate (SSGIR; 50% glucose solution) and basal glucose.
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC10</td>
<td>Area under the concentration curve between 0 and 10 minutes relative to administering adrenaline</td>
</tr>
<tr>
<td>ASBV</td>
<td>Australian Sheep Breeding Value</td>
</tr>
<tr>
<td>CS</td>
<td>Condition Score</td>
</tr>
<tr>
<td>CW</td>
<td>Carcase Weight</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual-energy X-ray absorptiometry</td>
</tr>
<tr>
<td>DXA fat</td>
<td>Total fat tissue mass measured by dual-energy X-ray absorptiometry</td>
</tr>
<tr>
<td>DXA lean</td>
<td>Total lean tissue mass measured by dual-energy X-ray absorptiometry</td>
</tr>
<tr>
<td>DXA bone</td>
<td>Total bone mineral mass measured by dual-energy X-ray absorptiometry</td>
</tr>
<tr>
<td>EBV</td>
<td>Estimated Breeding Value</td>
</tr>
<tr>
<td>EMD</td>
<td>Eye Muscle Depth - The depth of the <em>m. longissimus lumborum</em> muscle at the C-site, defined as a point between the 12th and 13th ribs and 45mm from the dorsal midline</td>
</tr>
<tr>
<td>FAT</td>
<td>Subcutaneous fat depth at the C-site, defined as a point between the 12th and 13th ribs and 45mm from the dorsal midline</td>
</tr>
<tr>
<td>HWT</td>
<td>ASBV for weight at hogget age (15 months old)</td>
</tr>
<tr>
<td>GLUT1</td>
<td>Glucose transporter-1</td>
</tr>
<tr>
<td>GLUT4</td>
<td>Glucose transporter-4</td>
</tr>
<tr>
<td>HEMD</td>
<td>ASBV for eye muscle depth at hogget age (15 months old)</td>
</tr>
<tr>
<td>HFAT</td>
<td>ASBV for C-site fat depth at hogget age (15 months old)</td>
</tr>
<tr>
<td>HCFW</td>
<td>ASBV for clean fleece weight at hogget age (15 months old)</td>
</tr>
<tr>
<td>HFD</td>
<td>ASBV for mean fibre diameter at hogget age (15 months old)</td>
</tr>
<tr>
<td>HFDCV</td>
<td>ASBV for the coefficient of variation of fibre diameter at hogget age (15 months old)</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Insulin-like growth factor -I</td>
</tr>
<tr>
<td>IIR</td>
<td>Insulin infusion rate</td>
</tr>
<tr>
<td>LW</td>
<td>Liveweight</td>
</tr>
<tr>
<td>NEFA</td>
<td>Non-esterified fatty acid</td>
</tr>
<tr>
<td>SSGIR</td>
<td>Steady-state glucose infusion rate</td>
</tr>
<tr>
<td>Urea N</td>
<td>Plasma urea nitrogen concentration</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile fatty acid</td>
</tr>
</tbody>
</table>
Chapter 1 General Introduction

The Merino sheep forms the foundation of the Australian sheep industry. Through selective breeding for over two centuries, Australian pioneers developed the Merino to be the world’s premier wool-producing breed. While wool production has been the focus, the genetics of the Merino have also accounted for almost three quarters of animals slaughtered for meat production. There is now considerable interest in improving the meat production characteristics of the Australian Merino because in Merino flocks the income from meat is now equal to or above that from wool. This shift in focus toward meat production in Merino flocks has resulted in a desire to improve growth rates and increase the level of muscling in Merino selection programs to improve carcass value. In addition, reproduction and maternal performance have also become important because in systems with a significant focus on meat production, reproductive and maternal traits are key drivers of profitability (Ercanbrack and Knight 1998). Fortunately there is considerable scope to improve growth, muscling, reproduction and maternal traits because they have a high level of variation within the Australian Merino population due to previously low selection pressure (Clarke et al. 2003). However the heritability of these traits is mixed: growth and carcass traits have medium to high heritability in the Merino allowing rapid genetic gain; yet reproductive traits display low heritability and genetic gain in these traits will be considerably slower (Fogarty et al. 2003; Greeff et al. 2003; see Safari et al. 2005) but steady gain is still possible (Cloete et al. 2003). Importantly concurrent genetic gain in all of these traits can be achieved using a well-designed breeding program and the
successful implementation of such a program will result in an Australian Merino sheep that is more profitable under current and future price scenarios for meat and wool products.

Considering the low heritability of reproductive traits and their importance for profitability of meat-focussed production systems, it is imperative that other production traits under selection do not have a negative influence on reproductive performance. Two major traits of interest in meat animals are total carcass lean tissue and the proportion of carcass fat. Breeding programs that focus on these traits by selecting to maximise growth and carcass muscling and reduce carcass fat will change body composition. In addition, in Merino sheep lower fatness is a result of decades of selection for increased clean fleece weight due to a negative correlation between fleece weight and fatness (Adams et al. 2005; Adams et al. 2006a; Refshauge et al. 2006b; Fogarty et al. 2003). Changes to body composition may be expected to alter the reproductive performance of breeding ewes since total amounts of muscle and fat tissue are linked to reproduction (Lambe et al. 2005). Permanent changes to the body composition of Merino ewes may change their ability to conceive, their likelihood of producing multiple lambs, their milk production and their ability to recover reserves after lactation. In addition, selection for carcass fat and muscling and growth traits is completed using measurements taken at a young age and it is not known how this early selection correlates with their performance later in life as breeding ewes. It is also unknown how genetic propensity for carcass and growth traits interacts with nutrition in terms of maternal performance. In order to design selection programs for Merinos
that will result in improvements in profit it is important to understand the effects of selection for growth, muscling and leanness on maternal performance and to develop knowledge of the associated biology and the influence of the production environment.

Almost all Merino ewes in Australia are managed under extensive conditions where there is an annual cycle of supra-maintenance and sub-maintenance nutrition because of highly seasonal rainfall and therefore pasture growth. There is likely to be a biological limit to production in this variable environment due to the inevitable association between production and fitness and the effects of selection on the relative partitioning of available energy to the production of meat, wool, milk, increasing body fat reserves or to a growing foetus. It is therefore likely that Merino breeding programs that breed toward animals with improved productivity in terms of wool production, carcass muscling and growth rate will encounter issues associated with reduced fitness. Early determination of the effects at a mechanistic level of selection for the above traits on the whole body energy metabolism will allow refined definition of breeding objectives and highlight expected nutritional requirements of high performing animals. Therefore the following review will focus on the maintenance of energy balance in ruminants and the impact of reproduction on these processes. It will also discuss how particular sheep selection strategies may impact on energy balance and the known or likely related impacts on reproduction. It should be noted that protein balance will largely be ignored.
Chapter 2 Literature Review

To design sheep breeding programs that are sustainable for the long term it is important to understand the biological implications of selection strategies that are economically desirable. This review aims to identify known implications and determine the gaps in current knowledge. First of all, this review will focus on the energy metabolism of ruminants and particularly on the storage and use of energy in the form of fat and glycogen. Secondly, this review will discuss a number of hormonal axes that act to mobilise or store energy and thereby maintain energy homeostasis and thirdly the homeorhetic processes that are invoked when animals are pregnant or lactating will be considered. Fourthly, the strategies that can be used to measure and change body composition and growth in the Australian sheep industry will be discussed. And finally, the impact of these and other selection strategies as well as nutrition on body composition and reproductive performance will be discussed. Where possible this review will focus on Merino sheep since this thesis focuses particularly on that breed.

2.1 Ruminant energy metabolism

2.1.1. Digestion of carbohydrate

The digestive process of ruminants involves fermentation of feed stuffs by many billions of micro-organisms resident in the rumen. Due to this process the end products of digestion passing into the blood stream are different than those seen in
monogastric animals. Typically greater than 90% of carbohydrate in feedstuffs is fermented to volatile fatty acids (VFA) for most diets (Leng 1970; Bergman 1973; Armstrong 1993). The VFA make up 50-80% of the metabolisable energy intake in ruminants and are absorbed into the blood stream from the reticulo-rumen and omasum (Annison and Armstrong 1970). While the VFA are directly used by many cells, glucose is required for: the brain and central nervous system; as a precursor to muscle glycogen; for fat turnover and synthesis; and in large quantities for pregnant or lactating animals (Bergman 1973). Due to the importance of glucose, its concentration in blood and the pathways involved in its metabolism are under tight homeostatic control. Unlike other mammals, ruminants must endogenously synthesise the majority of their glucose requirements. They are therefore heavily reliant on hepatic and renal gluconeogenesis, predominantly from propionate, but also from lactate, glycerol and amino acids, to meet their glucose needs (Katz and Bergman 1969; Leng 1970; Lindsay 1970). Gluconeogenesis has a higher importance in ruminant than in non-ruminant animals since forage-fed ruminants absorb only negligible amounts of glucose from the digestive tract and must synthesise the large majority of their glucose requirements.

2.1.2. Gluconeogenesis

The ability to synthesise glucose is common to all mammals however it is of particular importance to ruminants. Gluconeogenesis occurs predominantly in the liver; however around 15-30% of glucose appearance is released from gluconeogenesis in the kidneys in pregnant and lactating sheep (Bergman et al. 1974; van der Walt et al. 1983). The rate
of gluconeogenesis in the liver is largely controlled by the supply of gluconeogenic substrate and an increase in substrate supply results in a higher rate of gluconeogenesis (Bergman et al. 1970; Lindsay 1978; Jenssen et al. 1990; Chu et al. 1997; Müller et al. 1997; Gustavson et al. 2003). Elevation of gluconeogenic substrate levels also results in an increase in kidney glucose output (Stumvoll et al. 1997; Meyer et al. 2003). The close link between gluconeogenic substrate supply and gluconeogenesis is displayed by the linear correlation between energy intake and the rate of gluconeogenesis in ruminants (Schmidt and Keith 1983). Altered physiological state can also change the rate of gluconeogenesis and pregnant ewes carrying twin fetuses have a glucose production rate that is 42% higher than non-breeding ewes fed the same amount (Wilson et al. 1983). The reliance on VFA as an energy source and gluconeogenesis for all glucose requirements results in ruminants having a unique energy metabolism.

Changes to body composition can also change the rate of gluconeogenesis. Humans that are obese have two fold higher rates of gluconeogenesis and lower rates of glycogenolysis compared to lean humans (Müller et al. 1997; Buijs et al. 2004). The link between fatness and gluconeogenesis is possibly explained by elevated levels of circulating leptin in fatter individuals. Plasma leptin concentrations are generally positively correlated with the proportion of fat in the body in sheep (reviewed by Chilliard et al. 2005). Since leptin stimulates gluconeogenesis in the liver of rats, the liver’s capacity for gluconeogenesis is increased in animals with higher proportions of fat which may be through the actions of leptin (Nemecz et al. 1999; Frühbeck and
It is not clear whether leptin is causative in this relationship. Increased rates of gluconeogenesis as a result of obesity is of major consequence to humans it is unlikely to be of any significance in sheep however it may help to explain some observed physiological differences.

2.1.3. Storage of glucose as glycogen

Glycogen is the major storage form of carbohydrate in animals. The conversion of glucose to glycogen as an energy reserve occurs in most mammalian cells however, specialised glycogen storage occurs in the liver and skeletal muscle (Murray et al. 2000). In particular high amounts of glycogen are stored in muscles that have a higher oxidative metabolism (with more type I and type IIA myofibres; Pethick and Rowe 1996). Stores of glycogen in muscle and liver have different roles and different products of glycogenolysis (Murray et al. 2000). The glycogen stored in the liver is used as a reservoir of glucose for release into the blood to be used by other tissues when glycaemia is low as a result of fasting and the associated reduced rate of gluconeogenesis. During these times of glucose demand, glycogen is converted into glucose-1-phosphate which can form glucose-6-phosphate through a reversible reaction. The presence of glucose-6-phosphatase in the liver allows glucose-6-phosphate to be converted through to glucose and released into the blood.

The role of glycogen in skeletal muscle is different to the liver as it is thought to be less influenced by nutrition and more by the effects of stress or by the energy demands of
muscle (Murray et al. 2000). Glycogenolysis in muscle tissue also results in the release of glucose-6-phosphate however as there is a deficiency in glucose-6-phosphatase in muscle, glucose-6-phosphate goes through glycolysis and is converted to pyruvate (Leenanuruksa and McDowell 1985). Unless there are high energy demands, the excess pyruvate will be converted to lactate, released into the bloodstream and transported back to the liver for gluconeogenic re-conversion to glucose (Murray et al. 2000). The conversion of glycogen to lactate through the glycolysis pathway occurs in all mammalian cells and can occur in either aerobic or anaerobic conditions (Leenanuruksa and McDowell 1985). In muscle cells stress-induced adrenaline release activates glycogenolysis and glycolysis through the β-adrenergic receptors resulting in the release of second messenger cAMP which stimulates glycogenolysis and depresses glycogenesis thus mobilising muscle glycogen (Williams et al. 1984; Leenanuruksa and McDowell 1985; Murray et al. 2000). The size of the glycogenolytic response to stress is known to differ between sheep breeds and Merinos are known to be more likely to mobilise muscle glycogen in response to stress than breeds such as the Poll Dorset (Gardner et al. 1999). Merinos also have lower myoglobin concentrations and citrate synthase activity in muscle suggesting a greater dominance of glycolytic metabolism compared to cross-bred lambs (Gardner et al. 1999). Glycogen storage and release is a fundamental component of the ability of mammals to store excess glucose in the fed state and mobilise glucose when required either through direct release from the liver or through increasing hepatic gluconeogenic substrate supply as a result of lactate release from muscles.
All animals maintain a careful balance of energy intake, expenditure, storage and mobilisation to be able to meet their metabolic requirements throughout the production cycle. The deposition and mobilisation of fat is the key mechanism by which animals maintain this balance and it enables them to cope with adjacent periods of supra- and sub-maintenance nutritional conditions. Fat is stored in the form of triacylglycerol which contains less than 15% water and therefore meets the requirements of an energy store of being light and energy dense (Vernon and Houseknecht 2000). Triacylglycerol is formed from the esterification of glycerol formed from glucose with non-esterified fatty acids (NEFA) either sourced from the diet or synthesised de novo predominantly from acetate (Pethick et al. 1981; Pethick et al. 2005). While triacylglycerol can form in the cytosol of all cells the majority of storage occurs in specialised fat cells known as adipocytes which make up adipose tissue (Pond 1992). Growth of adipose tissue involves both hyperplasia (increase in adipose cell number) and hypertrophy (increase in adipose cell size). The periods of hyperplasia are generally confined to pre-natal and early post-natal stages but can be still occurring up until 11 months of age in sheep (Hood 1982). In ruminants hypertrophic growth of adipose tissue is the form of adiposity displayed when animals are fattened (Hood 1982). Adipose tissue predominantly has glucose transporter 4 (GLUT4) as the mechanism of glucose transport into the cells. Since transport by GLUT4 is regulated by insulin, glucose transport into fat cells is regulated by insulin. Once transported into the adipocyte, glucose is then converted to glycerol to become
the backbone of a triacylglycerol molecule. While glucose is an important precursor for fatty acid synthesis in most mammals, acetate is the most important fatty acid precursor in ruminants due to their reliance on VFAs for energy metabolism and the need to spare glucose. In ruminants, adipose tissue is the major site of fatty acid synthesis which is different from most other species where the liver synthesises most fatty acids (Vernon 1980). Due to the reduced reliance on glucose, ruminant adipose tissue is less responsive to insulin than tissue from other species (Hood 1982). The storage of energy in the light and compact form of triacylglycerol in sheep enables them to handle variation in nutrition throughout a breeding cycle and importantly allows them to store energy for use during late pregnancy and lactation.

2.1.5. Fat depots

The deposition of adipose tissue occurs in specific depots that are common for all mammals (Pond 1992). The main depots of fat in ruminants are subcutaneous, intermuscular, intramuscular, and abdominal (made up of omental, perirenal and mesenteric depots). The proportion of fat in these depots, both as a proportion of the total body weight or of the weight of the total fat depot, changes with the age and bodyweight of the animal (Pethick and Dunshea 1996). In the growing animal, adipose tissue depots develop in a defined order of abdominal, intermuscular, subcutaneous and ultimately intramuscular (Haugebak et al. 1974). The abdominal depot is the largest containing around one third of total body fat followed by the subcutaneous depot with around one quarter in Merino sheep of mature size (Thompson et al. 1987).
However, the relative size of fat depots can change depending on breed. In contrast to Merino sheep, the subcutaneous depot is larger than the abdominal depot in Scottish Blackface sheep (Lambe et al. 2003). Clearly then fat deposition has a genetic component can be altered by selection in a particular direction.

The relative size of the fat depots in different breeds or lines seems dependent on previous selection strategies. In cattle it has been shown that animals selected for muscling and draft usage tend to deposit more fat in intermuscular depots; animals selected for milk production deposit fat in the internal depots of intermuscular, perirenal and abdominal; and those selected for early maturity and meat production deposit more fat in the subcutaneous depot (Shahin and Berg 1985). Different fat depots have different lability with abdominal fat depots being the most labile in sheep, followed by the subcutaneous depot. These two depots are preferentially mobilised over inter-muscular fat (Lambe et al. 2003) and are therefore the most important in terms of animals being able to cope with short to medium term nutritional restriction. The amount of fat in the subcutaneous depot is well correlated with the amount of fat in the internal fat depot (Kirton and Johnson 1979) so selection for reduced subcutaneous fatness is also likely to result in reduced amount of internal fat. The subcutaneous depot remains the most important depot in sheep as it is both important for the wellbeing of the sheep and for the retail value of the sheep meat.

2.1.6. Fat mobilisation
Under times of nutritional, metabolic or psychological stress fat stores are mobilised to provide energy for fundamental functions (Dunshea et al. 1988; Boisclair et al. 1997; Pethick et al. 2005). In response to under-nutrition or stress fat tissue is mobilised by the lipolysis of triacylglycerol into its component glycerol and NEFA molecules in a reaction catalysed by hormone sensitive lipase (Langfort et al. 1999). However, not all of the NEFA mobilised from lipolysis is released into the blood. Some of this NEFA is re-esterified into triacylglycerol molecules without being released (Dunshea et al. 1990). Therefore the changes in plasma concentrations of NEFA do not represent the total rate of lipolysis however the two are closely correlated (Dunshea et al. 1990). The rate of re-esterification is proportional to the animal’s energy balance. Animals in negative energy balance have lower rates of re-esterification (Dunshea et al. 1990). Ultimately the rate of release of NEFA’s is lower in periods of positive energy balance as a result of both lower rates of lipolysis and higher rates of re-esterification.

The release of NEFA into the blood from stored triacylglycerol provides a readily accessible form of energy. Some NEFA is directly oxidised in various tissues in the sheep (Leat and Ford 1966; Pethick et al. 1983) while some undergoes partial oxidation in the liver to form ketones which can be readily oxidised by extra-hepatic tissues (Pethick et al. 2005). The oxidation of NEFA and ketones by tissues throughout the body results in the sparing of glucose for tissues that have an absolute requirement for glucose such as the central nervous system, uterus, or mammary gland (Pethick et al. 2005). As well as the effects of under-nutrition, the mobilisation of fat and release of NEFA is very sensitive to short term stresses or excitement which stimulate the
adrenergic axis and the excitement of feeding or handling can rapidly elevate NEFA levels (Pethick and Dunshea 1996; Boisclair et al. 1997). Controlled doses of adrenaline demonstrate that the lipolytic response to adrenaline is rapid but also very short lived (Boisclair et al. 1997). Lipolysis results in the rapid mobilisation of stored energy whether as a result of fasting or stimulation of the adrenergic axis and allows glucose to be spared for higher priority uses.

2.1.7. Muscle energy metabolism

Muscle is the major consumer of glucose in the body and glucose makes up more than 50% of the substrates used by muscle at rest, while muscle cells also derive energy from ketone bodies, fatty acids and triacylglycerol (Pethick and Vernau 1984). There are two routes of glucose use in muscles, oxidative and glycolytic metabolism. Oxidative metabolism results in the complete oxidisation of glucose to carbon dioxide and water and by definition requires aerobic conditions. Due to the requirement for oxygen, muscle fibres which are predominantly oxidative have higher proportions of myoglobin which is a protein that stores and transfers oxygen. The myoglobin proportion gives meat its characteristic red colour, and oxidative muscles are redder than muscles that are predominantly glycolytic which are whiter. Glycolytic metabolism results in glucose going through glycolysis and being converted to pyruvate, ultimately resulting in the release of lactate from muscle cells a process which occurs under anaerobic conditions. Muscle fibres which are predominantly glycolytic have a paler appearance due to the lower concentration of myoglobin.
Muscle appearance and metabolism are altered profoundly by changes in the proportions of muscle fibre types present.

Muscle fibres are generally classified as type I, type IIA and type IIX in ruminants as well as some intermediate types that won’t be discussed here (Reggiani and Mascarello 2004). These three muscle fibre types are classified based on their contractile properties and dominant metabolic pathway. Firstly, Type I muscle fibres have medium levels of muscle glycogen and are dependent on oxidative metabolism and are known as slow-twitch oxidative. Secondly, type IIA fibres have high levels of glycogen and have both oxidative and glycolytic metabolism occurring and are known as fast-twitch oxidative glycolytic. Lastly, type IIX fibres have low levels of muscle glycogen and are primarily dependent on glycolytic metabolism and are known as fast-twitch glycolytic (Pethick and Rowe 1996; Brandsetter et al. 1998). Within the fibre type groups there is also a range in the proportion of oxidative and glycolytic metabolism that occurs (Gardner et al. 2006; Greenwood et al. 2006). This can alter the storage and mobilisation of glycogen since predominantly glycolytic muscles have lower amounts of glycogen but they are more susceptible to stress induced mobilisation of glycogen (Pethick and Rowe 1996). The predominant mode of metabolism in a muscle fibre alters both glucose use and glucose uptake.

Muscle cell glucose uptake is completed by two specific transport proteins, glucose transporter 1 (GLUT1) and glucose transporter 4 (GLUT4) (Dühlmeier et al. 2005). Glucose transport by GLUT1 is largely independent of insulin and provides a
mechanism for basal glucose supply to the muscle cell. The transport of glucose by GLUT4 is regulated by insulin and the concentration of GLUT4 in muscles is closely associated with the sensitivity to insulin of those muscles (Megeney et al. 1993; Dühlmeier et al. 2005). The concentration of GLUT4 is associated with the metabolic properties of the muscle, Dühlmeier et al. (2005) reported that the content of GLUT4 is three times higher in oxidative muscles than glycolytic muscles in cows and pigs. Similarly, Goodyear et al. (1991) reported that the content of GLUT4 is higher in oxidative muscles than glycolytic muscles in rats. Furthermore, Duehlmeier et al. (2007) showed GLUT4 levels 1.5 to 6.3 times higher in oxidative rather than glycolytic muscles across a range of ruminant and monogastric species. However, in direct contrast, the results of Hocquette et al. (1995) suggest that GLUT4 expression is higher in glycolytic muscles in ruminants. It is unclear as to why this discrepancy exists however it seems likely that a higher predominance of oxidative metabolism will be associated with higher GLUT4 expression and therefore higher sensitivity to insulin.

2.2. Hormone regulation of growth and body composition

2.2.1. Growth Hormone

Growth hormone is produced by the pituitary gland and has important roles in partitioning of nutrients between functions and is therefore thought to provide a homeorhetic signal to partition nutrients to the dominant function (Bauman et al. 1982; Bauman 2000). Growth hormone’s effects on fatness, growth rate and milk production are important for animal production. Growth hormone is also important during
periods of under-nutrition and circulating growth hormone concentrations rise under times of low feed availability (Adams et al. 1996a). An important adaptation to under-nutrition is also the un-coupling of the GH – IGF-I axis allowing for both reduced lean growth and a simultaneous increase in fat mobilisation (McGuire et al. 1995). A lot of the information known about the actions of growth hormone has been discovered by exogenous treatment of animals. Administration of exogenous growth hormone results in higher deposition of lean tissue and lower deposition of fat tissue across a range of species including ruminants (Bauman et al. 1982; Pell et al. 1990). Furthermore, exogenous growth hormone treatment also results in higher, and more feed efficient, liveweight gain across species (Etherton and Kensinger 1984). Similar to results from exogenous treatment, transgenic sheep that had high growth hormone concentrations as a result of an extra growth hormone gene, grew faster, had less fat, heavier bones, similar muscle weights and heavier internal organs than non-transgenic equivalents (Adams et al. 2002b; Adams et al. 2006b). In addition, sheep that had lower concentrations of growth hormone as a result of being immunised against growth hormone releasing hormone, grew slower, were fatter and had lighter organs than non-immunised controls (Adams et al. 1996b). The most consistent effect of higher circulating levels of growth hormone, either as a result of exogenous supply or endogenous release, is the increase in the rate of lipolysis.

Growth hormone is associated with the net tissue mobilisation that occurs during lactation in ruminants and plasma concentrations of growth hormone are known to be higher in lactation than in the non-breeding state (Hart et al. 1978; Hatfield et al. 1999).
Furthermore, Hart *et al.* (1978) reported that high yielding dairy cows have greater circulating levels of growth hormone during lactation than low yielding equivalents. In addition, treating lactating dairy cows with exogenous growth hormone not only increases milk production but induces a state of insulin resistance in peripheral tissues, partly explaining the catabolic effect of growth hormone (Sechen *et al.* 1990). In support, treatment of non-breeding sheep and cattle with exogenous growth hormone decreases the response to insulin in peripheral tissues and reduces the ability of insulin to reduce gluconeogenesis (Dunshea *et al.* 1995; Rose and Obara 1996). Selection for increased growth and milk production by selecting for higher total weight of lamb weaned resulted in an increase in plasma growth hormone concentration in lactating ewes (Head *et al.* 1996). In addition selection for increase rate and efficiency of liveweight gain in Targhee sheep resulted in an increase in circulating growth hormone concentrations (Dodson *et al.* 1983). Selection for changes in body composition has also changed the secretion of growth hormone. Selecting sheep for lower fatness resulted in a higher growth hormone concentration in two separate selection experiments (Carter *et al.* 1989; Francis *et al.* 1997, 1999). Furthermore, single trait selection for increased fatness resulted in a large reduction in mature size in sheep which is further indirect evidence of a reduction in growth hormone concentration associated with selection for increased fat (Morris *et al.* 1997). The nutrient partitioning effects of growth hormone are clearly evidenced through both its effects during pregnancy and the correlated increase in growth hormone concentration as a result of selection for increased gain of lean tissue. Growth hormone is associated with lipolysis.
not through its direct actions but via changing the lipolytic sensitivity of adipose to the homeostatic hormones insulin and the catecholamines.

2.2.2. Insulin-like growth factor I

One of the many roles of growth hormone in energy metabolism is to stimulate the synthesis in the liver of the hormone Insulin-like growth factor I (IGF-I) (Breier et al. 1986). IGF-I is important for the regulation of both energy and protein metabolism. Infusion of IGF-I results in lower protein degradation, higher protein gain and also higher glucose uptake in the hind-limb of sheep (Oddy and Owens 1996). IGF-I is therefore associated with the anabolic state. Plasma IGF-I was found to be higher in sheep selected for higher weaning weight compared to those selected for lower weaning weight (Oddy 1993). Furthermore, circulating IGF-I concentrations were found to be positively correlated with growth rate in sheep (Carter et al. 1989; Hegarty et al. 2006b). However, there was no difference in IGF-I concentration in ewes or lambs from a line selected for increased total weight of lamb weaned that were also heavier than unselected controls (Head et al. 1996). Similarly, there was no difference in IGF-I concentration in lambs from lines selected over 10 generations for higher weaning weight compared with un-selected controls (Medrano and Bradford 1991). However, there was a positive correlation between IGF-I and body size at various ages (Medrano and Bradford 1991). Furthermore there is a negative genetic correlation between IGF-I and a variety of growth traits in cattle (Moore et al. 2005), but a positive correlation in
mice (Blair et al. 1990). The reasons for the differences between various studies are unknown but may be associated with differences in nutrition since IGF-I synthesis is known to be affected by nutrition (Breier et al. 1986). It is clear that IGF-I plays an important role in growth however selection for higher growth doesn’t consistently result in higher circulating levels of IGF-I.

Part of the difficulty of interpretation of the relationship between IGF-I levels and the expected change in the animal may be explained by the role of associated binding proteins. The release of Insulin-like Growth Factor Binding Protein -3 (IGFBP-3) is also stimulated by Growth Hormone resulting in higher circulating levels (Hodgkinson et al. 1991). IGFBP-3 is the major carrier of IGF-I and has an important role in modulating the actions of IGF-I on target tissues (Baxter 1993). In addition, when IGF-I is bound to IGFBP-3 the active life of IGF-I in plasma is extended by over fifty-fold (Davis et al. 1989). Considering the key role that the binding proteins have on the effects of IGF-I it is likely that differences in the presence of binding proteins would have a major impact on the measured relationships of IGF-I with other factors.

There has been considerable interest in IGF-I as a potential physiological marker for feed efficiency. Feed efficiency is difficult and expensive to measure and therefore IGF-I measurement is an attractive option as it can be measured on a single blood sample and is relatively inexpensive. IGF-I also has positive genetic correlation with ultrasound scan fat and a negative genetic correlation with scan muscle depth in beef cattle (Robinson and Oddy 2004; Moore et al. 2005). Furthermore, in juvenile pigs, IGF-
I is genetically positively correlated with back fat, feed intake and feed conversion ratio (Bunter et al. 2005). Selection based on IGF-I has been used in the beef industry (Stick et al. 1998; Moore et al. 2005) however its success has been limited. Similar to inconsistent changes in IGF-I associated with selection for growth attempts to use IGF-I has a physiological marker for efficient lean growth have not revealed a consistently positive result.

2.2.3. Insulin

Insulin is a key hormone associated with maintaining homeostasis and homeorhesis in all mammals. This includes ruminants even though they are less sensitive to insulin than monogastrics due to their lower reliance on glucose (Bauman and Currie 1980; Weekes et al. 1983; Pethick and Dunshea 1996). The general role of insulin is to stimulate the anabolic state and to lower blood glucose by inhibiting lipolysis, glycogenolysis, gluconeogenesis and proteolysis and stimulating lipogenesis, the uptake and use of glucose by peripheral tissues, and the uptake and assimilation of amino acids into proteins (Prior and Smith 1982). The main action of insulin is to maintain the concentration of glucose in the blood within the desired range and it is the only hormone that’s actions result in blood glucose being lowered. Muscle and fat tissue are the major insulin sensitive tissues yet account for less than 20% of total glucose use. Tissues with an obligate requirement for glucose such as the central nervous system take up glucose without stimulation by insulin and account for over 80% of glucose uptake and use (Weekes 1991; Weekes et al. 2000). Insulin is released
in response to higher blood glucose and it acts to facilitate uptake and utilisation of glucose in muscle and adipose tissue. The reduction in blood glucose in response to increased insulin concentration is also achieved by insulin acting to inhibit gluconeogenesis in the liver (Brockman 1983; Bergman et al. 1989) and enhance glycogenesis in muscle (Mandarino et al. 1987) and liver (Brockman 1983). The primary action of insulin on glucose uptake is the stimulation of glucose transport through the translocation of glucose transporter 4 (GLUT4) to the plasma membrane in insulin sensitive tissues (Weekes 1991). As well as its impacts on glucose, insulin also stimulates amino acid uptake into muscle cells and stimulates protein synthesis and inhibits protein breakdown (Goldberg et al. 1980). Within adipose tissue, insulin also stimulates lipogenesis, the oxidation of glucose, the uptake of fatty acids and also inhibits lipolysis (Baldwin et al. 1973; Prior and Smith 1982). Therefore, while the roles of insulin are varied, they are all associated with the net storage of energy and protein and inhibition of the mobilisation of energy reserves.

Insulin acts to stimulate the net anabolic state and changes to the relative sensitivity or responsiveness to insulin between tissues and depots can alter the partitioning of nutrients between them (Pethick and Dunshea 1996). The actions of insulin result from its binding to its specific cellular receptor followed by a cascade of post-receptor events. Possible mechanisms by which differential responsiveness of tissues to insulin is controlled are tissue or depot changes in receptor affinity for insulin or changes in the concentration of insulin receptors. In growing lambs receptor affinity for insulin varies between tissues. The insulin receptor concentration also differs between tissues.
with perirenal and subcutaneous adipose depots having higher receptor concentrations than muscle and liver (McGrattan et al. 2000). Due to the differing response to insulin between tissues and the net anabolic effect of insulin it is closely associated with growth and body composition. A clear demonstration of the association between insulin and growth is provided by Oddy (1993) who showed that sheep selected over several generations for increased weaning weight had greater muscle response to insulin than sheep selected for lower weaning weight. This difference was associated with greater insulin receptor density in the muscle of sheep selected for increased growth (Shutt et al. 1991). As in other species, overfatness in sheep can lead to lower sensitivity and responsiveness to insulin in peripheral tissues (McCann et al. 1987; Bergman et al. 1989; Francis and Bickerstaffe 1996). Changes to the response to insulin in specific tissues can have marked changes to nutrient partitioning between tissues.

2.2.4. Adrenaline

Stressful situations result in the stimulation of the adrenal medulla by the sympathetic nervous system which ultimately results in the release of adrenaline into the blood. Adrenaline exerts its effects on target tissues by binding to both α- and β-adrenergic receptors in cell membranes which stimulates a cascade of reactions. While adrenaline impacts on a range of organs in the body and on protein metabolism, this review will focus on the actions of adrenaline that result in the net mobilisation of energy stores (Leenanuruksa and McDowell 1985; Chapa et al. 1996). There are three main changes to energy metabolism as a result of an increase in the concentration of circulating
adrenaline. Firstly, adrenaline promotes lipolysis resulting in an increase in non-esterified fatty acid (NEFA) and glycerol concentrations in blood (Bassett 1970; Blum et al. 1982). Most of the NEFA released into the blood in response to adrenaline is released from lipolysis within adipose tissue where 95% of an animal’s fat is stored (Jocken and Blaak 2008). These lipolytic effects of adrenaline on adipose tissue are mediated by β-adrenergic receptors (Blum et al. 1982; Ferlay et al. 2001). Secondly, adrenaline stimulates glycogenolysis in muscles resulting in increases in the concentration of lactate in blood. Lactate is a gluconeogenic precursor and is preferentially used by the liver when abundant (Fattor et al. 2005). And thirdly, adrenaline stimulates an increase in blood glucose as a net result of the stimulation of glycogenolysis in the liver and gluconeogenesis in both liver and kidney (Bassett 1970). The immediate increase in glucose production from the liver is attributed to a direct stimulation of liver glycogenolysis, this glycogenolytic effect is short-lived and with time the glucose output from glycogenolysis is lower than that from gluconeogenesis (Chu et al. 1997). The increase in gluconeogenesis in response to adrenaline is thought to be mainly an indirect effect of increased gluconeogenic substrate supply (Jenssen et al. 1990; Chu et al. 1997). Increases in adrenaline concentrations also stimulate glucagon secretion which further promotes glycogenolysis and gluconeogenesis (Bassett 1971). Adrenaline actions result in the mobilisation of energy storages and an increase in the circulating fatty acids and glucose which can be readily oxidised.

The response to adrenaline can be modified by nutrition and physiological state. The lipolytic response to catecholamines in adipose tissue is increased when sheep are
in fasting or under low nutrition (Fröhli and Blum 1988; Chilliard et al. 2000). In addition, increases in maternal tissue sensitivity and responsiveness to adrenaline are partly responsible for the net catabolic states of late pregnancy and early lactation particularly in adipose tissue (Vernon and Finley 1985; Guesnet et al. 1987; McNamara 1988). The higher response of maternal tissues to adrenaline in lactation is at least partially a result of higher cellular density of β-adrenergic receptors (Jaster and Wegner 1981; Vernon et al. 1995). Adrenaline is a key hormone associated with the catabolic state and a relative change to the sensitivity to adrenaline is a key mechanism by which homeorhetic control over dominant processes is achieved. This review has focussed primarily on the measured impacts of exogenous supply of adrenaline. The considerably more important natural mediator of the processes described is the local release of noradrenaline from the sympathetic nervous system (Bray 1986). This system plays a key role in the metabolism of many tissues including fat, liver and muscle.

2.2.5. Leptin

Leptin is predominantly produced by adipocytes and has wide ranging effects on energy homeostasis. Its physiological functions include regulation of feed intake, body temperature and energy expenditure (Houseknecht et al. 1998). Leptin acts to increase tissue energy expenditure and fat lipolysis. It also acts to increase insulin sensitivity and glucose utilisation in muscles and increase fatty acid oxidation in muscle, liver, and fat (Chilliard et al. 2005). Not all of the roles are completely understood, however,
it is known to affect the reproductive axis in sheep (Adam et al. 2003). Since leptin is predominantly secreted by adipocytes, the concentration of leptin in the blood is closely correlated with the proportion of fat in the body, but when animals have less than 20% fat in the body the correlation between leptin and fat is not evident or is weaker (Chilliard et al. 2001; Chilliard et al. 2005; Delavaud et al. 2007). Since ewe liveweight and condition score are indicators of fatness they are also positively correlated with leptin concentration (McFadin et al. 2002). While leptin concentrations reflect long-term nutritional history through body fatness which can explain 35 to 50% of the variation, short-term changes to nutrition also change leptin concentrations and can explain 15-20% of the variation in leptin concentration (Chilliard et al. 2001; Chilliard et al. 2005; Delavaud et al. 2007). The roles of leptin in energy homeostasis are generally to inhibit energy storage and stimulate energy use.

2.3. Adaptation to pregnancy and lactation

2.3.1. Accumulation of maternal tissues during early pregnancy

Early pregnancy is normally associated with a period of storage of tissues especially fat in sheep as in most mammals (Vernon et al. 1981). Early pregnancy is characterised by enhanced whole body responsiveness to insulin which is the major reason that net storage and accumulation of adipose tissue occurs as demonstrated in rats by Ramos et al. (2003). Lipolysis is down regulated and lipogenesis is favoured over this period under normal nutritional conditions, the situation is reversed in late pregnancy and lactation when adipose stores are required to meet the increasing energy demands.
Animals are thought to be following a genetically predetermined level of tissue storage throughout pregnancy (Friggens 2003).

2.3.2. Mobilisation of maternal tissues during late pregnancy

Glucose is the primary limiting nutrient for fetal growth and around half of maternal glucose supply is directed toward the uterus in late pregnancy (Wilson et al. 1983; Bell 1995). This increased requirement for glucose must be met by enhanced gluconeogenesis since forage-fed ruminants absorb negligible amounts of glucose from the digestive tract (Bergman et al. 1970). Therefore late pregnancy poses a significant challenge to glucose metabolism in the ewe. The large demand for glucose from the gravid uterus requires considerable adaptation in glucose partitioning and utilisation and a systematic shift toward glucose sparing in maternal tissues (Bauman and Currie 1980). Sometime around day 100 of pregnancy, there is a switch from accumulation of fat to mobilisation of fat (Vernon et al. 1981). The change is at least partly a result of a decrease in circulating insulin levels and the number of insulin receptors (Vernon et al. 1981) resulting in reduced responsiveness and sensitivity to insulin in muscle, adipose and liver tissue (Leturque et al. 1987; Sano et al. 1991; Petterson et al. 1993; Petterson et al. 1994). This adaptation results in reduced glucose use and enhanced tissue mobilisation in peripheral tissues (Oddy et al. 1985; Hay et al. 1988) and increased rates of gluconeogenesis in the liver (Wilson et al. 1983). Since the sensitivity to insulin in adipose tissue is reduced, and sensitivity and responsiveness to adrenaline is also increased, late pregnancy results in higher lipolysis which is demonstrated by
increased concentrations of circulating NEFA and reduction in the amount of fat in depots (Guesnet et al. 1987; Bell 1995; Lambe et al. 2003; Pethick et al. 2005). These changes enable greater uptake of glucose by the gravid uterus as uterine uptake of glucose is not insulin dependent (Hay et al. 1984). Therefore the glucose requirements of the fetus in late pregnancy are met by a combination of lower glucose use in peripheral tissues, and a higher supply of gluconeogenic substrate. This increased supply is a result of both increased feed intake (often capped either by restricted space for the rumen or availability of pasture) and the mobilisation of maternal tissues allowing enhanced gluconeogenesis.

2.3.3. Mobilisation of maternal tissues during lactation

The ability to provide young with an easily digested and high-energy source of food regardless of current location or environmental conditions is thought to be the main evolutionary advantage of lactation (Friggens 2003). Lactation is characterised by a net catabolic state with fat storages that were built up in early pregnancy being mobilised to provide energy and substrate for milk production (Vernon et al. 1981; Joseph and Foot 1990). This mobilisation of fat reserves occurs irrespective of current nutritional conditions (Friggens 2003) suggesting there is an evolutionary disadvantage to maintaining body fat above a required level in lactation. The mobilisation is achieved through an increase in the responsiveness and sensitivity to adrenaline in adipose tissue in lactating compared with non-breeding animals (Vernon and Finley 1985; Guesnet et al. 1987; Vernon et al. 1995; Theilgaard et al. 2002). The lactating mammary
gland also has a large demand for fatty acids and the mobilisation of fat stores in early lactation helps to meet this demand (Pethick et al. 2005). The onset of lactation in the ewe results in a doubling of the total amount of glucose required in comparison to the pregnant state (Bergman and Hogue 1967). To meet this large demand for glucose there is (i) a large increase in food intake and total digestive tract size (including the liver) with an associated large increase in gluconeogenesis in the liver (Wilson et al. 1983); and (ii) glucose utilisation in other tissues is diminished through a reduction in the responsiveness to insulin in both muscle and adipose tissue initiated via growth hormone (Vernon and Taylor 1988; Faulkner and Pollock 1990; Vernon et al. 1990). There is also a reduced ability of insulin to lower hepatic gluconeogenesis in lactating ewes that are losing weight (Weekes 1991). These changes result in decreased glucose utilisation by these tissues, sparing glucose for preferential use by the mammary gland (Bergman and Hogue 1967). The onset of lactation requires a systematic shift of nutrient partitioning toward the new priority of milk production.

Body reserves account for around 30% of the energy required for milk production in the first month of lactation in high producing dairy cows (Bauman and Currie 1980). This mobilisation of energy has been somewhat encouraged because loss of body condition in early lactation is positively correlated with milk yield in dairy cows (Domecq et al. 1997b). Similarly, sheep also mobilise fat to meet the requirements of lactation and Merino and Corriedale ewes use around 1.5kg and 3.2kg of fat in the first five weeks of lactation for single bearing and twin bearing ewes respectively. This only meets around 10% of the ewes required energy for lactation and maintenance (Joseph
and Foot 1990) but these breeds are not prolific milk producers. The degree of energy mobilisation in lactation is both driven by energy intake and genetic drive to produce milk (Friggens et al. 2004). On a matched ration high milk producing goats lost 19% of initial weight while lower milk producing goats only lost 7% of initial weight in the first 10 weeks of lactation (Cronjé et al. 2000). Furthermore, Brand and Franck (2000) showed that differences in milk production and lamb growth rate between Merino breeds were at least partly attributable to the greater mobilisation of tissues in lactation resulting in greater liveweight loss. There are also changes to protein metabolism in lactation with fractional synthesis rate of protein in muscle of lactating ewes being considerably lower than pregnant or dry ewes (Liu et al. 1999), actively freeing up amino acids for use by the mammary gland. Lactation results in the mobilisation of tissue, in particular fat tissue, the degree of mobilisation depends on a range of nutritional and genetic factors.

2.3.4. Re-building maternal tissues in late lactation and post weaning.

Considering the positive correlation of ewe condition at mating on the number of lambs conceived it is important for ewes to re-build maternal tissues in the interval between lamb weaning and the following mating. This re-building process starts in late lactation when the intake of pasture by the lamb has increased and correspondingly lamb milk intake and ewe milk production have declined (Langlands 1977). This accretion of maternal tissues will be more rapid after the lambs are weaned (Ferguson et al. 2004b). Depending on nutrition during lactation and available feed in
the interval between weaning and mating ewes may not recover liveweight by the following mating (Ferguson et al. 2004b). For example, Waters et al. (2000) showed the effects on liveweight and condition score of previous reproduction lasts for up to 10 months and that recovery from liveweight and condition loss during lactation is unlikely to have occurred prior to the next breeding season. Furthermore, Lambe et al. (2004) reported that ewes that reared twins had less carcass fat in the following year compared with ewes that either reared a single lamb or didn’t rear a lamb. Genetic differences in a ewe’s ability to re-build maternal tissues prior to the next mating may be important for ewe performance in an extensive production system.

2.4. Measuring and breeding for changes to growth and body composition in sheep

Body composition in sheep is important for two reasons. Firstly, the proportion of fat in the body of breeding ewes is important to cope with times of nutritional stress and is also important for reproductive success. Secondly, the fat content in lamb carcasses is very important for their marketability and processing efficiency and therefore carcass fatness has an intermediate optimum. Both too little and too much fat in the carcass results in consumer disapproval based on poor eating quality (too lean) or health concerns of excessive fat. It is suggested that the optimum amount of fat in a carcass is when an animals is between 70 and 75% of its mature weight (Bradford 2002). The fat proportion in sheep and lamb carcasss is assessed and selected for in a variety of ways.
2.4.1. Measuring body composition using dual-energy x-ray absorptiometry

Measurement of body composition in sheep has traditionally been done by manual dissection or by mincing the entire body and measuring its components chemically (Little and Sandland 1975; Thompson et al. 1985; Aziz et al. 1992; Ryan et al. 1993). These methods are labour intensive, expensive, and require the animal to be slaughtered. However, total body fat can be relatively simply and cheaply estimated by dual-energy x-ray absorptiometry (DXA) which can be used to either scan live anaesthetised animals or carcasses (Kelly et al. 1998; Pearce et al. 2009). The DXA uses two X-ray beams of different energy levels to estimate the body composition. It works on the theory that when X-ray beams pass through material the beam is attenuated in a way that is proportional to the composition of the material (Lukaski 1993). Bone, fat and lean tissue all result in a different attenuation (reduction in intensity) and these differences can therefore be used to predict the body composition of the animal under test (Lukaski 1993).

The DXA method has been validated against chemical fat data in live pigs and pig carcasses and has high accuracy (Suster et al. 2003). DXA has also been validated in sheep and is more accurate when predicting the total fat content of sheep carcasses than live sheep (Pearce et al. 2009). Part of this reduction in accuracy is likely to be due to loss of internal fat at slaughter which has not been accounted for. DXA provides a useful research tool to estimate body composition of live sheep, further work is required for its validation in sheep where internal fat depots are also accounted for.
2.4.2. Condition scoring

Condition scoring of sheep is the subjective assessment of the loin, backbone and lumbar processes to determine the fat and muscle tissue that is present using a scale of 1 to 5 (Jefferies 1961). The condition score of sheep can be assessed with good repeatability (van Burgel et al. 2004). Condition score is used to estimate the proportion of fat in the body of a sheep and therefore its nutritional status. Condition score is well correlated with ewe body composition and a more accurate indicator of the proportion of fat in the body than ewe liveweight (Russel et al. 1969; Bocquier et al. 1999). As well as correlating with the total amount of fat, ewe condition score is also strongly correlated to the amount of muscle tissue in breeding Merino ewes (Yates and Gleeson 1975). Condition score is used as a fast and reliable assessment of ewe fatness.

2.4.3. GR tissue measurement for carcass grading

The GR site is defined as the depth of tissue over the 12th rib at a point 11cm from the mid-line of the carcass (Kirton and Johnson 1979). The GR site is commonly used to grade carcasses to determine suitability for particular markets. It is well correlated with the proportion and total amount of fat in the carcass particularly when carcass weight is taken into account (Kirton and Johnson 1979; Dunshea et al. 2007). GR tissue depth is also a good indicator of the total lean tissue in a carcass when carcass weight is accounted for (Dunshea et al. 2007). GR tissue depth is reliably used in the Australian
sheep industry to grade the suitability of carcasses to markets and therefore carcass price.

2.4.4. C-site muscle and fat depth

The depth of fat and muscle tissue at the C-site, defined as a point between the 12th and 13th ribs and 45mm from the dorsal midline, over the *m. longissimus lumborum* muscle (Pålsson 1939), is used in the Australian sheep industry to select animals for muscling and fatness. Importantly, C-site measurements can be routinely measured by ultrasound and are therefore very useful for animal selection purposes. Fat depth at the C-site either measured on the carcass or by ultrasound is well correlated with the proportion and total amount of fat in lambs (Kirton and Johnson 1979; Junkuszew and Ringdorfer 2005). The depth of muscle at the C-site (known as eye muscle depth) is well correlated with overall muscle development in sheep and is used to select for improved lean meat yield (Hegarty *et al.* 2006a; Gardner *et al.* 2010). Selection of sheep based on C-site fat and muscle depths has proven to be a dependable means of changing the proportion of fat and muscle in sheep.

2.4.5. Australian Sheep Breeding Values

In order to achieve genetic change in the desired direction within the Australian industry it is necessary to have a practical and accurate genetic evaluation system in place. Sheep Genetics was formed to deliver across-flock genetic evaluation for the
Australian sheep industry. It has two main components, LAMBPLAN™ for terminal and maternal breeds and MERINOSELECT™ for Merino and Merino-based breeds. Sheep Genetics collates and analyses performance values, pedigree information and relevant environmental and management information from participating breeders. The analysis is completed using OVIS software and delivers Australian sheep breeding values (ASBVs) for a range of growth, carcass, wool, reproduction and parasite resistance traits (Brown et al. 2007). The OVIS software combines phenotypic information on the individual with phenotypic information on all known relatives. The software also uses common sires used between flocks to generate genetic linkage across flock. The software also makes standard allowance for known permanent environmental effects such as birth type and age of dam. All of this information is combined with the relevant genetic parameters and used to generate an across flock ASBV for all the traits the animal has been measured for. The Australian industry is able to use the ASBVs provided by Sheep Genetics to realise genetic gain and breed more profitable sheep.

In the Merino sheep industry there has recently been an increase in the emphasis on improving the carcass and reproductive traits due to a relative increase in prices for meat and lower prices for wool (Curtis and Dolling 2006). Carcass traits in the Merino have similar heritability and genetic variation as they do in other breeds and therefore substantial genetic gain is possible (Clarke et al. 2003). Three carcass traits often selected for improvement are growth, muscling and fatness and since Merinos tend to be slower growing than meat breeds, measurement and selection is usually done at
yearling or hogget age. Selection for growth is based on an ASBV calculated on weight at hogget age (HWT), while selection for muscling or fatness at hogget age are based on ASBVs calculated from ultrasound measurements of the depth of fat (HFAT) and muscle (HEMD) at the C-site. The ASBVs are calculated from both the measurements of the individuals and measurements of relatives and also measurements of correlated traits (Brown et al. 2007). Selection of Merinos based on these carcass ASBVs is likely to change the body composition of Merinos (Hegarty et al. 2006a) and at present there is little information about the impact this strategy may have on reproductive traits.

2.5. The impact of ewe nutrition and selection strategy on body composition and maternal traits.

Fitness is a central concept in animal breeding and genetics but can be defined in a number of ways. We define it here as ‘the ability of an animal to contribute to the next generation within a production environment where available nutrition fluctuates widely throughout the year’. It is likely that fitness will be enhanced in animals that carry adequate tissue reserves in the form of fat and protein to buffer periods of relative under-nutrition in the non-breeding, pregnant and lactating states resulting in more lambs in a ewe’s lifetime. There is evidence that selection for higher production can result in lower fitness if the higher production of the improved genotype is not matched by improvements in the available nutrition (Beilharz et al. 1993; Rauw et al. 1998; Knap 2005). For example Ercanbrack and Knight (1998) found that selection for higher lifetime reproductive performance (higher fitness under our definition) resulted
in correlated responses of lower wool production. Furthermore, Adams et al. (2007) showed that sheep with higher wool production had lower body reserves of nutrients when nutrition was limited, suggesting lower fitness in these high wool producing ewes. Therefore, it is important to understand the impact of particular breeding strategies on ewe body composition and its associations with fitness as reproductive performance can significantly impact on system profitability and the sustainability of breeding programs.

2.5.1. Ewe nutrition

Changes to body composition can impact on the ability of ewes to reproduce and successfully rear lambs. Changes to ewe nutrition and the associated changes in body composition can be used as a model for permanent changes to body composition achieved through animal selection. The inclusion of ewe nutrition here is for the purpose of comparison with genetic changes. The interaction between ewe nutrition and genetic potential is also of considerable importance. The influence of this interaction can make interpretation of results from both nutrition and selection experiments difficult.

2.5.1.1. Body composition

In growing animals, the proportion of fat in the body is determined mainly by the stage of maturity with some effects of genotype and nutrition (Bellof and Pallauf 2004;
Lewis et al. 2004; Lewis and Emmans 2007; Ponnampalam et al. 2007a). As the animals approach maturity the proportion of fat in the body is increased (Ponnampalam et al. 2007b). Once mature the relative proportions of fat and muscle in the body of ewes is largely a consequence of their short- and long-term ‘relative’ nutritional history during the dry, pregrnant and lactating periods, while genotype is also important (Little and Sandland 1975; Bocquier et al. 1998). The most significant changes in body composition in response to nutrition is the change in both the total amount and proportion of fat tissue in the body with up to 80% of this tissue being used in times of under-nutrition (Bocquier et al. 1998). Through the breeding season fat and muscle tissue of sheep follows an oscillating pattern of tissue accretion and mobilisation in line with energy requirements and available nutrition (Ball et al. 1996) and body composition is therefore in a constant state of change.

2.5.1.2. Fertility and fecundity

Reproduction is closely linked to both the current level of body reserves and the current rate of reserve mobilisation or accretion. During periods of un-favourable conditions mammals depress fertility and produce fewer young (Friggens 2003). However, it is difficult to un-ravel the joint negative effects of reduced body reserves and increased energy mobilisation rate on reproduction, however the combined effect is greater than each effect on its own (Wright et al. 1992). In sheep, liveweight is closely linked to nutrition and liveweight is often a good indicator of the total level of fat reserves (Russel et al. 1969). Correspondingly, liveweight is positively correlated with
ovulation rate in Merinos and other sheep breeds (Killeen 1967; Morley et al. 1978; Adalsteinsson 1979). A clear example of this effect is provided by Donnelly et al. (1982) who showed that decreasing ewe nutrition by increasing the stocking rate resulted in reduced ewe body weight and lower numbers of lambs born (Donnelly et al. 1982). In addition, weight loss around the time of mating has a negative impact on ewe and cow ovulation (Gunn and Maxwell 1989; Domecq et al. 1997a). In addition to liveweight, condition score is also a good indicator of fat reserves in sheep and cattle and condition score at mating is well correlated with subsequent ovulation and reproductive rate (Gunn et al. 1969; Gunn et al. 1972; Adalsteinsson 1979; Domecq et al. 1995; Pryce et al. 2001). Both short- and long-term nutrition interact to impact on fertility and fecundity in breeding ewes.

2.5.1.3. Lamb birthweight and survival

The perinatal mortality of lambs is both a considerable economic loss and a welfare issue for the Australian sheep industry. Most lamb losses occur within 48 hours of birth and are typically as a result of starvation, exposure, damage during parturition or predation, with prevailing weather significantly contributing to these predisposing factors (Donnelly 1984; Jordan and Le Feuvre 1989). Lamb mortality is closely related to lamb birthweight with both light and very heavy lambs having compromised survival (Fogarty et al. 1992; Fogarty and Hall 1995; Ferguson et al. 2004a). Ewe nutrition during pregnancy can have a large effect on lamb birthweight and on lamb survival particularly if nutrition is restricted in late pregnancy. In general increases in
ewe nutrition during pregnancy result in higher lamb birthweights, higher total body fatness and an improved likelihood of survival (Donnelly 1984; Holst et al. 1986; Jordan and Le Feuvre 1989; Jordan and Mayer 1989; Fogarty et al. 1992; Kelly et al. 1992; McNeill et al. 1997; Everett-Hincks et al. 2004; Ferguson et al. 2004a). Therefore, lambs born from ewes that have lower dry matter intake and lose weight during pregnancy have a lower chance of survival than those that are from ewes that have sufficient intake and either maintain or gain weight over pregnancy (Kelly 1992; Brand and Franck 2000). However feed restriction to ewes during early and mid pregnancy may have no effect on lamb birthweight or viability if nutrition in late pregnancy is above requirements (McClymont and Lambourne 1958; Fogarty et al. 1992; Holst et al. 1992; Holst and Allan 1992). Furthermore, ewes that have been well fed in early pregnancy and then underfed in late pregnancy are able to mobilise fat in late pregnancy and produce heavier lambs than ewes that are underfed in early pregnancy (McNeill et al. 1999). Similarly, ewe condition score at parturition which is an indicator of ewe nutrition throughout pregnancy is closely correlated with lamb survival (King et al. 1990). It is clear that nutritional restriction during pregnancy is likely to negatively impact on lamb birthweight and survival although the size of the impact depends on the timing and severity of the nutritional restriction and on subsequent nutrition.

2.5.1.4. Ewe milk production and lamb growth to weaning

Ewe milk production is known to be correlated with lamb growth rate (Snowder and Glimp 1991) and increasing the dietary energy intake of ewes is known to increase ewe
milk production and/or lamb growth to weaning (Arnold et al. 1977; Langlands 1977; Jordan and Mayer 1989; Brand and Franck 2000; Sormunen-Cristian and Jauhiainen 2001; Paganoni et al. 2004). There is also a strong genetic correlation between milk production and calf weaning weight in cattle (Meyer et al. 1994; MacNeil et al. 2006). In addition, long term nutrition can also impact on milk production. In dairy cows, milk production is positively correlated with condition score at calving which is a good indicator of previous nutrition (Domecq et al. 1997b). The same result is seen in sheep and nutrition during mid-pregnancy can impact on ewe milk production and lamb growth (Kelly et al. 1992; Paganoni et al. 2004). Ewe milk production and lamb growth are closely linked to both short- and long-term nutrition.

2.5.2. Selection for wool traits

In Merino production systems, wool remains an important co-product of meat. While it is not the focus of this thesis, it is important to understand some of the impacts of selection for wool traits on body composition and fitness especially considering the large emphasis that has historically been applied to the wool traits of the Merino.

2.5.2.1. Body composition

Merino breeding has traditionally focussed on improving the quality and quantity of wool grown. This selection strategy has inadvertently altered the body composition of
the sheep under selection. Merino sheep selected for increased staple strength had higher protein reserves and a lower proportion of body fat than equivalents selected for low staple strength (Adams et al. 1997). These differences are potentially due to differences in protein metabolism between these genotypes (Adams et al. 2000). Body composition is also changed by selection for higher fleeceweight. Ewes that had higher breeding values for fleeceweight had lower proportion of fat tissue and more lean tissue than equivalents with low breeding values for fleeceweight when fed on a poor quality diet (Adams et al. 2005; Adams et al. 2006a). Furthermore, ewes selected for high phenotypic fleece weight were in lower fat score at joining than low fleeceweight equivalents (Refshauge et al. 2006b). These results are supported by a negative genetic correlation that exists between fleece weight and fat at the GR site in Australian Merinos (Fogarty et al. 2003). This reduction in fatness associated with fleeceweight is potentially as a result of energy lost due to a higher whole-body protein turnover rate or reduced voluntary feed intake on low quality roughage in high fleeceweight sheep (Adams et al. 2002a; Adams et al. 2004). In addition, there is evidence that selection for lower fibre diameter results in increased fatness because it has been shown that superfine bloodlines produce fatter carcasses than medium and broad wool bloodlines (Hopkins et al. 2005a), however this is likely to be a direct result of differences in mature size between bloodlines rather than a correlation between fibre diameter and carcass fatness.
2.5.2.2. Fertility and fecundity

It is generally accepted that selection for increased wool production results in a reduction in reproductive performance. Increasing the proportion of fleece weight to body weight resulted in a reduction in the total weight of lamb weaned across three different breeds (Herselman et al. 1998). In addition, others have observed a negative genetic and/or phenotypic correlation between the number of lambs weaned and greasy fleece weight (Burfenning et al. 1989; Ercanbrack and Knight 1998; Bromley et al. 2001). Furthermore, Merino ewes with more skin wrinkle produced more wool but produced 17% less lambs as a result of lower fertility and lower lamb survival (Drinan and Dun 1965). Similarly, ewes selected for high phenotypic fleece weight weaned fewer lambs than low fleeceweight equivalents (Refshauge et al. 2006a). As well as the effects of fleeceweight, there is also a well-known association of reduced fibre diameter with a reduction in liveweight and reproductive performance in Merino sheep (see Adams and Cronje 2003). It is clear that selection for wool traits and in particular fleeceweight results in a reduction in the number of lambs weaned from Merino ewes.

2.5.2.3. Lamb birthweight and survival

In addition to changes to fertility and fecundity, selection for wool traits can also alter lamb survival. Lamb birthweight and survival were higher in a flock selected for improved staple strength in comparison to a flock selected for lower staple strength (Thompson et al. 2006). The increase in lamb survival was related to an improved
capacity of the neonate to switch its metabolism from fetal to milk substrates and not to changes in energy stored as fat or brown fat (Thompson et al. 2006). This improved capacity to switch metabolism results in lambs having increased levels of glucose and NEFA and reduced levels of blood urea nitrogen at birth and prior to suckling. This suggests these lambs are less reliant on energy from amino acid oxidation and more dependent on glucose and NEFA and therefore more able to adapt to milk substrates (Thompson et al. 2006). These results provide a clear example of how selection for a production trait can have a correlated impact on metabolic processes, in this example the impact was a positive one but that is not always the case.

2.5.2.4. Ewe milk production and lamb growth to weaning

There are generally positive genetic and phenotypic correlations between fleeceweight and liveweight and also between fibre diameter and liveweight (Safari et al. 2005). Selection for increased fleeceweight is therefore likely to increase weaning weight and mature liveweight while selection for lower fibre diameter will have the opposite effect.

2.5.3. Selection for higher growth

Selection for higher growth based on breeding values for weight at a defined age has been shown to be an effective means of increasing the rate of lamb growth (Hall et al. 2002). However the selection strategy can result in a range of associated changes.
2.5.3.1. Body composition

Higher growth rate is usually associated with leaner animals than lower growth animals of the same age. However, when these animals reach maturity and are on supra-maintenance feeding the opposite is true (Scholtz et al. 1990). Selection for increased growth rate ultimately results in selection for an increase in mature size and voluntary feed intake resulting in greater rates of fat deposition after the animal has reached mature size. Mice selected for growth alone showed a 60% increase in total body fat over an un-selected control. However, mice selected for increased protein accretion showed lower total body fat than the control (Nürnberg et al. 1998). To counter the negative effects of increased feed intake (mature mass and obesity) when selecting for growth, selection under a feed restricted environment allows gain in growth rate to be made without these negative effects and results in an improvement in feed efficiency (Scholtz et al. 1990). However, selection for high growth does not always result in higher fatness. In sheep, Thompson et al. (1987) showed that selection for higher weaning weight increased mature size and total amount of fat but did not change the proportion of fat. Selection for higher weaning weight also resulted in a greater total amount of muscle but had no effect on its proportion or distribution in the body (Perry et al. 1988). Under some circumstances, selection for growth results in higher fatness in the mature animal while in others there is no change to the proportion of fat in the body.
2.5.3.2. Fertility and fecundity

Selection for higher growth ultimately results in ewes of higher mature liveweight, which is positively correlated with ovulation rate in many sheep breeds (Killeen 1967; Morley et al. 1978; Adalsteinsson 1979). Therefore selection for higher growth is likely to result in an increase in reproductive rate and while genetic and phenotypic correlations between growth traits and ewe reproduction are highly variable they are generally positive (Safari et al. 2005). In agreement, selection for increased weaning weight resulted in an increase in mature liveweight and a correlated increase in ovulation rate and number of lambs weaned in Targhee sheep (Quirke et al. 1985; Sakul et al. 1999; Bradford et al. 1999). Interestingly, in a separate selection line in the same experiment, breeding for litter size resulted in an increase in litter size (Sakul et al. 1999), ovulation rate (Quirke et al. 1985), fertility and total weight of lamb weaned with no correlated increase in weaning weight or mature liveweight (Bradford et al. 1999). In mice, selection for growth resulted in a higher ovulation rate but lower fertility and pre-natal survival (Armbrust and Eisen 1994). Similarly, in Targhee sheep, selection for increased weaning weight over a 20 year period resulted in no net gain in the total weight of lamb weaned because of a correlated reduction in fertility and lamb viability in weight selected animals over an unselected control. This result was achieved in both low nutrition and high nutrition environments (Lasslo et al. 1985). This decline in reproduction resulting from single trait selection was evident after five generations. A further 10 years of selection in these lines did result in an increase in total weight of lamb weaned in the low nutrition environment but ewe fertility and lamb viability
remained lower than an un-selected control (Bradford et al. 1999). Therefore, there is evidence for both negative and positive relationships of reproduction and growth rate.

These apparent discrepancies can possibly be explained by the notion that in natural populations a peak in fitness will be close to the mean liveweight of that population and it depends on where the animals under scrutiny sit in the wider genetic pool as to whether increasing size will be negatively or positively associated with fitness (Scholtz et al. 1990). The apparent discrepancy between studies may also be due to the fact that the relationship between size and ovulation rate is slightly positive, while the correlations between weight and fertility and lamb viability are negative (Bradford 2002). A separate explanation may be that total body weight and ovulation rate are only correlated on some occasions as it is actually total weight of lean tissue that seems to be important (Armbrust and Eisen 1994). There is evidence that selection for increased growth rate could have both positive and negative effects on ewe fertility and fecundity, the reasons for this discrepancy remain unclear.

2.5.3.3. Lamb birthweight

Increasing growth is likely to increase lamb birthweight since there are generally positive genetic and phenotypic correlations between growth traits and lamb birthweight (reviewed by Safari et al. 2005). This finding is also support by a close correlation between sire breeding values for growth and birthweights in cattle (Garrick et al. 1989). Similarly, there is also a small positive genetic correlation between the total
weight of lamb weaned and lamb birthweight (Bromley et al. 2001). However, Hopkins et al. (2007a) reported that there was no effect of a yearling weight estimated breeding value of the sire on lamb birthweight. This finding is not consistent with the rest of the literature and is likely to be specific to the dataset examined. Based on the genetic correlation in sheep and cattle it is expected that selection for increased growth will result in an increase in lamb birthweight.

2.5.3.4. Ewe milk production and lamb growth to weaning

Quantitative genetics separates the influence of the dam on the growth of her lamb into two components, (i) the maternal environment provided by the dam such as milk production (maternal effects), and (ii) the contribution of her own growth potential to the growth potential of the lamb (direct additive effects). Most relevant studies indicate a negative genetic correlation between direct additive and maternal effects for early growth traits. The negative correlation exists in Merinos although the correlation is partly driven by sire by year interactions (Konstantinov and Brien 2003). A similar negative correlation between direct and additive effects has been shown in Simmental and Angus cattle and in Coopworth sheep (Garrick et al. 1989, Lee and Pollak 1997; Lewis and Beatson 1999). The finding in cattle is again partly explained by the effect of sire by year interactions (Robinson 1996). The existence of such a negative correlation is suggestive of the presence of an antagonism between growth potential and maternal characteristics although this is not seen in practice (eg. Pattie 1965a).
Selection strategies that result in an increase in mature liveweight also result in improved milk production. Selection for weaning weight in Merino sheep resulted in an increase in mature weight, higher ewe milk production and greater lamb growth to weaning (Pattie 1965a, 1965b). The increase in milk production in these selection lines was proportional to the increase in mature weight (Thompson et al. 1987). Similarly, Näsholm and Danell (1996) showed an increase in ewe mature weight and maternal ability in response to selection for higher lamb growth. Furthermore, ewes selected for high phenotypic bodyweight weaned heavier lambs than those selected for low bodyweight (Refshauge et al. 2006a). In addition, both selection for increased total weight of lamb weaned and the addition of a growth hormone transgene resulted in higher lamb growth, higher ewe mature weight, higher growth hormone concentration and higher milk production (Head et al. 1996; Ercanbrack and Knight 1998; Adams and Briegel 2005). However, the amount of milk produced is not only a function of the ewe genotype because the genetic growth potential of the lamb and therefore it’s demand for milk also partly drives the amount of milk produced (Moore 1966). It is clear that selection for higher growth potential results in an increase in mature ewe liveweight and a corresponding increase in ewe milk production and lamb growth rate.

2.5.4. Selection for higher muscling

2.5.4.1. Muscle growth, fibre type, glucose and glycogen metabolism

In selection programs that aim to increase muscling it is expected that increases will be made to muscle fibre size rather than muscle fibre number (Rehfeldt et al. 2000). Selection or mutations that result in higher muscling also results in a greater
proportion of glycolytic muscle fibre types (type IIx) and therefore glycolytic capacity in the skeletal muscle of ruminants in most (Hocquette et al. 1998; Wegner et al. 2000; Gardner et al. 2006; Greenwood et al. 2006) but not all studies (Gardner et al. 2007). Muscles that are more glycolytic store less glycogen and are more sensitive to stress-induced depletion of glycogen than muscles which are predominantly oxidative (Pethick and Rowe 1996). In support, Merinos are known to have less glycolytic and more oxidative muscle fibres than more muscular meat breeds such as the poll dorset (Greenwood et al. 2007). The change in muscle metabolism associated with selection for muscling is likely to change the energy metabolism of breeding ewes.

2.5.4.2. Body composition

Selection for muscling is undertaken to increase the proportion of lean meat in the carcass of animals. There is evidence that selection for muscling does increase carcass lean proportion (Hopkins et al. 2007b) and that selection for ultrasound eye muscle depth results in an increase in lean growth capacity and a higher muscle to bone ratio (Larsgard and Kolstad 2003; Cake et al. 2007). However, the effect of selection for muscling on body composition is not clear as reports are contradictory. There are two components that make the correlation between muscling and body composition confusing. Firstly, the effect of selection for muscling on total body fat is reported as being anything from strongly negative to moderately positive. Secondly, the link between selection for increased muscling and actually increasing total muscle amount is not always clear. The genetic and phenotypic correlations between eye muscle depth
and subcutaneous fat depth at the C-site are generally moderately positive across a range of breeds of sheep (Gilmour et al. 1994; Bishop 1993; Clarke et al. 2003; Ingham et al. 2003; Larsgard and Kolstad 2003; Nsoso et al. 2004; Karamichou et al. 2007). Considering the strong phenotypic correlation between subcutaneous fat and total carcass fat (Dunshea et al. 2007), it is reasonable to expect that selection for increase eye muscle depth would result in an increase in total body fat. In support, Southdown sheep bred for higher fat had higher levels of muscularity and higher muscle to bone ratios then sheep bred for low fat (Kadim et al. 1989; Abdullah et al. 1998). Furthermore, Lambe et al. (2005) reported positive genetic correlations between the amount of carcass muscle tissue and both the amount of carcass fat and internal fat. However, there is also evidence that selection for muscling reduces fatness. The correlation between eye muscle depth and total body fat was not evident in cross-bred lambs (Ponnampalam et al. 2007b). Furthermore, the correlation between eye muscle depth and total body fat was negative when lambs were grown on a high level of nutrition (Hegarty et al. 2006a). Similarly, Hopkins et al. (2005b) reported a negative correlation between post weaning muscle depth ASBV and intramuscular fat and Fogarty et al. (2003) reported a negative genetic correlation between C-site fat depth and eye muscle depth. In addition there is a negative correlation between muscle fibre number and carcass fatness (Rehfeldt et al. 2000). Also selection for protein accretion or physical ability results in a reduction in total body fat in mice (see Nürnberg et al. 1998). The effect on body composition of selecting for higher muscling is not clear with literature suggesting a range of possibilities, further work is required to elucidate the reasons for the apparent differences.
Some information about the potential impacts of selection for improved muscling on body composition can be gleaned from sheep that carry the callipyge mutation. These sheep exhibit an extreme muscling phenotype and have 42% higher muscle weights (Jackson et al. 1997b) and 21% less total body fat (Jackson et al. 1997a) than sheep without the mutation. Sheep with the callipyge mutation display hypertrophy of the fast twitch muscle fibres and therefore have greater reliance on glycolytic metabolism (Cockett et al. 2001). The difference in carcass fat levels associated with the callipyge mutation is related to lipogenic enzyme activity in adipose tissue. In comparison to normal lambs, those expressing the callipyge gene show a depression in lipogenic enzyme activity which is evident across adipose tissue depots and over a range of liveweights (Rule et al. 2002). It is not known whether the lower enzyme activity is a result of down regulation or increased competition for substrate from muscle. The dominance of type IIX fibres in callipyge sheep may help to explain the measured reduction in fatness in these sheep. The most convincing evidence of the direct effect of the proportion of type IIB fibres (similar metabolism to type IIX fibres) on total body fatness comes from a line of transgenic mice (Izumiya et al. 2008). Activation of the transgene resulted in a rapid increase in the proportion of type IIB fibres. Once the proportion of type IIB fibres had been increased, the previously obese mice rapidly mobilised fat tissue. There was an increased muscle uptake and use of glucose associated with the glycolytic metabolism of type IIB fibres. As a result of the increased use of glucose by muscle, fat was mobilised and fatty acid oxidation was increased as shown by increased plasma β-hydroxy butyrate (Izumiya et al. 2008).
These mutant models may help to explain the negative correlation between muscling potential and fatness but the reason for the positive correlation between muscling and fatness on some occasions remains unknown but may be associated with the relative maturity of tissues when measured.

2.5.4.3. Fertility and fecundity

In an extensive study into the impacts of changing body composition on maternal traits Lambe et al. (2005) demonstrated a positive genetic correlation between both the proportion of muscle and total amount of muscle and the number of lambs born. In addition, in Merino sheep that had been selected for or against multiple rearing ability for four or five generations, the positive selection line weaned more lambs but also had greater loin muscle and hindquarter weights at slaughter (Cloete et al. 2004). This results suggests that selection for improved maternal performance resulted in a correlated increase in carcass muscling. Furthermore, Safari et al. (2008) and Huisman and Brown (2009) reported positive genetic and phenotypic correlations between scanned eye muscle depth and reproduction traits in Merino ewes. In direct contrast, Larsgard and Kolstad (2003) reported a lower average litter size in sheep selected for increased muscle depth however the difference disappeared as selection continued so may have been an artefact of the source population. It seems that selection for muscling will increase the number of lambs born in Merino ewes.
2.5.4.4. Lamb birthweight and survival

There is limited information about the association between selection for muscling and lamb birthweight and survival. Larsgard and Kolstad (2003) reported lower lamb birthweights from ewes selected for increased ultrasonic muscle depth compared with an unselected control line. Simm et al. (2002) also reported a slight negative genetic correlation between eye muscle depth and lamb birthweight. However, Hopkins et al. (2007b) reported no effect of sire breeding value for muscling on lamb birthweight. The correlation between ewe muscling and lamb birthweight requires further investigation.

2.5.4.5. Ewe milk production lamb growth to weaning

The information available on the impact of selection for muscling on ewe milk production and lamb growth is limited. Lambe et al. (2005) showed a positive genetic correlation between the total amount of muscle tissue in ewes and lamb growth to marking and weaning. However, there is also evidence of a reduction in lamb growth to weaning in sheep selected for increased ultrasonic muscle depth (Larsgard and Kolstad 2003; Hopkins et al. 2007b). The reason for this discrepancy is unknown but may be associated with differences between the effects of muscling on the maternal capability of the ewe and the effects of muscling on the growth potential of the lamb. The concept requires further investigation.
2.5.5. Selection for lower fatness

2.5.5.1. Body composition

Selection for lower or higher subcutaneous fat depth by ultrasound measurement is effective at changing the amount of fat in all fat depots in the desired direction (Kadim et al. 1989; Cameron 1992b; Cameron and Bracken 1992; Karamichou et al. 2006; Hopkins et al. 2007b). Changes in fat weights in the various depots are as a result of differences in the size rather than the number of adipocytes (Kadim et al. 1989). Changes to body composition as a result of selection for or against fat are a result of the amount of fat changing without an associated change in lean tissue. Mice selected for higher fat had the same amount of lean tissue but around 50% more fat than a line selected for low fat (Hastings et al. 1991). Selection for reduced fatness may be also expected to change the energy metabolism of animals. In sheep selected for leanness or fatness the lean line produced a higher relative change in the concentrations of NEFA and triacylglycerides after fasting for three days than the fat line (Cameron 1992a). The differences in plasma concentrations seem to support the theory that sheep selected for higher lean proportion synthesise protein in preference to fat deposits under normal conditions and use fat as an energy source rather than catabolising protein under fasted conditions (Cameron 1992a). Also of interest, animals with lower breeding values for fatness (animals that are genetically leaner) have less oxidative and more glycolytic capacity in muscle potentially altering their energy metabolism (Gardner et al. 2007). Selection strategies that aim to change body composition by reducing the
amount of fat are effective; the impact on energy metabolism requires further investigation.

2.5.5.2. Fertility and fecundity

There is a minimum amount of fat in the body necessary for normal reproductive function and therefore selection for reduced fatness in ewes may alter their reproductive success. Safari et al. (2008) found negative genetic and phenotypic correlations between fat and reproduction traits in Merinos. However, in contrast Gernand et al. (2008) found positive genetic and phenotypic correlations between ultrasound fat depth and litter size in Merinos. Interestingly, within their dataset in the ewes that were mated under restricted nutrition there is a strong positive correlation between fatness and reproduction whereas in the ewes that were mated under adequate nutrition there are negative correlations between fatness and reproduction. This finding suggests that the impact of fatness on reproduction is dependent on available nutrition. Selection for increased litter size in pigs results in changes to fat deposition within the body. In a selection experiment that had a line selected intensively for litter size and an un-selected control line, improvements in litter size resulted in higher levels of ultrasound measured back fat and carcass fat and associated reductions in carcass lean content (Estany et al. 2002a; Estany et al. 2002b). In support, divergent selection lines for fatness in mice resulted in higher fecundity as a result of higher ovulation rate and neonatal survival in the fat line compared with the lean line (Hastings et al. 1991). However, in contrast selection for increased fatness
resulted in lower conception rates and lower number of lambs born in Coopworth sheep. This reduction was associated with a reduction in mature ewe size which may be the causative mechanism (McEwan et al. 2001). Selection for increased fatness while holding body weight constant may have produced a different result. As expected, selection against fatness may be expected to impair the reproductive potential of breeding ewes although it is likely to depend on the production environment.

2.5.5.3. Lamb birthweight and survival

The impacts of selection for divergence in fatness on lamb birthweight and survival are inconclusive. Lambs from ewes selected for higher fat were slower to stand and suckle than lambs from ewes selected for reduced fat and since time to suckle was positively correlated with survival to weaning this suggests that lambs from genetically fat ewes have compromised survival (Dwyer et al. 2001). This experiment was conducted indoors and under conditions of good nutrition. Conversely, in a separate experiment lambs from ewes selected for increased fatness had lighter birthweights but higher survival than lambs from either un-selected control ewes or ewes selected for less fat. Selection for reduced fat had a positive effect on lamb birthweight but negative effect on lamb survival (Morris et al. 1997; McEwan et al. 2001). However, in contrast, Lambe et al. (2005) demonstrated that sheep that had proportionately more internal fat produced lambs of higher birthweight. The reasons for these apparent discrepancies may be associated with available nutrition during pregnancy. It is reasonable to expect that genetically fat animals provide a better maternal environment to the fetus and
neonate when nutrition is limiting. Evidence from pig experiments supports the notion that genetic fatness in the dam is supportive of the neonate. In pigs from lines where breeding values for piglet survival had been calculated, piglets with high survival breeding values had higher carcass fatness and higher levels of liver glycogen than low survival equivalents (Leenhouwers et al. 2002). Furthermore, piglets from obese selection lines had 17% higher survival rates from birth to weaning despite lighter birthweights than those from lean selection lines (Mersmann et al. 1984). Selection strategies that reduce fatness may have detrimental effects on neonatal survival but it is likely to depend on nutritional conditions.

2.5.5.4. Ewe milk production and lamb growth to weaning

As lactation usually involves the mobilisation of significant amounts of adipose tissue, selection for reduced fat may impair ewe milk production, yet there is limited information available to define any correlation. There is evidence that the amount of both internal fat and carcass fat are positively correlated with lamb growth to weaning in Scottish Blackface sheep (Lambe et al. 2005), suggesting a positive influence of fatness on lactation. However, in contrast pigs from lines selected for leanness produced more milk than pigs selected for higher fatness (Mersmann et al. 1984). The impact of genetic fatness on ewe milk production and lamb growth requires further investigation.
2.6. Conclusions

To improve the profitability of Merino sheep production systems Merino ewes need to be able to produce both quality wool and lambs with adequate carcass characteristics. In addition, this outcome needs to be balanced with the partitioning of sufficient energy to mechanisms that enable them to survive and reproduce so that they contribute to the next generation and therefore retain fitness under fluctuating environmental conditions. The key way that ewes maintain fitness is to maintain fat storages to buffer against nutritional shortfalls or physiological requirements. Merino breeding programs that breed toward animals with improved productivity in terms of wool production, carcass muscling, leanness and growth rate will result in changes in to ewe body composition and will change the ewes reproductive performance and therefore alter the fitness for purpose of the animals. Early determination of the effects at a mechanistic level of selection for the relatively new carcass traits of growth, muscling and leanness on composition and the whole body energy metabolism will allow refined definition of breeding objectives. Although genetic correlations exist between all of these traits there remains a gap in the knowledge of the mechanisms that ultimately determine the trade off between traits and the biological limits to selection within a production environment. Merino production profitability is likely to be driven by the total weight of lamb weaned per ewe mated. Ercanbrack and Knight (1998) found that the overall genetic improvement in total weight of lamb weaned was attributable to a range of component traits including; prolificacy (37%), lamb survival (27%), lamb weaning weight (17%), fertility (12%) and ewe viability (7%).
important for the sheep industry to understand the impact of selection for carcass traits on these important component traits.

2.7. General Aims

The general aim of this thesis is to quantify the impacts in Merino ewes of selection for: higher growth and muscling to capitalise on the higher value of meat; and lower fatness as a result of selecting for higher wool weight. The thesis aims to define the impact of these selection strategies on ewe body composition, fertility, fecundity and milk production and on the birthweight, survival and growth of their lambs. The aim is also to investigate changes in intermediary metabolism associated with these selection strategies.
2.8. Hypotheses

The hypotheses tested in this thesis cover four areas:

2.8.1. Measurement of body composition and its impact on maternal performance

i. DXA can be used to accurately predict total body fat in live Merino ewes

ii. Ewes with higher liveweight and condition score produce heavier lambs at birth that grow faster as a result of higher milk production

2.8.2. Impact of breeding values on body composition

iii. Fatness breeding values are positively correlated with total body fatness measured by DXA.

iv. Ewes with higher HWT ASBVs have higher total body fatness measured by DXA

v. Ewes with higher HEMD ASBVs will have less total body fat measured by DXA

2.8.3. Impact of breeding values on reproduction and lamb growth

vi. Selection for fleece weight, muscling and growth will have correlated effects on ewe fecundity, lamb birthweight and lamb survival.

vii. Ewes with higher HFAT breeding values have lambs of higher birthweight, produce more milk and wean heavier lambs when under restricted nutrition.

viii. Ewes with higher HEMD breeding values will have lower milk production and lighter lambs at birth and weaning
ix. Ewes with higher HWT breeding values have higher milk production and lambs with higher weights at birth and weaning

2.8.4. Impact of breeding values on regulatory hormones and intermediary metabolism

x. Growth hormone, IGF-I and insulin concentrations will be increased with higher HWT and decreased with higher HFAT and higher HEMD ASBVs in Merino ewes across the breeding cycle; and that these differences will be more pronounced in lactation when growth hormone levels are elevated.

xi. That lambs from high HEMD ewes will have a more mature metabolism at birth

xii. That the response of glucose output to adrenaline will be decreased as the breeding value for fatness of ewes is decreased

xiii. That the glycogenolytic response to adrenaline in muscle will decrease as the muscling breeding value of ewes is increased

xiv. That the lipolytic response to adrenaline will be higher in ewes with higher fatness breeding values

xv. That breeding value effects on response to adrenaline across all tissues will not be evident in late pregnancy and lactation.

xvi. That the uptake of glucose in response to insulin:

   a. is higher in ewes bred for high muscling
   b. is lower in ewes bred for high fat
   c. these breeding differences will no longer be present during pregnancy and lactation.
Chapter 3 Implications of selection for meat and wool traits on maternal performance in Merinos.

Part of this chapter has been published:


3.1 Introduction

While decades of research and selection has been aimed at improving performance for wool traits and more recently meat traits in the Australian Merino, relatively little attention has been given to the implications of these strategies on maternal performance. Australian sheep producers are fortunate to have at their disposal a performance recording system for sheep known as MERINOSELECT™. This system provides Australian sheep breeding values (ASBVs) for a range of important traits (Brown et al. 2007). This system can deliver rapid genetic gain in production traits in breeders’ flocks. However, this gain needs to be achieved in a way that ensures fitness is not adversely affected, since selection for high performance has resulted in reduced fitness across a range of species (Rauw et al. 1998). We define fitness as ‘the ability of an animal to contribute to the next generation within a production environment where available nutrition fluctuates widely throughout the year’. Reductions in fitness can affect enterprise profitability especially in production systems where animal nutritional needs are not always met. In both pure Merino production systems and systems where the Merino is used as the maternal component for first-cross lamb
production, ewe maternal traits contribute significantly to enterprise profit. Maternal performance encompasses a range of fitness-related traits including: fertility, fecundity, milk production and lamb survival and growth. The impacts of production traits on the components of maternal performance are largely unknown. Testing the impacts of ASBVs for production traits in breeding ewes will elucidate any useful or potentially harmful correlations. The ASBVs that are considered important are those that are commonly used to either improve productivity or to improve consumer acceptability of wool and meat products. Those of interest are hogget-age clean fleece weight (HCFW), mean fibre diameter (HFD), the co-efficient of variation of fibre diameter (HFDCV), live weight (HWT) and eye muscle (HEMD) and fat depth (HFAT) over the eye muscle (*m. longissimus lumborum*) on the 12th rib, 45mm from the midline (C-site). It is important to understand any correlations that exist between these production traits under selection and components of maternal performance to define selection strategies that maximise farm profit.

Selection for improved growth and carcass characteristics is expected to alter maternal performance. Selection for higher growth by selecting for higher HWT is expected to increase the number of lambs weaned. Selection for higher growth ultimately results in higher mature weight (Thompson *et al.* 1987) and since body weight is positively correlated with ovulation rate and fecundity (Killean 1967; Morley *et al.* 1978), ewes with higher HWT are expected to produce more lambs. Ewes with higher HWT are also expected to have heavier lambs at birth and weaning since there are generally positive genetic and phenotypic correlations between growth traits and birth and
weaning weights (Safari et al. 2005). Ewes with higher HEMD are expected to have a higher fecundity since Lambe et al. (2005) reported strong positive genetic correlations between the weight and proportion of muscle in ewes and the number of lambs weaned. High HEMD ewes are also expected to have lighter lambs at birth, since Larsgard and Kolstad (2003) reported lower lamb birthweights but unchanged lamb survival in ewes selected for higher eye muscle depth in comparison to an unselected control line. Furthermore, they reported a positive genetic correlation between the weight of muscle in ewes and the growth of lambs to weaning. The maternal performance of ewes with high HFAT is difficult to predict, some authors have reported positive associations between fatness and reproduction traits in pigs and mice (Estany et al. 2002a; Hastings et al. 1991; Gernand et al. 2008), however others have reported negative correlations between fatness and reproductive performance in sheep (McEwan et al. 2001; Dwyer et al. 2001; Safari et al. 2008). The difference in these findings is potential a result of the prevailing nutritional conditions with genetic fatness having a different effect on reproduction under different conditions (Gernand et al. 2008). Ewes were generally managed at or above condition score three in the study population (data not shown) and therefore we may not expect HFAT to impact on maternal performance.

Selection for wool traits using ASBVs is likely to have both positive and negative impacts on ewe maternal performance. Selection for higher HCFW is expected to result in lower numbers of lambs born and weaned per ewe joined based on the generally negative correlation between fleece weight and ewe reproduction across a
range of studies and breeds (Herselman et al. 1998; see Safari et al. 2005). Ewe reproduction may also be impaired by selection for lower HFD as there are generally positive correlations between fibre diameter and reproduction traits (see Safari et al. 2005). However, selection for low HFDCV is expected to have the opposite effect on the number of lambs weaned due to its association with lamb survival. Thompson et al. (2006) reported higher lamb survival from ewes selected for high staple strength. Since staple strength and the co-efficient of variation of fibre diameter are negatively correlated (Safari et al. 2005), selection for low HFDCV is expected to increase staple strength and also increase lamb survival. The wool trait of most interest is clean fleece weight considering the widespread selection pressure placed on it and its potential to reduce fitness.

This chapter tests the hypotheses that: i) ewes with higher HWT will have more lambs that are heavier at birth and weaning; ii) ewes with higher HEMD will have more lambs that are lighter at birth; iii) ewes with higher HCFW will have lower lamb survival; and iv) ewes with lower HFDCV will have higher lamb survival.

3.2 Materials and methods

An analysis of data collected on-farm over six production years was used to test the effects of ASBVs for production traits on ewe fecundity, lamb survival to weaning and lamb weight at birth and weaning.
3.2.1. Animal data

This analysis utilised information collected by Merinotech (WA) Pty. Ltd. from their nucleus flock of Merino ewes based at “Yarrak” near Kojonup in South-West Western Australia. All ewes used in the analysis had full pedigree information and ASBVs for HWT, HEMD, HFAT, HFD, HCFW and HFDCV (generated on the 7th Nov 2006). The ranges of ASBVs for ewes included in the data set were HWT (-5.2 to 12.6kg), HEMD (-1.9 to 3.6mm), HFAT (-1.9 to 2.2mm), HFD (-1.9 to 2.3µm), HCFW (-9.3 to 32.5%) and HFDCV (-4.2 to 3.6%). Ewes included in the analysis were aged between 2 and 9 years and some ewes were present in more than one of the years studied. Due to restricted numbers in each age group, ewes over 7 years old were grouped together for the analysis. Six years (2000 to 2005) of ewe lambing records were used in the analysis, however lamb birthweight was only available for the four years from the 2002 to 2005 drops. Ewe fecundity was determined as pregnant ewes that either did or did not give birth to multiple lambs and there were a total of 2368 records analysed for this trait. Approximately 35% of the ewes gave birth to multiple lambs on average. Lamb survival was determined as lambs born that were either alive or not at weaning and a total of 3408 records were available for analysis. There were 2415 and 2686 records available for lamb weights at birth and weaning.
3.2.2. Animal management and measurement

In each production year, ewes were run as a single mob except for 5 weeks when they were allocated to between 10 and 12 different groups for natural mating, however during this mating period care was taken to ensure nutrition was similar between groups. Ewes were managed as per normal farm practice for the remainder of the year and were managed at commercial stocking rates of approximately 12 dry sheep equivalents (maintenance requirements of a non-breeding sheep at 45kg) per winter-grazed hectare. Ewes were closely monitored at lambing and lambs were tagged and weighed (weighed in 2002 to 2005 only) within 12h of birth. At birth, sex and lamb birth type were recorded and the lamb’s identification was matched to the ewe’s identification so that the sire and dam of each lamb was known. At lamb weaning lambs were recorded as either present or missing (presumed dead) and were weighed.

3.2.3. Statistical analysis

A linear mixed model approach was used to analyse birthweight and weaning weight. Fixed effects included in the model were ewe age (2 to 7 years and older), year, sire, birth or rear type (single or multiple) and Sex. Ewe tag was modelled as a random effect. Covariates included in the analyses were HWT, HEMD, HFAT, HFD, HCFW and HFDCV. The proportion of pregnant ewes giving birth to multiple lambs was analysed using a Generalised Linear Regression model, using a binominal variate structure. Ewe age and year were included in the model as fixed effects. Covariates
included were: HEMD, HFAT, HWT, HCFW, HFD and HCVFD. The same approach was used to analyse lamb survival data with lamb sire, birth type and sex included as fixed effects and ewe tag included as a random effect in the original model. The analysis was completed with and without birthweight included in the model as a covariate. All analyses were completed using Genstat 11.1 (VSN International 2008), and first and second order interactions were included in the original models and the final models were determined by stepwise removal of non-significant terms ($P>0.05$).

3.3 Results

3.3.1. Ewe fecundity

Of ewes that lambed, the proportion of ewes that had multiple lambs was higher in ewes with higher HEMD, with an increase of around 8% in multiple births across the range of HEMD values in the study flock (Table 3.1). For each 1mm increase in ewe HEMD, the proportion of multiple births increased by 2.1%. Likewise, ewe HWT was positively related to the proportion of multiple births, with an increase of 12% across the range of HWT values in the study flock. There was no effect of HFAT or any wool traits on the probability of multiple births. However, ewe age at lambing had a significant effect ($P<0.001$) on the proportion of ewes bearing multiple lambs, with ewes at their first lambing at 2 years of age having a lower proportion of twins than older ewes (Table 3.1). Year also had a significant effect on ewe fecundity ($P<0.001$).
Table 3.1 Generalised linear regression model parameter estimates for the effect of birth type, ewe age, year, and ewe Australian sheep breeding value (ASBV) for hogget-age eye muscle depth (HEMD), weight (HWT) and clean fleece weight (HCFW) for the proportion of ewes having multiple lambs and the proportion of lambs surviving to weaning. Parameters for factors are differences compared with the reference level of ewe age = 2 years old, year = 2000 and in the case of lamb survival, birth type = single.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Proportion of multiple lambs</th>
<th>Proportion of lambs surviving</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate ± se</td>
<td>Significance</td>
</tr>
<tr>
<td>Constant</td>
<td>-2.22±0.18</td>
<td>***</td>
</tr>
<tr>
<td>Birth type - twin</td>
<td>-</td>
<td>ns</td>
</tr>
<tr>
<td>Ewe age - 3</td>
<td>1.03±0.13</td>
<td>***</td>
</tr>
<tr>
<td>Ewe age - 4</td>
<td>1.46±0.14</td>
<td>***</td>
</tr>
<tr>
<td>Ewe age - 5</td>
<td>1.72±0.15</td>
<td>***</td>
</tr>
<tr>
<td>Ewe age - 6</td>
<td>1.52±0.17</td>
<td>***</td>
</tr>
<tr>
<td>Ewe age - 7+</td>
<td>1.36±0.19</td>
<td>***</td>
</tr>
<tr>
<td>Year - 2001</td>
<td>0.92±0.17</td>
<td>***</td>
</tr>
<tr>
<td>Year - 2002</td>
<td>0.57±0.17</td>
<td>***</td>
</tr>
<tr>
<td>Year - 2003</td>
<td>0.68±0.17</td>
<td>***</td>
</tr>
<tr>
<td>Year - 2004</td>
<td>0.56±0.17</td>
<td>***</td>
</tr>
<tr>
<td>Year - 2005</td>
<td>0.66±0.17</td>
<td>***</td>
</tr>
<tr>
<td>Ewe HEMD (per mm)</td>
<td>0.10±0.05</td>
<td>*</td>
</tr>
<tr>
<td>Ewe HWT (per kg)</td>
<td>0.06±0.02</td>
<td>**</td>
</tr>
<tr>
<td>Ewe HCFW (per kg)</td>
<td>-</td>
<td>ns</td>
</tr>
<tr>
<td>Ewe HCFW x birth type - twin</td>
<td>-</td>
<td>ns</td>
</tr>
</tbody>
</table>

Significant effect: ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001

3.3.2. Lamb birthweight

The significant main effects and interactions for the fixed effects and breeding value covariates on birth and weaning weight are shown in Table 3.2. Lamb birthweight increased (P<0.01) with ewe HWT ASBV, resulting in an increase in lamb birthweight...
of 0.4kg across the range of HWT values in the flock studied. For each 1kg increase in HWT ASBV lamb birthweight increased by 0.03±0.01kg. In addition, there was a general negative correlation ($P<0.05$) between ewe HCFW and lamb weight at birth, with birthweight decreasing by 0.2kg across the range of HCFW values in the flock. For each 1% increase in HCFW ASBV lamb birthweight decreased by 0.006±0.003kg. For this and all results reported in this chapter the figures show are means ±s.e. Within the model there was an interaction between Year and HCFW ($P<0.01$), which revealed that the negative correlation between ewe HCFW and BWT was only evident in three of the four years however in 2004 there was a positive correlation. There was also a negative correlation between ewe HEMD and lamb birthweight (Table 3.2). For each 1mm increase in ewe HEMD, birthweight was 0.09±0.03kg lower resulting in a reduction of birthweight of 0.35kg across the range of HEMD values in the study flock. There was no effect of HFAT, HFDCV or HFD on lamb birthweight. Lamb birthweight differed ($P<0.01$) between years and was the lowest in 2005 (4.04±0.08kg) and highest in 2004 (4.27±0.06kg). Lamb sire also had a significant effect on lamb birthweight (Table 3.2). The birthweight of single lambs (4.99±0.04kg) was higher ($P<0.001$) than that of twin born lambs (4.14±0.04kg) and male lambs (4.33±0.05kg) were heavier at birth ($P<0.001$) than females (4.03±0.05kg). In addition, the age of the ewe was an important determinant ($P<0.001$) of lamb birthweight. Lamb birthweight increased with ewe age and was lowest in 2 year old ewes (3.55±0.06kg) that were lambing for the first time and highest in the ewes that were 7 years old or older (4.56±0.07kg) and increased with ewe age.
Table 3.2  $F$ values for the effect of lamb birth type or rear type, sex, sire, ewe age at birth, year of birth, and ewe Australian sheep breeding values (ASBVs) for hogget-age eye muscle depth (HEMD), weight (HWT), clean fleece weight (HCFW), coefficient of variation of fibre diameter (HFDCV) and significant interactions between terms on lamb weight at birth and weaning.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Lamb birthweight</th>
<th></th>
<th>Lamb weaning weight</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF, DDF</td>
<td>$F$ value</td>
<td>Sig.</td>
<td>NDF, DDF</td>
</tr>
<tr>
<td>Birth/rear type</td>
<td>1, 2361</td>
<td>313.8 ***</td>
<td></td>
<td>1, 2618</td>
</tr>
<tr>
<td>Sex</td>
<td>1, 2361</td>
<td>137.0 ***</td>
<td></td>
<td>1, 2618</td>
</tr>
<tr>
<td>Sire</td>
<td>36, 2361</td>
<td>4.1 ***</td>
<td></td>
<td>53, 2618</td>
</tr>
<tr>
<td>Ewe age</td>
<td>5, 2361</td>
<td>86.6 ***</td>
<td></td>
<td>5, 2618</td>
</tr>
<tr>
<td>Year</td>
<td>3, 2361</td>
<td>4.3 **</td>
<td></td>
<td>5, 2618</td>
</tr>
<tr>
<td>Ewe HEMD</td>
<td>1, 2361</td>
<td>6.6 **</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Ewe HWT</td>
<td>1, 2361</td>
<td>8.3 **</td>
<td></td>
<td>1, 2618</td>
</tr>
<tr>
<td>Ewe HCFW</td>
<td>1, 2361</td>
<td>4.0 *</td>
<td></td>
<td>1, 2618</td>
</tr>
<tr>
<td>Ewe HFDCV</td>
<td>-</td>
<td>- ns</td>
<td></td>
<td>1, 2618</td>
</tr>
<tr>
<td>Ewe HCFW x Year</td>
<td>3, 2361</td>
<td>3.8 **</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom
Significant effect: ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

3.3.3. Lamb weight at weaning

Lamb weight at weaning increased ($P<0.001$) with ewe HWT by 0.33±0.05kg per kg HWT resulting in a 3.3kg increase in weaning weight across the range of HWT values in the study flock. In addition, weaning weight was also higher in lambs from ewes with low HFDCV values. The negative association ($P<0.001$) between HFDCV and lamb weaning weight meant that weaning weight increased by 0.31±0.11kg for each 1% reduction in HFDCV. This effect resulted in a change of around 2kg in weaning
weight across the range of HFDCV values in the study flock. Another wool trait, HCFW, also had an effect \((P<0.001)\) on lamb weaning weight. For each 1% increase in ewe HCFW, lamb weaning weight increased by 0.07±0.02kg, resulting in a 1.2kg increase in weaning weight across the range of HCFW values in the study. There were no effects of ewe HFAT, HEMD or HFD on lamb weight at weaning, however year of birth had a significant impact on lamb weaning weight ranging from a minimum of 24.5±0.7kg in 2000 and a maximum of 30.5±0.5kg in 2003 (Table 3.2). The age of ewes also had a significant impact \((P<0.001)\) on lamb weaning weight with both young ewes (2 year old, 26.2±0.2kg) and old ewes (6 year old, 28.7±0.3kg; and 7 year old or older, 28.5±0.4kg) rearing lighter lambs than three to five year old ewes (29.0-29.4kg). Lambs reared as singles (30.9±0.2kg) were over 4kg heavier \((P<0.001)\) than those reared as twins (26.1±0.2kg) and males (29.8±0.2kg) were heavier \((P<0.001)\) at weaning than females (27.3±0.2kg).

3.3.4. Lamb survival

Lamb survival was analysed with birth type \((P<0.001)\), ewe age \((P<0.05)\) and year \((P<0.001)\) included in the final model (Table 3.1). Lambs born as twins and those from 2 year old ewes had lower rates of survival to weaning. The proportion of single lambs that survived to weaning was negatively correlated with HCFW (-0.47% per % HCFW; \(P<0.01\)) resulting in 10% lower survival in single born lambs from high HCFW ewes, however this correlation was not evident \((P>0.05)\) in twin-born lambs (Figure 3.1). When birthweight was included in the model it was significant \((P<0.001)\), however the
correlation between lamb survival and HCFW and the interaction with birth type remained significant.

Figure 3.1 Predicted response (±s.e.) of lamb survival to the Australian sheep breeding value (ASBV) for percentage increase in ewe hogget-age clean fleece weight (HCFW) for single and twin born lambs.

3.4 Discussion

The survival of lambs from ewes with higher HCFW breeding values was reduced and therefore our hypothesis that high HCFW would result in lower lamb survival is supported. This finding is supported by published estimates of a negative correlation between fleece weight and the number of lambs weaned (Safari et al. 2005). The result is also supported by those of Herselman et al. (1998) and Refshauge et al. (2006a) who both reported lower number of lambs weaned in high fleece weight ewes. In my
study, the negative association was only evident in single born lambs, the reason for the difference between birth types is not known. Ewe HCFW was also negatively related to lamb birthweight in this data set, which does not agree with published genetic correlations between these two traits (Safari et al. 2005). When birthweight was included in the analysis it was positively correlated with lamb survival, however the relationship between lamb survival and HCFW and the interaction with birth type remained significant. The impact of HCFW on lamb survival is therefore independent of reductions in birthweight associated with HCFW in this data set. High HCFW genotype sheep have been shown to have impaired energy reserves, although only when these sheep are underfed (Adams et al. 2006a). The negative correlation between ewe HCFW and birthweight was evident in three of the four years, however in 2004 there was a positive correlation. Interestingly, of the four years in the data set, 2004 had the highest mean birthweight suggesting better nutrition during pregnancy for that year. This would be expected if the negative influence of HCFW on whole body energy metabolism is not seen under good nutritional conditions, as shown by Adams et al. (2007). A negative association between HCFW and lamb survival is important to the Australian sheep industry for both economic and animal welfare reasons. Lamb survival is a trait that encompasses both the maternal ability of the ewe and the innate ability of the lamb to survive, further work is required to elucidate whether the reduced survival in lambs from high HCFW is a result of ewe energy status and fetal and lamb nourishment or due to reduced lamb vigour.
The proportion of multiple births was higher and lamb birthweight was lower from ewes with higher HEMD, therefore our hypothesis that ewes with higher HEMD would have higher fecundity and lighter lambs is supported. The result of high HEMD ewes having higher fecundity is supported by the results of Cloete et al. (2004) who reported that in Merino sheep selected for or against the ability rear multiple lambs, the positive selection line weaned more lambs but also had greater loin muscle and hindquarter weights at slaughter. In addition, Safari et al. (2008) and Huisman and Brown (2009) both report positive genetic and phenotypic correlations between eye muscle depth and litter size. Furthermore, positive genetic and phenotypic correlations between muscle proportion and litter size have been found in Scottish Blackface ewes (Lambe et al. 2005). Higher HEMD results in an increase in lean mass and while fatness has been implicated in affecting fertility it seems likely that lean mass is a greater contributor to fecundity. Ewes with higher HEMD also had lighter lambs at birth and this negative correlation between ewe HEMD and lamb birthweight was evident even when the increase in multiple births associated with HEMD is taken into account as birth type was included in the model. This result is supported by Larsgard and Kolstad (2003) who also reported that lamb birthweight was lower in ewes selected for high eye muscle depth. Reductions in lamb birthweight are normally associated with lower lamb survival (Fogarty et al. 1992), however in the present study there was no reduction in lamb survival in lambs from high HEMD ewes. These results show that selection for increase muscling in Merino ewes by selection for higher HEMD will result in higher ewe fecundity and lower lamb birthweight without the accompanying reduction in lamb survival.
Ewes with higher HWT values had higher fecundity, and ewe HWT was positively correlated with lamb weight at birth and weaning. Therefore our hypothesis that ewes with higher HWT will have more lambs that are heavier at birth and weaning is supported. The increase in fecundity in ewes with higher HWT is supported by evidence of a positive correlation between body weight and ovulation rate in sheep (Killeen 1967; Morley et al. 1978). Furthermore, selection for increased weaning weight resulted in an increase in mature size and a correlated increase in ovulation rate and number of lambs weaned (Quirke et al. 1985; Sakul et al. 1999; Bradford et al. 1999). Therefore, selection strategies that result in an increase in mature size such as selection for higher HWT are likely to result in an increase in ewe fecundity. Ewes with higher HWT also had lambs of higher birthweight, this result is consistent with the known positive genetic correlation between lamb birthweight and growth traits (see Safari et al. 2005). Increasing lamb birthweight associated with selection for higher HWT is of some concern as ewes carrying high birthweight lambs are more prone to dystocia and can result in both lamb and ewe mortality (Fogarty et al. 1992). There was no effect of HWT on lamb survival in this study. As well as birthweight being higher in high HWT ewes, lamb weaning weight was also higher. Lambs from high growth ewes would be expected to have a greater potential for rapid growth as their mature weight is higher (Thompson et al. 1987). In addition, ewes selected for high growth produce more milk (Pattie 1965a) which would further aid greater growth to weaning in lambs from high HWT ewes. Ewes with higher HWT values have improved maternal performance
through higher numbers of lambs born and faster growth to weaning. The increase in 
lamb birthweight associated high HWT and its potential to increase the incidence of 
dystocia needs to be considered when designing breeding programs. Consideration 
also needs to be given to the associated increase in mature ewe size of selection for 
higher HWT because although the bigger ewes are more productive they also have 
higher maintenance requirements. Future work should investigate the possibility of 
selecting for early growth while maintaining adult liveweight constant.

The HFDCV of the ewes had no effect on lamb survival, therefore our hypothesis that 
ewes with lower HFDCV values would have higher lamb survival is rejected. The 
hypothesis was based on the results of Thompson *et al.* (2006) that showed that ewes 
selected for high staple strength had higher lamb survival, and the knowledge that the 
coefficient of variation of fibre diameter is negatively correlated with staple strength 
(Safari *et al.* 2005). It is not known why this correlation was not found in the present 
study population but it may be associated with differences in nutrition through 
pregnancy associated with the different environments experienced by the flock studied 
by Thompson *et al.* (2006) and the flock in our study. The interaction between HFDCV, 
nutritional environment and lamb survival requires further investigation.

While there was no effect of HFDCV on lamb survival, ewes with lower HFDCV had 
heavier lambs at weaning. This result is supported by generally negative correlations 
between coefficient of variation of fibre diameter and growth traits in sheep (Safari *et 
al.* 2005). Lamb weight at weaning was also higher in ewes with higher HCFW which 

78
also agrees with generally positive correlations between fleece weight and live weight at various ages (see Safari et al. 2005). There was no effect of HFAT or HFD on any of the traits studied in this analysis.

This work, undertaken in a commercial setting in a relatively harsh Mediterranean environment climate, points to the benefits of selecting for muscle in Australian Merino sheep which has never been a widespread activity. Moreover the results here point to the need to be careful setting breeding objectives with a large emphasis on selection for fleece weight because of the associated impacts on fitness. These results highlight the need to understand the impacts of selection for performance traits on maternal traits, and for a balanced approach to designing breeding strategies. While rapid genetic gain in performance is very achievable, care must be taken to ensure fitness traits are not adversely affected. Merino production profitability is increasingly reliant on animal sales, and reductions in maternal performance may reduce enterprise profitability.
Chapter 4 Dual-energy X-ray absorptiometry accurately predicts total body fat in live adult Merino ewes with diverse muscling and fatness breeding values.

4.1 Introduction

The body composition of sheep is usually measured either by manual dissection or by mincing of the entire body and measuring its components chemically (Little and Sandland 1975; Thompson et al. 1985; Aziz et al. 1992; Ryan et al. 1993). These methods are labour intensive, expensive, and require the animal to be slaughtered. However the measurement of body composition in live animals is required for both breeding programs and experiments that monitor changes to body fat across time. The body composition of live animals can be predicted by both X-ray computed tomography and the dilution of deuterium oxide (Young et al. 1996; Bocquier et al. 1999), but their use is cost-prohibitive in most circumstances. Body composition can be simply and cheaply estimated by Dual-energy X-ray absorptiometry (DXA; Kelly et al. 1998) and the method has been validated against chemical fat data in live pigs and pig carcasses with high accuracy (Dunshea 2003; Suster et al. 2003). However, DXA is more accurate when predicting the total fat content of sheep carcasses than live sheep (Dunshea et al. 2007; Pearce et al. 2009). However, these studies have not accounted for the removal of internal fat at slaughter when defining correlations between DXA measured fatness in live sheep with the amount of fat in the carcass. In sheep the weight of carcass fat is closely correlated with the weight of omental and kidney fat (Kirton and Johnson 1979). Inclusion of internal fat depot weights in correlations between live sheep DXA results and carcass fat will improve the accuracy of prediction of total fat in live sheep.
Increasing lean meat and reducing fat are important aims in many meat sheep breeding programs. These aims are achieved by selection based on estimated breeding values (EBVs) calculated from the depth of the eye muscle (m. longissimus lumborum; EMD) and subcutaneous fat (FAT) at a point on the 12th rib, 45mm from the midline (C site; Pálsson 1939). The measurement is obtained by ultrasound in live animals.

Selection for lower FAT breeding values not only lowers the amount of subcutaneous fat (Hall et al. 2002) it also lowers the amount of fat in the whole body (Hopkins et al. 2007a). The effects of selection for higher muscling on body composition are less clear. Progeny from rams selected for higher EMD breeding values had higher muscling and less total body fat than their low EMD counterparts (Hegarty et al. 2006a). However, the correlation between EMD and fatness was only evident when lambs were reared under high nutrition and the correlation between EMD and total body fat was not evident in a similar experiment, with a larger number of sires and progeny (Ponnampalam et al. 2007b). Therefore it is not clear what the expected correlation between EMD and whole body fatness is the sheep population but this study will confirm the association in adult Merino ewes. However it is highly likely that FAT EBVs will be positively correlated with total body fatness.

We tested the hypotheses that in adult Merino ewes: DXA can be used to accurately predict total body fat in live animals; and FAT EBVs are positively correlated with total body fatness.
4.2 Materials and Methods

Merino ewes were scanned by DXA and then slaughtered. The DXA values were then compared with the sum of the weight of kidney fat, omental fat and total amount of chemical fat in the ground carcass. Measures of live animal and carcass fatness were also compared with DXA derived values.

The experiment was approved and monitored by the CSIRO Floreat Animal Ethics Committee.

4.2.1 Animal Details

The animals used in this experiment were 44 Merino ewes that were approximately 34 months old at slaughter. The origin of the animals and previous experiments conducted with them are detailed in Adams et al. (2007). Briefly, the ewes were sourced from a flock of 432 ewes from the Department of Agriculture and Food Western Australia’s Katanning Merino resource flocks (Greeff and Cox 2006). Performance information collected for each ewe, along with pedigree information, was used to generate EBVs for muscle depth (EMD EBV), fat depth (FAT EBV) and weight (WT EBV), using BLUP mixed-method methodology (Groeneveld 1990). The EBVs for muscle and fat depth at the C site were estimated with adjustment for liveweight at scanning. Prior to this experiment the ewes had been managed on senesced pasture (subterranean clover and volunteer annual grasses) with oat grain supplementation at
the CSIRO Yalanbee Research Station near Bakers Hill, Western Australia (31°46'S, 116°29'E).

4.2.2 Live animal measurements

The sheep were weighed and condition scored (Jefferies 1961) and transported to the DXA facility on 8 May 2006 (Day 0). The following day body composition was estimated by DXA using a Norland XR-26 Fan Beam X-Ray Whole Body Densitometer (Inderlec Medical Systems Baulkham Hills, Australia) after the sheep had been off feed and water for between 18 and 24h. Details regarding calibration of the DXA, anaesthesia of sheep and the scanning process are described by Pearce *et al.* (2009). Briefly, sheep were anaesthetised using inhalation of nitrous oxide to induce and isoflurane to hold anaesthesia. Sheep were then placed on the DXA unit in sternal recumbancy. The scan was completed using the Whole Body scan mode of the DXA scanner and a regional analysis was performed on the image produced to provide total bone mass (DXA bone), total lean mass (DXA lean) and total fat mass (DXA fat). Following DXA scanning, sheep were returned to the Yalanbee research station and were fed at maintenance in a small paddock.

4.2.3 Carcass Measurements

On day 13 sheep were removed from feed and water and weighed 12h later. Sheep remained off feed and water and were slaughtered on day 14 by captive bolt gun
followed by exsanguination. Skin, head, legs and viscera were removed. Fat was
dissected from around the intestines and rumen and weighed and recorded as omental
fat. The kidneys and surrounding fat were removed from the carcass and the fat that
was dissected from the kidneys was weighed and recorded as kidney fat. The weights
of kidney fat and omental fat were combined to determine internal fat. The hot carcass
was weighed and the tissue depth at the GR site (tissue depth over the 12th rib, 110mm
from the centre of the spine) was measured using a GR knife.

The carcass was split along the midline and the left side was discarded. The right side
of the carcass was weighed and the fat and muscle tissue was dissected from bone.
Total bone and total tissue were then weighed separately. The tissue and bone were
then recombined and mixed before mincing through a commercial mincer (Nolex B55
grinder). The tissue was minced once through a coarse die mincing plate and three
times through a fine plate. Between each mincing the minced tissue was thoroughly
mixed. Moisture loss throughout the mincing process was determined by weighing
the total tissue both prior to and after mincing. The difference was taken into account
for later calculations of dry matter content of the components.

After the post-mincing weight was taken, 3 sub-samples were collected and frozen at
-20°C for later chemical analysis. A portion of each sub-sample was weighed accurately
and dried at 102°C for 24h and weighed again after cooling in a desiccator. The
moisture content was determined by difference. The triplicate samples were then
ignited in a muffle furnace set at 650°C for 6h, cooled in a desiccator and reweighed.
Ash proportion was determined by difference. A portion of each sub-sample was freeze-dried for calculation of total fat content. An accurately weighed 2g portion of the freeze-dried sub-samples (3 per sheep) was placed in 60mL of hexane:isopropanol (3:2) and tumbled at 60 revolutions per minute for 2h. The mixture was allowed to stand for 24h before filtering onto weighed filter paper, drying for 12h at 80°C and then reweighing after cooling in a desiccator. The proportion of fat was determined as the loss of weight after extraction (Ryan et al. 1993). A representative sample of internal fat from each sheep was accurately weighed, freeze-dried and re-weighed to determine the dry matter percentage of the internal fat and these values were adjusted accordingly.

Moisture percentage, chemical fat proportion and ash proportion were used to calculate total carcass fat and total carcass ash. Total lean soft tissue weight in the carcass was determined by subtracting bone weight (determined by dissection) and total carcass fat (determined chemically) from the total tissue weight. The weight of total body fat was taken as the total carcass fat (determined chemically) added to the dry weight of internal fat.

4.2.4 Statistical Analysis

The correlations between carcass components and related measures were analysed using general linear models in SAS software (SAS version 9.1, SAS Institute, Cary, NC, USA). The correlations between DXA fat and total body fat and carcass fat were
analysed with and without the inclusion of liveweight as a covariate. Similarly, the correlation of DXA lean and carcass lean and the correlation of DXA bone with carcass bone were analysed with and without the inclusion of liveweight as a covariate. The effects of WT EBV, EMD EBV, and FAT EBV on liveweight, carcass weight, total fat, internal fat, carcass fat, carcass lean, carcass bone, GR depth, condition score, and carcass ash were determined by general linear models with and without the inclusion of liveweight or carcass weight included as a co-variate where appropriate. Terms that were not significant ($P>0.05$) were removed. The relationships of liveweight, carcass weight, GR tissue depth and condition score with total fat, carcass fat, and carcass lean were analysed using general linear models. Where liveweight or carcass weight was fitted within the model results were interpreted as changes in proportions of tissue rather than absolute amount of tissue. This was determined as a better method of estimating changes in tissue proportion than calculating the proportion and the calculated tissue proportions. Models were fitted with and without this inclusion so that total tissue changes could be assessed along with changes in tissue proportion.

4.3 Results

4.3.1 Prediction of total body fat, lean and bone

DXA fat was well correlated ($P<0.001$) with both total body fat and carcass fat (Figure 4.1). The DXA measurement slightly over-estimated total body fat, particularly at high levels of fatness and was higher than carcass fat measurement across the range. This was expected since omental fat and kidney fat were removed from the carcass prior to
mincing and determination of the amount of carcass fat. The amount of carcass lean tissue was well correlated ($P<0.001$) with DXA lean (Figure 4.1). Values for DXA lean included organs and stomach contents and were over twice as high as for carcass lean content. The total weight of bones in the carcass was moderately correlated ($P<0.001$) with DXA bone (Figure 4.1). The values for DXA bone were considerably lower than the total weight of bone but are measured as bone mineral content on a dry weight basis. The correlations between DXA measurements and total body fat, carcass fat, carcass lean and bone weight were all improved by the inclusion of liveweight in the analysis (Table 4.1).
Figure 4.1 Relationships between Dual-energy X-ray Absorptiometry (DXA) determined tissue masses and weighed and chemically determined values for a) total fat (carcass plus internal fat), b) carcass fat, c) carcass lean and d) carcass bone weight. Equations for lines of best fit are presented in Table 4.1.
Table 4.1 Regression coefficients (± s.e.), model $F$ values and correlation coefficients for Dual-energy X-ray Absorptiometry (DXA) estimates of fat (DXA fat), lean tissue (DXA lean) and bone (DXA bone) compared with chemically determined and weighed measures of total fat, carcass fat, carcass lean and carcass bone. All terms are significant ($P<0.05$).

<table>
<thead>
<tr>
<th>Prediction Model</th>
<th>$F$ value</th>
<th>$r^2$ or $R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total fat</strong></td>
<td>2.22 (±0.36) + 0.71 (±0.03) DXA fat</td>
<td>533.9</td>
</tr>
<tr>
<td></td>
<td>-0.99 (±0.79) + 0.64 (±0.03) DXA fat + 0.07 (±0.02) LW</td>
<td>395.7</td>
</tr>
<tr>
<td><strong>Carcass fat</strong></td>
<td>2.48 (±0.38) + 0.45 (±0.03) DXA fat</td>
<td>193.4</td>
</tr>
<tr>
<td></td>
<td>0.45 (±0.95) + 0.41 (±0.04) DXA fat + 0.05 (±0.02) LW</td>
<td>109.5</td>
</tr>
<tr>
<td><strong>Carcass lean</strong></td>
<td>0.48 (±0.89) + 0.29 (±0.02) DXA lean</td>
<td>155.0</td>
</tr>
<tr>
<td></td>
<td>-0.63 (±0.92) + 0.21 (±0.04) DXA lean + 0.07 (±0.03) LW</td>
<td>93.8</td>
</tr>
<tr>
<td><strong>Carcass bone</strong></td>
<td>2.53 (±0.45) + 3.96 (±0.36) DXA bone</td>
<td>123.9</td>
</tr>
<tr>
<td></td>
<td>1.62 (±0.46) + 1.91 (±0.63) DXA bone + 0.06 (±0.02) LW</td>
<td>88.5</td>
</tr>
</tbody>
</table>

4.3.2 Prediction of carcass traits from estimated breeding values

Liveweight was correlated ($P<0.05$) with all breeding values (Table 4.2). Carcass weight was correlated ($P<0.05$) with all breeding values when liveweight was not included in the model, however the inclusion of liveweight resulted in the effects of the WT EBV and EMD EBV to be non-significant ($P>0.05$) and for the effect of FAT EBV on carcass weight to be the opposite (Table 4.2).
Total body fat was positively correlated with WT EBV, yet there were no correlations of EMD EBV and FAT EBV with total body fat. When liveweight was included in the model the effect of WT EBV was no longer significant ($P>0.05$) but there was a positive correlation between FAT EBV and total carcass fat (Table 4.2). The effects of EBVs and liveweight on total body fat were the same as those for internal fat although the coefficients were different. The effects of EBVs and liveweight were also similar to the effects on carcass fat, with the exception that the effect of WT EBV was still significant when liveweight was included in the model but the coefficient changed from a positive correlation to a negative correlation between WT EBV and carcass fat.

The weight of lean tissue in the carcass was positively correlated ($P<0.05$) with WT EBV and EMD EBV and negatively correlated with FAT EBV. When liveweight was included in the model the effect of EMD EBV was no longer significant ($P>0.05$). The weight of bone in the carcass was positively correlated ($P<0.05$) with WT EBV and EMD EBV but negatively correlated with FAT EBV. When liveweight was included in the model the effects of EMD EBV were no longer significant ($P>0.05$).

The depth of tissue at the GR site was positively correlated ($P<0.05$) to the FAT EBV and to liveweight when included in the model. Condition score was positively correlated with WT EBV, however when liveweight was included in the model its effects were significant ($P<0.05$) and the effect of WT EBV was not significant ($P>0.05$). Total carcass ash was not correlated with any EBVs however it was correlated ($P<0.05$) with liveweight.
Table 4.2 Predicted means and regression coefficients (± s.e.), model F values and correlation coefficients for models of carcass traits predicted by 15 month old weight (WT EBV), depth of eye muscle (EMD EBV) and subcutaneous fat at the C site (FAT EBV) estimated breeding values. Coefficients are shown with and without carcass weight (CW) or liveweight (LW) included. Only significant terms (P<0.05) are included.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean (±s.e.)</th>
<th>Regression Coefficients (±s.e.)</th>
<th>LW/CW</th>
<th>F value</th>
<th>r² or R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intercept</td>
<td>WTEBV</td>
<td>EMDEBV</td>
<td>FATEBV</td>
<td></td>
</tr>
<tr>
<td>Live weight (kg)</td>
<td>56.0 (±4.18)</td>
<td>54.0 (±0.76)</td>
<td>1.2 (±0.20)</td>
<td>0.6 (±0.33)</td>
<td>-1.7 (±0.56)</td>
</tr>
<tr>
<td>Carcass weight (kg)</td>
<td>26.5 (±2.10)</td>
<td>25.6 (±0.38)</td>
<td>0.6 (±0.10)</td>
<td>0.4 (±0.16)</td>
<td>-0.5 (±0.28)</td>
</tr>
<tr>
<td></td>
<td>LW included</td>
<td>0.4 (±1.55)</td>
<td>ns</td>
<td>0.3 (±0.14)</td>
<td>0.5 (±0.03)</td>
</tr>
<tr>
<td>Total fat (kg)</td>
<td>10.3 (±2.18)</td>
<td>9.6 (±0.36)</td>
<td>0.4 (±0.09)</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LW included</td>
<td>-4.4 (±2.28)</td>
<td>ns</td>
<td>0.8 (±0.2)</td>
<td>0.3 (±0.04)</td>
</tr>
<tr>
<td>Internal fat (kg)</td>
<td>2.7 (±0.82)</td>
<td>2.4 (±0.14)</td>
<td>0.1 (±0.03)</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LW included</td>
<td>-2.7 (±0.89)</td>
<td>ns</td>
<td>0.3 (±0.08)</td>
<td>0.1 (±0.02)</td>
</tr>
<tr>
<td>Carcass fat (kg)</td>
<td>7.6 (±1.11)</td>
<td>7.2 (±0.26)</td>
<td>0.2 (±0.06)</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CW included</td>
<td>-6.9 (±1.66)</td>
<td>-0.2 (±0.06)</td>
<td>ns</td>
<td>0.6 (±0.13)</td>
</tr>
<tr>
<td>Carcass lean (kg)</td>
<td>11.5 (±0.98)</td>
<td>11.0 (±0.18)</td>
<td>0.3 (±0.05)</td>
<td>0.2 (±0.08)</td>
<td>-0.5 (±0.13)</td>
</tr>
<tr>
<td></td>
<td>CW included</td>
<td>3.6 (±1.51)</td>
<td>0.1 (±0.05)</td>
<td>ns</td>
<td>-0.4 (±0.12)</td>
</tr>
<tr>
<td>Carcass bone (kg)</td>
<td>7.5 (±0.48)</td>
<td>7.3 (±0.09)</td>
<td>0.2 (±0.02)</td>
<td>0.1 (±0.04)</td>
<td>-0.3 (±0.06)</td>
</tr>
<tr>
<td></td>
<td>CW included</td>
<td>3.3 (±0.67)</td>
<td>0.1 (±0.02)</td>
<td>ns</td>
<td>-0.2 (±0.05)</td>
</tr>
<tr>
<td>GR depth (mm)</td>
<td>12.5 (±3.75)</td>
<td>12.5 (±0.57)</td>
<td>ns</td>
<td>1.0 (±0.45)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CW included</td>
<td>0.6 (±4.47)</td>
<td>ns</td>
<td>0.9 (±0.42)</td>
<td>0.5 (±0.17)</td>
</tr>
<tr>
<td>Condition Score</td>
<td>3.4 (±0.36)</td>
<td>3.3 (±0.06)</td>
<td>0.04 (±0.01)</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CW included</td>
<td>1.3 (±0.38)</td>
<td>ns</td>
<td>ns</td>
<td>0.1 (±0.01)</td>
</tr>
<tr>
<td>Carcass Ash (kg)</td>
<td>1.2 (±0.2)</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CW included</td>
<td>0.5 (±0.26)</td>
<td>ns</td>
<td>ns</td>
<td>0.03 (±0.01)</td>
</tr>
</tbody>
</table>
Ewe condition score explained more variation (54.6%) in total body fat than did liveweight (41.7%), however the combination of condition score and liveweight together explained more variation (61.1%) than either trait on its own. Tissue depth at the GR site explained the same amount of variation (54.6%) as condition score, however the combination of liveweight and GR tissue depth explained more variation (76.2%) in total body fat than the combination of liveweight and condition score (Table 4.3).

The variation in carcass fat was explained to similar degrees by carcass weight (58.6%), condition score (53.8%) and GR tissue depth (55.4%). The combination of carcass weight and condition score explained more variation (68.6%) than either of the components. The combination of carcass weight and GR tissue depth explained the greatest amount of variation (82.1%) in carcass fat of the traits and combinations examined (Table 4.3).

Carcass weight explained 64.6% of the variation in carcass lean tissue, however condition score only explained 9.1% of the variation in total carcass lean tissue. The combination of carcass weight and condition score explained more variation (72.5%) than carcass weight on its own. There was no independent effect ($P>0.05$) of GR tissue on the total amount of lean tissue in the carcass, however the combination of carcass
weight and GR tissue depth explained more variation (82.6%) than either carcass weight combined with condition score or carcass weight on its own (Table 4.3).

Table 4.3 Regression coefficients (± s.e.), model F values and correlation coefficients for models of carcass traits predicted by GR depth, condition score and liveweight.

All terms are significant (P<0.05) unless marked otherwise.

<table>
<thead>
<tr>
<th>Prediction Model</th>
<th>F value</th>
<th>r² or R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total fat (kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-4.03 (±2.62) + 0.26 (±0.05) LW</td>
<td>30.1</td>
<td>0.42</td>
</tr>
<tr>
<td>-5.92 (±2.29) + 4.84 (±0.68) CS</td>
<td>50.6</td>
<td>0.55</td>
</tr>
<tr>
<td>-8.78 (±2.41) + 0.13 (±0.05) LW + 3.59 (±0.8) CS</td>
<td>32.2</td>
<td>0.61</td>
</tr>
<tr>
<td>4.29 (±0.89) + 0.48 (±0.07) GR</td>
<td>49.4</td>
<td>0.55</td>
</tr>
<tr>
<td>-5.42 (±1.74) + 0.19 (±0.03) LW + 0.4 (±0.05) GR</td>
<td>64.1</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>Carcass fat (kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-3.25 (±1.42) + 0.41 (±0.05) CW</td>
<td>59.5</td>
<td>0.59</td>
</tr>
<tr>
<td>-3.26 (±1.56) + 3.25 (±0.46) CS</td>
<td>49.0</td>
<td>0.54</td>
</tr>
<tr>
<td>-5.6 (±1.41) + 0.27 (±0.06) CW + 1.82 (±0.5) CS</td>
<td>44.8</td>
<td>0.69</td>
</tr>
<tr>
<td>3.5 (±0.6) + 0.33 (±0.05) GR</td>
<td>51.0</td>
<td>0.55</td>
</tr>
<tr>
<td>-3.24 (±0.96) + 0.3 (±0.04) CW + 0.23 (±0.03) GR</td>
<td>91.5</td>
<td>0.82</td>
</tr>
<tr>
<td><strong>Carcass Lean (kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.18 (±1.18) + 0.39 (±0.04) CW</td>
<td>76.7</td>
<td>0.65</td>
</tr>
<tr>
<td>7.44 (±1.98) + 1.2 (±0.59) CS</td>
<td>4.2</td>
<td>0.09</td>
</tr>
<tr>
<td>3.06 (±1.19) + 0.5 (±0.05) CW - 1.46 (±0.43) CS</td>
<td>54.0</td>
<td>0.73</td>
</tr>
<tr>
<td>11.84 (±0.81) - 0.03 (±0.06) GR</td>
<td>0.2</td>
<td>ns</td>
</tr>
<tr>
<td>1.16 (±0.85) + 0.47 (±0.03) CW - 0.18 (±0.03) GR</td>
<td>95.1</td>
<td>0.83</td>
</tr>
</tbody>
</table>

4.4 Discussion

The total body fat measured by DXA was closely correlated with internal fat, carcass fat and total body fat measured by a combination of dissection and chemical methods. Therefore, our hypothesis that DXA can be used to accurately predict total body fat in live animals is supported. This finding is supported by previous studies which have
shown that DXA can accurately predict the amount of fat and lean in pig and sheep carcasses (Clarke et al. 1999; Marcoux et al. 2005; Mercier et al. 2006; Dunshea 2003; Dunshea et al. 2007). In addition the DXA method has been shown to be accurate in predicting carcass lean and fat tissue from live sheep (Pearce et al. 2009) and whole animal fat and lean tissue in live pigs (Suster et al. 2003). Although the results of this and other studies show good correlations between DXA derived values and chemically or gravimetrically derived values for fat, these correlations are not perfect. DXA values therefore need to be corrected to determine the actual amounts of lean or fat tissue to enable comparisons of body composition between different studies and methods of measurement. However in most circumstances where animals are being compared within a group or across time on the same DXA instrument, it is more important to be able to accurately rank animals on total body fat and lean tissue than to be able to determine the absolute quantities of these tissues. However if the measure is being used for energetic calculations it is important to be able to estimate the absolute quantities of these tissues. Measurement of whole body fat in the live animal by DXA is a reliable method of determining total body fatness in adult Merino ewes for the purposes of comparison within a group of individuals or across time. DXA can therefore be used as a non-destructive and relatively cheap method of serial measurement of body composition.

The FAT EBV was positively correlated with internal fat, carcass fat and total body fat and therefore our hypothesis that FAT EBV would be positively associated with fatness traits is supported. This finding is supported by studies that have shown that selection
for back fat depth using ultrasound measurement is successful at changing the proportion of fat in the body of sheep (Kadim et al. 1989; Cameron and Bracken 1992). The change in fatness is not only evident at the subcutaneous depot but ewes with genetically higher subcutaneous fat levels also had higher internal fat amounts. This result is supported by the results of Kirton and Johnson (1979) who reported a strong positive correlation between subcutaneous fat depth and omental and kidney fat weights. Furthermore, ewes with high FAT EBVs had lower weights of carcass lean tissue and lower weights of carcass bone. So ewes with high FAT EBVs have a greater proportion of fat but not a greater total amount of fat tissue. These results are supported by Cameron (1992b) and Lewis et al. (1996) who showed that rams selected for lower subcutaneous fat depth, higher growth and higher eye muscle depth produced lambs that had more lean tissue and less fat tissue than lambs from unselected equivalents. This and other studies have shown that selection for a change in fatness based on breeding values calculated from C-site fat depth may not change the total amount of fat in the body, but it will change the proportion of fat in the body of sheep.

The EMD EBV was not correlated with either internal fat, carcass fat, or total body fat. In support of this result, Ponnampalam et al. (2007a, 2007b) also found no effect of EMD breeding values on carcass fatness. By contrast, Southdown sheep bred for lower fat had lower levels of muscularity and lower muscle to bone ratios then sheep bred for high fat (Abdullah et al. 1998) and this result is in better agreement with positive
genetic and phenotypic correlations between EMD and FAT across multiple breeds (Bishop 1993; Gilmour et al. 1994; Clarke et al. 2003; Nsoso et al. 2004; Karamichou et al. 2007; Greeff et al. 2008). However, Hegarty et al. (2006a) demonstrated a negative correlation between muscling breeding value and carcass fatness. Furthermore, much lower whole body fat levels have been found in sheep with the Callipyge mutation (that exhibit muscle hypertrophy) compared to non-mutant equivalents (Jackson et al. 1997a). While there was no effect of EMD EBV on fatness in this study, ewes with higher EMD EBVs did have a higher amount of lean tissue (muscle) in the carcass. This result is supported by a similar finding by Lewis et al. (1996), while Ponnampalam et al. (2007a) found no effect of EMD breeding values on the weight of individual muscles in lamb carcasses. There was a slight positive correlation between EMD EBV and carcass bone weight in the current study. This is contrary to the work of Cake et al. (2007) who showed a negative correlation between bone length and EMD breeding value and Ponnampalam et al. (2007b) who demonstrated a negative correlation between DXA bone and EMD breeding value. In this population there was no correlation between EMD EBV and measures of body fatness, this result combined with those in the literature suggest that selection for muscling can have a positive, negative or no effect on total body fatness.

Ewes with higher WT EBVs had greater amounts of internal fat, carcass fat and total fat, however they did not have greater proportion of body fat since including liveweight in the model either removed the correlation between WT EBV and fatness or changed it to a negative correlation. In support of this result, Thompson et al. (1985)
found that selection for growth resulted in an increase in the total amount of fat but not
the proportion of fat in Merino sheep. Ewes with higher WT EBVs also had higher
total carcass lean and carcass bone. The increase in carcass bone is supported by the
results of Thompson et al. (1985) who also reported increased bone proportion in
Merino sheep selected for increased weaning weight. Ewes with higher WT EBVs had
a higher amount of total fat, total lean tissue and total bone but there was no change in
the proportion of the various tissues associated with the WT EBV.

The combination of tissue depth at the GR site and carcass weight were reliable
indicators of both total amount of lean tissue in the carcass and the total amount of fat
tissue in the carcass in Merino ewes. The combination of GR depth and carcass weight
explained more of the variation in carcass fat and carcass lean than either trait on its
own. This result is supported by Dunshea et al. (2007) who also found that the
combination of GR depth and carcass weight explained the majority of the variation in
the amounts of carcass fat and carcass lean. The combination of liveweight and GR
tissue depth also explained the majority of variation in total body fat in the live animal
a result supported by Kirton and Johnson (1979). In live sheep, total body fat was
moderately well predicted by condition score while a combination of liveweight and
condition score explained more of the variation in total body fat than either trait on its
own. Condition score on its own remains a useful determinant of body fatness and
nutritional status of breeding ewes, and explains more variation in total body fatness
than liveweight on its own, a result supported by Russel et al. (1969) and Yates and
Gleeson (1975). While the combination of GR tissue depth and liveweight explained
more variation than condition score and liveweight, it is difficult to reliably predict GR
tissue depth with sufficient accuracy. The accepted industry practice of valuing carcass
based on GR tissue depth and carcass weight seems wise based on the high
correlations of these two traits with total carcass lean tissue and fatness. In addition,
the accepted industry practice of condition scoring to determine the total body fatness
and therefore nutritional status of ewes (Curnow et al. 2010) is supported by our
results.
Chapter 5 Merino ewe muscling and growth breeding values impact on lamb birthweight and growth and on ewe fatness and milk production.

5.1 Introduction

There is now considerable interest in improving the carcass attributes of the Australian Merino. This can be achieved by selecting for animals with higher growth potential and improved muscling. The most effective means to achieve this is through the use of the genetic evaluation system MERINONSELECT™ provided by Sheep Genetics (Brown et al. 2007). This system provides Australian sheep breeding values (ASBVs) for a range of traits including age adjusted weight (HWT) and weight adjusted eye muscle depth (HEMD) at hogget age as indicators of growth and muscling. It is clear that selection for growth and muscling in this way will improve the value of the Merino as a meat animal (Hopkins et al. 2007a). However, it is also important to consider the effects that these selection strategies have on body composition and reproductive performance. Selection for higher HWT is unlikely to change body composition since selection for growth results in an increase in the total size of the muscle and fat depots but not in a change in their relative proportions at maturity (Thompson et al. 1987; Perry et al. 1988). However, selection for muscling is likely to change body composition. The aim of selection for increased muscling is to increase the proportion of lean meat in a carcass, which has proven successful (Hopkins et al. 2007a). Furthermore, progeny of sires with high eye muscle depth ASBVs had lower whole body fatness than progeny from sires with low eye muscle depth ASBVs (Hegarty et al. 2006a). Similarly, Hopkins et al. (2005a) reported a negative correlation between post-
weaning eye muscle depth ASBV and intramuscular fat levels. In addition, sheep with the callipyge mutation have an extreme muscling phenotype and have around 20% less body fat than non-mutant equivalents (Jackson et al. 1997a). Selection for muscling is therefore likely to reduce animal fatness while selection for higher growth is unlikely to change ewe body composition.

Differences in growth and body composition between genotypes may also be confirmed by hormone levels. Since the growth promotant effects of growth hormone are largely mediated by IGF-I, plasma concentrations of IGF-I can be positively related to growth (Oddy 1993) and may give an insight into any role of growth hormone. In addition, leptin concentration is well associated with fatness (Chilliard et al. 2001) and leptin levels may confirm any differences in body composition detected by other means. In addition the protein status of the animals under investigation may be reflected in levels of urea nitrogen (the product of amino acid breakdown) and plasma albumin, which is the largest pool of blood borne protein and represents an important store of labile protein (Lowrey et al. 1962). The known impacts of selection for growth and muscling on body composition have mostly been studied in young sheep from terminal breeds or their crosses and the impact of these selection strategies on the body composition and hormone levels of Merino ewes across the breeding cycle is currently unknown.

Selection for increased growth and muscling may change the maternal performance of a Merino ewe. Selection for growth results in an increase in mature size resulting in
several changes to maternal performance (Pattie 1965a). An important component of maternal performance is lamb birthweight because it is associated with lamb survival. While lamb birthweight is largely controlled by maternal nutrition (Kelly *et al.* 1996), sire and dam genetics also play a role (Chapter 3). Selection for increased HWT is likely to result in an increase in lamb birthweight since there are generally positive genetic and phenotypic correlations between growth traits and lamb birthweight (Safari *et al.* 2005; Chapter 3). Selection for higher HWT is also likely to result in higher ewe milk production and lamb growth considering the general across species relationship between mature size and milk production (Taylor 1973). Selection for higher lamb growth rate has resulted in an increase in mature weight and higher milk production across a number of studies (Näsholm and Danell 1996; Pattie 1965a; Head *et al.* 1996). As well as the impact of selection for growth, the impact of selection for muscling on maternal performance is also of interest. There is evidence that selection for increased ultrasound eye muscle depth can result in lower lamb birthweights but greater reproductive rate (Simm *et al.* 2002; Larsgard and Kolstad 2003; Chapter 3). There is also evidence that selection for muscling results in slower lamb growth (Larsgard and Kolstad 2003; Hopkins *et al.* 2007b). However there is no known relationship between muscling and milk production, although the reduced lamb growth rate does suggest lower milk production in high muscled ewes. In summary, selection of Merino ewes with higher HWT breeding values is expected to increase lamb birthweight, ewe milk production, and lamb growth rate, and selection for muscling is expected to decrease lamb birthweight and decrease lamb growth rate.
In this chapter we test the hypotheses: i) that increasing the HWT ASBVs of Merino ewes results in no change to body composition, higher milk production, heavier lambs at birth and weaning and higher plasma IGF-I levels; ii) that increase the HEMD ASBVs of ewes results in a lower proportion of fat, lower plasma leptin levels, lower milk production and lighter lambs at birth and weaning.

5.2 Material and Methods

A group of Merino ewes with a range in both HEMD and HWT ASBVs were monitored throughout a breeding cycle for changes in liveweight, condition score, body composition and concentrations of hormones and metabolites. Lamb birthweight and weaning weight as well as ewe milk production and quality were recorded. The experiment was approved and monitored by the CSIRO Floreat Animal Ethics Committee.

5.2.1 Animals

The 1.5 year old Merino ewes used for the experiment were from the Billandri Poll Merino stud. One hundred ewes were selected from a flock of 780 which had not previously lambed and had pedigree and ASBVs for HEMD and HWT available. The selected ewes were chosen to ensure a wide divergence in both HEMD and HWT ASBVs. This experiment was conducted on “Billandri” (34°28’15” S; 117°36’50” E) near Kendenup in Western Australia. All experimental animals were grazed in a single
paddock for the duration of the experiment. Pasture available to the ewes was an annual pasture of subterranean clover with volunteer annual grasses. The ewes were supplementary fed with a mixture of oat and lupin grain in autumn until pasture availability was sufficient. Ewes were weighed and condition scored (Jefferies 1961) regularly.

The timing of ovulation was synchronised using progesterone intra-vaginal sponges (Chronogest®, Intervet Aust. Bendigo, Australia) and ewes were artificially inseminated with semen from a single ram on 7th February 2006 (day 0). The same ram and two other rams that were half siblings and had similar ASBVs were introduced to the ewe flock on day 14 and removed on day 35 to mate those ewes that did not conceive to the artificial insemination. Ewes had pregnancy, fetus age and fetus number determined by ultrasound on day 89 post-conception. All ewes that had either conceived to artificial insemination or in the first 20 days following artificial insemination remained in the experiment. At lambing, ewes were regularly monitored and lambs were weighed and tagged within 12h of birth. Lambs were weighed at weaning on day 282 when they were approximately 18 weeks old. Single blood samples were collected from the ewes by jugular venipuncture into heparinised blood tubes just prior to conception (day -14), during mid-pregnancy (day 92), during late-pregnancy (day 141), during lactation (day 212) and at weaning (day 282). Samples were centrifuged and the plasma was harvested and frozen at -20°C for later laboratory analysis.
Ewe milk output was measured according to the method developed by McCance (1959) and refined by Bencini et al. (1995) and was measured on three occasions in lactation on days 178, 198 and 212. At each occasion ewes were removed from their lambs and restrained in a head bail with access to lupin seed to keep them content while milking. Ewes were administered with an intra-muscular injection of 1IU of synthetic oxytocin (Syntocin, Troy Laboratories, Smithfield, Australia) and then milked with a milking machine operated at a vacuum pressure of 40kPa and a pulsation rate of 100/min. Milk collected from the first milking was discarded. Ewes were kept separate from their lambs for 4h and the milking process was repeated. The total weight of milk collected at the second milking and the time between milkings was recorded. An estimate of milk production per day was calculated by scaling the milk weight recorded in approximately 4h to a 24h time period. A 10mL milk sample was kept from each ewe for later determination of composition. Milk composition was determined using a Milko Scan 133 (Foss Electric, Hillerød, Denmark) calibrated for sheep milk. The Milko Scan measures the infra red absorption at wavelengths characteristic of the components to be analysed and provided the proportions of fat, protein, and lactose in the milk sample.

5.2.2 Sample analysis

Plasma concentrations of insulin-like growth factor-1 (IGF-I) were measured on two sub-samples from each sample using the double antibody radioimmunoassay described by Breier et al. (1991). The samples were measured in two assays, the
proportion of tracer bound without competitor (B₀) was 43.6% and the non-specific binding (NSB) was 2.2%. The 50% maximal displacement on the standard curve was 14.7µg/L and the minimum detectable concentration was 1.7µg/L. Control samples included in the assay with average values of 4.7, 8.3, 15.0, 33.8, and 65.0µg/L had intra-assay CVs of 22.3, 12.1, 12.1, 11.5, and 10.0% and inter-assay CVs of 24.0, 17.0, 11.4, 12.2, and 15.6%. Plasma concentrations of leptin were measured on two sub-samples from each sample using the double antibody radioimmunoassay described by Blache et al. (2000). All samples were measured in a single assay with NSB of 1.7% and B₀ of 26.9%. The minimum detectable concentration was 0.07µg/L and 50% maximal displacement on the standard curve was at 1.1µg/L. Control samples included in the assay with average values of 0.51, 0.98 and 1.77µg/L had an intra-assay CV of 6.9%, 5.3% and 6.1%.

Plasma urea nitrogen and plasma albumin were measured in duplicate on single samples collected using the Infinity™ Urea Liquid stable reagent (Thermo Electron Co., Melbourne, Australia) in a Cobas Mira Autoanalyser (Hoffman-La Roche Diagnostica, Basal, Switzerland). Plasma Albumin was measured using a DMA Albumin kit from Thermo Electron Co., Melbourne, Australia (Cat. No.: TR36026) with the Cobas Mira Autoanalyser.

5.2.3 Body composition measurement

Body composition of ewes was measured just prior to conception (day -5) during late pregnancy (day 133), during lactation (day 217) and post-weaning (day 336) by Dual-
energy x-ray absorptiometry (DXA) using a Norland XR-26 Fan Beam X-Ray Whole Body Densitometer (Inderlec Medical Systems Baulkham Hills, Australia). Details regarding calibration of the DXA, anaesthesia of sheep and the scanning process are described by Pearce et al. (2009). Briefly, sheep were anaesthetised using inhalation of nitrous oxide to induce and isoflurane to maintain anaesthesia. There were then placed on the DXA unit in sternal recumbancy. The scan was completed using the Whole Body Scan mode and a regional analysis was performed to provide total bone mass (DXA bone), total lean mass (DXA lean) and total fat mass (DXA fat).

5.2.4 Statistical Analysis

All traits were analysed using linear mixed effects models in SAS software (SAS version 9.1, SAS Institute, Cary, NC, USA). The analysis included the fixed effect of physiological state (conception, mid-pregnancy, late-pregnancy, lactating, non-breeding) for each of liveweight, condition score, urea nitrogen concentration, albumin concentration, IGF-I concentration, and leptin concentration. The covariates included were HFAT, HWT and HEMD. Animal tag was used as a random term. Lamb birthweight and weaning weight were analysed with the fixed effects of sex (male, female), and lamb birth type (single, multiple). The covariates included were ewe HFAT, HWT, and HEMD. Milk production, fat, protein, and lactose were analysed with the fixed effect of day of milking (178, 198 and 212), and covariates of HFAT, HWT, HEMD, and condition score. All first order interactions were included in the starting model, and removed in a stepwise process if non-significant ($P>0.05$). Models were run with and without the inclusion of ewe liveweight and condition score.
5.3 Results

5.3.1 Ewe liveweight and condition score

Mean ewe live weight varied by around 20kg across the experiment and was lowest in mid pregnancy and highest at lamb weaning (Figure 5.1). Across all times ewe liveweight was positively associated with HWT and for each 1kg increase in HWT, ewe liveweight increased by 1.72±0.18kg (Table 5.1). For this and all results reported in this chapter the figures shown are means ±s.e. In addition, ewe liveweight was negatively associated with HEMD and for each 1mm increase in HEMD, ewe liveweight decreased by 0.85±0.31kg (Table 5.1). The condition score of ewes was lowest during early pregnancy and highest at lamb weaning (Figure 5.1). Ewe condition score was positively associated ($P<0.001$) with HEMD (Table 5.1).

Table 5.1 $F$ values for the effect of time, the number of lambs born and reared (rear type) and breeding values for weight (HWT) and eye muscle depth (HEMD) at hogget age, on ewe liveweight and condition score.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Liveweight</th>
<th>Condition score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF, DDF</td>
<td>$F$ value</td>
</tr>
<tr>
<td>Time</td>
<td>12, 1049</td>
<td>184.3***</td>
</tr>
<tr>
<td>Rear type</td>
<td>2, 1049</td>
<td>9.5***</td>
</tr>
<tr>
<td>HWT</td>
<td>1, 1049</td>
<td>86.7***</td>
</tr>
<tr>
<td>HEMD</td>
<td>1, 1049</td>
<td>7.7**</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom  
* $P<0.01$; **, $P<0.05$; ***, $P<0.01$
5.3.2 Ewe body composition

Dual-energy x-ray absorptiometry (DXA) was used to measure the total amount of fat (DXA fat) and lean tissue (DXA lean) in the body at conception, prior to lambing, during lactation, and following lamb weaning (Table 5.2). DXA fat was positively associated with both HWT ($P<0.001$) and HFAT ($P=0.06$). For each 1kg increase in HWT, DXA fat increased by $0.38\pm0.09$kg and for each 1mm increase in HFAT, DXA fat increased by $0.68\pm0.36$kg. There was no effect of HEMD on DXA fat (Table 5.3). When liveweight was included in the model it was positively associated ($F=32.2; P<0.001$) with DXA fat and for each 1kg increase in liveweight DXA fat increased by $0.16\pm0.03$kg. Furthermore, in the presence of this covariate the relationship between HFAT and DXA fat remained, however the association between HWT and DXA fat was no longer evident ($P>0.05$). In addition, when liveweight was included in the...
model, there was a significant effect (F= 2.7; \( P<0.05 \)) of HEMD which varied with the time of the DXA measurement. At conception and pre-lambing, HEMD and DXA fat were not associated; however the two traits were positively associated at the measurement during lactation (increase of 0.22±0.25kg DXA fat for each 1 mm increase in HEMD). Furthermore, there was a strong correlation between DXA fat and HEMD at the post-weaning measurement (increase of 0.62±0.27kg DXA fat for each 1mm increase in HEMD (Figure 5.2).

**Table 5.2 Liveweight, condition score, and total mass of fat, lean, and bone tissue measured by dual-energy x-ray absorptiometry (DXA) of ewes at conception, pre-lambing, lactation and post-weaning.**

<table>
<thead>
<tr>
<th>Time</th>
<th>DXA fat (kg)</th>
<th>DXA lean (kg)</th>
<th>DXA bone (kg)</th>
<th>Liveweight (kg)</th>
<th>Condition score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conception</td>
<td>6.1±0.2(^a)</td>
<td>36.4±0.3(^a)</td>
<td>0.94±0.01(^a)</td>
<td>48.6±0.4(^a)</td>
<td>2.9±0.04(^a)</td>
</tr>
<tr>
<td>Pre-lambing</td>
<td>3.9±0.3(^b)</td>
<td>41.6±0.4(^b)</td>
<td>0.98±0.01(^b)</td>
<td>53.4±0.5(^b)</td>
<td>2.8±0.05(^b)</td>
</tr>
<tr>
<td>Lactation</td>
<td>3.6±0.2(^b)</td>
<td>42.7±0.4(^b)</td>
<td>0.93±0.01(^a)</td>
<td>53.9±0.5(^b)</td>
<td>2.9±0.05(^b)</td>
</tr>
<tr>
<td>Post-weaning</td>
<td>4.7±0.3(^c)</td>
<td>40.5±0.4(^c)</td>
<td>1.04±0.01(^c)</td>
<td>53.7±0.5(^b)</td>
<td>3.0±0.05(^b)</td>
</tr>
</tbody>
</table>

\(^{abc}\) values within columns with different superscripts are different (\( P<0.01 \))
Figure 5.2 The effect of eye muscle depth (EMD) Australian sheep breeding value (ASBV) on dual-energy X-ray absorptiometry (DXA) measured fat tissue (liveweight included in the model).

DXA lean was positively associated ($P<0.001$) with HWT but negatively associated with HEMD (Table 5.3). For each 1 kg increase in HWT, DXA lean increased by 1.52±0.28kg and for each 1mm increase in HEMD, DXA lean decreased by 2.19±0.49kg. When liveweight was included in the model it was positively associated ($F=199.5; P<0.001$) with DXA lean. For each 1kg increase in liveweight DXA lean increased by 0.69±0.05kg. The addition of this covariate resulted in the relationship between HWT and DXA lean becoming non-significant ($P>0.05$). The negative association between HEMD and DXA lean remained significant ($P<0.01$) and varied with the time of measurement. At conception and at lambing, DXA lean and HEMD were not
associated ($P>0.05$). However, during lactation, for each 1mm increase in HEMD DXA lean decreased by $0.73\pm0.36$kg and following weaning for each 1mm increase in HEMD, DXA lean decreased by $1.09\pm0.35$kg (Figure 5.3).

Figure 5.3 The effect of eye muscle depth (EMD) Australian sheep breeding value (ASBV) on dual-energy X-ray absorptiometry (DXA) measured lean tissue (corrected to constant liveweight).
Table 5.3 $F$ values for the effect of physiological state, breeding values for weight (HWT), subcutaneous fat depth (HFAT), eye muscle depth (HEMD) at hogget age, and interactions of physiological state with HWT, HFAT and HEMD on total mass of fat, lean, and bone tissue measured by dual-energy x-ray absorptiometry (DXA).

<table>
<thead>
<tr>
<th>Effect</th>
<th>DXA fat</th>
<th></th>
<th>DXA lean</th>
<th></th>
<th>DXA bone</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF, DDF</td>
<td>$F$ value</td>
<td>NDF, DDF</td>
<td>$F$ value</td>
<td>NDF, DDF</td>
<td>$F$ value</td>
</tr>
<tr>
<td>State</td>
<td>3,178</td>
<td>15.7***</td>
<td>3,173</td>
<td>152.6***</td>
<td>3,175</td>
<td>30.4***</td>
</tr>
<tr>
<td>HWT</td>
<td>1,178</td>
<td>16.4***</td>
<td>1,173</td>
<td>46.7***</td>
<td>1,175</td>
<td>54.9***</td>
</tr>
<tr>
<td>HFAT</td>
<td>1,178</td>
<td>3.5*</td>
<td>ns</td>
<td>ns</td>
<td>1,175</td>
<td>2.1</td>
</tr>
<tr>
<td>HEMD</td>
<td>1,178</td>
<td>0.2</td>
<td>1,173</td>
<td>14.8***</td>
<td>1,175</td>
<td>0.6</td>
</tr>
<tr>
<td>State x HEMD</td>
<td>ns</td>
<td>ns</td>
<td>3,173</td>
<td>8.1***</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>State x HWT</td>
<td>ns</td>
<td>ns</td>
<td>3,173</td>
<td>3.5**</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>State x HFAT</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>3,175</td>
<td>5.27***</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom
Significant effect: ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

DXA bone varied across the physiological states (Table 5.2) and increased ($P<0.001$) as HWT increased (0.04±0.005kg DXA bone per kg HWT). DXA bone was negatively associated with HFAT during lactation (-0.06±0.002kg DXA bone per mm HFAT) and post-weaning (-0.05±0.002kg DXA bone per mm HFAT) but not during the other states (Table 5.3). When liveweight was included in the analysis it was positively associated (F=156.3; $P<0.001$) with DXA bone (0.02±0.001kg DXA bone per kg liveweight). The inclusion of liveweight in the analysis resulted in the effects of HFAT being no longer significant but HWT remained as a significant term.
5.3.3 Ewe milk production and quality

Ewe milk production averaged 1255±92g/day. Milk production was positively associated ($P<0.01$) with HWT and for each 1kg increase in HWT, milk production increased by 99±48g/day. Conversely, for each 1mm increase in HEMD milk production decreased by 82±42g/day (Table 5.4). When included in the model liveweight was positively associated ($F=6.0; P<0.05$) with milk production (26±11g/day milk production for each 1kg increase in liveweight). Including liveweight in the model resulted in the effects of HWT and HEMD on ewe milk production being no longer significant ($P>0.05$). The proportion of fat in the milk averaged 5.4±0.8% and there were no correlations between ewe ASBVs and proportion of fat in the milk. The proportion of protein in the milk averaged 5.1±0.1%, there were no effects of ewe breeding values on the percentage of protein in milk. Similarly, there were no effects of breeding values on the percentage of lactose in milk which averaged 5.2±0.1%.

5.3.4 Lamb birth and weaning weights

Lamb birthweight was higher ($P<0.001$) in single born lambs (5.29±0.11kg) than twin born lambs (3.89±0.17kg). Birthweights of male lambs (4.81±0.13kg) were higher ($P<0.05$) than those of female lambs (4.37±0.13kg). Lamb birthweight increased ($P<0.001$) with ewe HWT (0.16±0.05kg increase in birthweight per kg HWT) and decreased ($P<0.001$) with ewe HEMD (0.22±0.08kg decrease in lamb birthweight per mm HEMD). There was no effect of ewe HFAT on lamb birthweight. Lamb weaning weight was higher ($P<0.05$) in single born lambs (29.5±0.6kg) than twin born lambs.
Weaning weights of male lambs (29.2±0.9kg) were higher than those of female lambs (26.4±0.9kg). Lamb weaning weight increased \((P<0.01)\) with ewe HWT (0.9±0.3kg increase in weaning weight for each 1kg increase in ewe HWT). There were no effects of HFAT or HEMD on lamb weaning weights (Table 5.4).

Table 5.4 F values for the effect of breeding values for weight (HWT), and eye muscle depth (HEMD) at hogget age, lamb birth type, lamb sex on lamb weights at birth and weaning and for the effect of breeding values for weight (HWT), and eye muscle depth (HEMD) at hogget age, time of milking and interaction between time of milking and HWT on ewe milk production.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Lamb birthweight</th>
<th></th>
<th></th>
<th>Ewe milk production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF, DDF</td>
<td>F value</td>
<td>NDF, DDF</td>
<td>F value</td>
</tr>
<tr>
<td>HWT</td>
<td>1, 75</td>
<td>11.8***</td>
<td>1, 49</td>
<td>9.3***</td>
</tr>
<tr>
<td>HEMD</td>
<td>1, 75</td>
<td>7.7***</td>
<td>1, 49</td>
<td>1.8</td>
</tr>
<tr>
<td>Birth type</td>
<td>1, 75</td>
<td>52.4***</td>
<td>1, 49</td>
<td>5.2**</td>
</tr>
<tr>
<td>Sex</td>
<td>1, 75</td>
<td>6.3**</td>
<td>1, 49</td>
<td>ns</td>
</tr>
<tr>
<td>Time</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Time x HWT</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom
Significant effect: ns, not significant; *, \(P<0.05\); **, \(P<0.01\); ***, \(P<0.001\)

5.3.5 Ewe hormones and metabolites

Leptin concentration in plasma was highest at post-weaning and lowest during late-pregnancy and lactation (Table 5.5). Leptin concentration was positively associated with HFAT across all physiological states (0.12±0.06µg/L leptin per 1mm HFAT).
Leptin concentration was not associated \((P>0.05)\) with HEMD at conception, mid-pregnancy, lactation or post-weaning samplings, however at the late-pregnancy sampling leptin concentration decreased by 0.08±0.04µg/L for each 1mm increase in HEMD. When condition score was included in the model it was positively associated \((F=78.4; \ P<0.001)\) with leptin concentration \((0.43±0.05\mu g/L \text{ leptin per unit condition score})\). Leptin concentration was also positively associated \((F=14.2; \ P<0.001)\) with liveweight \((0.02±0.01\mu g/L \text{ leptin per kg liveweight})\) when included in the model.

The concentration of IGF-I was highest \((P<0.001)\) during late pregnancy and lowest during conception and post-weaning (Table 5.5). The concentration of IGF-I was positively associated \((P<0.05)\) with HEMD during mid-pregnancy \((4.32±1.48\mu g/L \text{ IGF-I per mm HEMD})\) but not during any other physiological state (Table 5.6). When condition score was included in the model it was positively associated \((F=33.8; \ P<0.001)\) with IGF-I concentration \((12.22±2.10\mu g/L \text{ IGF-I per unit condition score})\). Including condition score in the model resulted in the positive correlation between HEMD and IGF-I being non-significant \((P>0.05)\), however HWT was positively associated \((P<0.05)\) with IGF-I concentration during mid-pregnancy \((0.94±0.84\mu g/L \text{ IGF-I per kg HWT})\) and during late pregnancy \((1.51±0.88\mu g/L \text{ IGF-I per kg HWT})\). There was no significant effect of liveweight on IGF-I concentration when it was included in the model.
Table 5.5 Plasma concentration of leptin, insulin-like growth hormone-1 (IGF-I), urea nitrogen and albumin in ewes during conception, mid-pregnancy, late-pregnancy, lactation, and post-weaning.

<table>
<thead>
<tr>
<th>Time</th>
<th>Leptin µg/L</th>
<th>IGF-I µg/L</th>
<th>Urea Nitrogen mg/dL</th>
<th>Albumin g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conception</td>
<td>1.19±0.03a</td>
<td>14.6±1.3a</td>
<td>24.8±0.4a</td>
<td>37.7±1.1a</td>
</tr>
<tr>
<td>Mid-pregnancy</td>
<td>1.25±0.03a</td>
<td>29.8±1.4b</td>
<td>29.2±0.4b</td>
<td>37.7±1.1a</td>
</tr>
<tr>
<td>Late-pregnancy</td>
<td>0.85±0.04b</td>
<td>34.5±1.5c</td>
<td>18.8±0.5c</td>
<td>36.4±1.3a</td>
</tr>
<tr>
<td>Lactation</td>
<td>0.78±0.04b</td>
<td>21.6±1.7d</td>
<td>24.1±0.5a</td>
<td>41.5±1.4b</td>
</tr>
<tr>
<td>Post-weaning</td>
<td>1.48±0.04c</td>
<td>13.7±1.7a</td>
<td>12.5±0.5d</td>
<td>37.9±1.4ab</td>
</tr>
</tbody>
</table>

abcd values within columns with different superscripts are different (P<0.01)

There were no effects (P>0.05) of breeding values on plasma concentration of urea nitrogen. Neither condition score nor liveweight were associated (P>0.05) with the concentration of urea nitrogen. Plasma albumin concentration varied little throughout the year and was highest in lactation (Table 5.5). Plasma albumin concentration was positively associated with HWT during lactation (3.43±0.85g/L albumin per kg HWT) but not during any other physiological state (Table 5.6). Neither condition score nor liveweight had a significant effect (P>0.05) on plasma albumin concentration.
Table 5.6 $F$ values for the effect of physiological state, breeding values for weight (HWT), sub-cutaneous fat depth (HFAT), eye muscle depth (HEMD) at hogget age, and interactions of physiological state with HWT and HEMD on ewe plasma concentrations of leptin, insulin-like growth factor-I (IGF-I), urea nitrogen and albumin.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Leptin</th>
<th>IGF-I</th>
<th>Urea nitrogen</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF, DDF, $F$ value</td>
<td>NDF, DDF, $F$ value</td>
<td>NDF, DDF, $F$ value</td>
<td>NDF, DDF, $F$ value</td>
</tr>
<tr>
<td>State</td>
<td>4,273</td>
<td>71.5***</td>
<td>4,273</td>
<td>47.3***</td>
</tr>
<tr>
<td>HWT</td>
<td>1,273</td>
<td>0.7</td>
<td>1,273</td>
<td>1.8</td>
</tr>
<tr>
<td>HFAT</td>
<td>1,273</td>
<td>4.6*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>HEMD</td>
<td>1,273</td>
<td>0.2</td>
<td>1,273</td>
<td>4.2**</td>
</tr>
<tr>
<td>State x HEMD</td>
<td>4,273</td>
<td>2.5*</td>
<td>4,273</td>
<td>2.9*</td>
</tr>
<tr>
<td>State x HWT</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom
Significant effect: ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

5.4 Discussion

Lamb birthweight was positively associated with HWT and negatively associated with HEMD, supporting our initial hypotheses and results in Chapter 3. The positive correlation between HWT and birthweight was expected considering the general positive genetic and phenotypic correlations between growth traits and lamb birthweight (Safari et al. 2005). Likewise, the negative correlation between lamb birthweight and HEMD is supported by the results of Larsgard and Kolstad (2003) who found lower lamb birthweights from ewes selected for increased ultrasonic muscle depth compared with unselected ewes. Furthermore, Simm et al. (2002) reported a negative genetic correlation between eye muscle depth and lamb...
birthweight. Lambs with high eye muscle depth have been shown to have shorter bones and therefore smaller frames (Cake et al. 2007), and our results suggest that this effect of high muscling may also be evident at birth. An alternative explanation of lighter weight at birth in lambs from high HEMD ewes is related to the relative contribution of internal organs to liveweight. A positive correlation between muscling and dressing percentage was identified in slaughter lambs which suggests that high muscled lambs have lighter internal organs (Gardner et al. 2010). This could partially explain the lighter birthweight in high muscled lambs especially considering that the proportion of internal organs to bodyweight is highest when lambs are born (Butterfield 1988). Further work is required to elucidate the precise reason for lower birthweights in lambs from high muscled ewes. Importantly, lamb survival is maximised at an intermediate birthweight (Fogarty et al. 1992), thus the opposing effects of HWT and HEMD on birthweight can be used to manipulate the average birthweight of a flock and thus maximise lamb survival. Alternatively, selection strategies that aim to concurrently improve growth and muscling are likely to have no net effect on lamb birthweight and therefore lamb survival.

Ewe milk production was higher in ewes with high HWT and lower in ewes with high HEMD again supporting our hypotheses. These effects on milk production can be attributed to the impacts of selection for HWT and HEMD on mature liveweight. In this experiment HWT was closely associated with liveweight and therefore mature liveweight while HEMD was negatively associated with ewe liveweight. Increasing liveweight strongly aligned with greater ewe milk production, and importantly the
inclusion of liveweight in the analysis eliminated the significance of HWT and HEMD on milk production, confirming that the impacts of these ASBVs were directly associated with their effect on mature liveweight. This result aligns well with the known positive relationship between mature liveweight and milk production in sheep as well as other species (Taylor 1973; Thompson et al. 1987; Head et al. 1996). Therefore selection for muscling may decrease ewe milk production and selection for growth will increase it, in both cases due to their impact on mature liveweight which is directly related to total milk output.

In support of our initial hypothesis, ewes with high HWT breeding values weaned heavier lambs however, in contrast to our expectations, there was no decrease in weaning weight associated with the ASBV for HEMD. The increase in weaning weight associated with HWT is clearly anticipated as a result of both a likely higher mature weight of the lamb and therefore a more rapid growth path and also an improved milk supply to sustain growth. This result is supported by the findings of Pattie (1965a) who reported an increase in mature liveweight and milk production as a result of selection for higher weaning weights. The result is also supported by those of Lambe et al. (2005) who showed that the total weight of lean tissue and total weight of fat tissue in ewes was positively associated with the growth to weaning of their lambs. The reason that HEMD had no effect on lamb growth in this study is unclear. Considering the reduced milk supply and previous evidence for slower growth (Larsgard and Kolstad 2003; Hopkins et al. 2007b), a lower weight at weaning in high HEMD ewes was expected. Analysis of milk samples reveals that no differences can be
attributed to changes to milk composition as there were no effects of breeding value on the proportion of fat, protein and lactose in the milk. This result shows that although selection for higher muscling may result in lower weaning weights on some occasions, it is not always the case. As expected ewes with higher HWT breeding values weaned heavier lambs, unexpectedly there was no effect of HEMD on lamb weaning weight.

The proportion of DXA fat in ewes was not associated with the HWT ASBV and therefore our hypothesis that HWT would not be associated with body composition is supported. As expected DXA fat was positively associated with both HWT and liveweight. High HWT ewes were larger and therefore had a higher amount of both DXA fat and DXA lean but their relative proportion of fat remained unchanged and the inclusion of liveweight in the analysis resulted in the effect of HWT being no longer significant. In addition, both liveweight and HWT were also positively associated with DXA lean. These results demonstrate that selection for higher growth results in an increase in the total amount of fat and lean tissue in the body with no associated change in their relative proportions. This result is supported by the data of Thompson et al. (1987) and Perry et al. (1988) who showed that selection for increased weaning weight resulted in an increase in mature liveweight and the amount of both fat and muscle tissue but had no effect on the relative proportions of these tissues. Conversely, HFAT was positively associated with both the total amount of fat and the proportion of fat in the body given that the correlation between HFAT and DXA fat was evident with or without liveweight included in the analysis. This positive correlation is expected and is supported by previous work that has shown that selection for differences in
subcutaneous fat depth results in associated changes in fatness in all fat depots (Kadim et al. 1989; Cameron 1992b; Cameron and Bracken 1992). Selection of ewes with high HWT breeding values will result in an increase in total fat but not an increase in the proportion of fat in the body. The HFAT breeding value is associated with an increase in both the amount and proportion of fat in the body.

The total amount of fat in ewes increased with HEMD breeding value during lactation and post-weaning and therefore our hypothesis that ewes with higher HEMD breeding values will have less total body fat is rejected. The effect of HEMD on fatness during lactation and post-weaning was only evident when liveweight was included in the analysis and therefore HEMD was associated with an increase in the proportion of fat in the body. The effects of HEMD on the proportion of fat are directly mirrored in the effects of HEMD on DXA lean. There was no effect of HEMD on fatness at conception or in late pregnancy, however, the positive correlation between HEMD and ewe condition score across the physiological states also supports the finding of a positive correlation between HEMD and total body fat. This result is in contrast to those of Hegarty et al. (2006a) and Hopkins et al. (2005a), however these experiments were conducted on young growing animals that had not reached maturity which may explain the difference with the results here. In addition, Jackson et al. (1997a) reported a 20% reduction in fatness associated with the extremely high muscling sheep with the callipyge mutation, however it is not known to what extent these mutants are reflective of sheep with high eye muscle depth ASBVs. Alternatively, the positive correlation between HEMD and fatness is supported by the results of Kadim et al. (1989) and
Abdullah et al. (1998) who demonstrated that sheep bred for higher fatness had higher levels of muscularity and higher muscle to bone ratios than equivalents bred for lower fatness. In addition there are generally moderately positive phenotypic and genetic correlations between eye muscle depth and C-site fat depth (Safari et al. 2005). The correlation between muscling breeding values and body composition is variable, even in this study the correlation ranged from non-existent to positive depending on physiological state. Further work is required to elucidate the conditions under which the correlation between muscling and fatness are positive and those under which a negative correlation is expected. The positive correlation found here is important since it means that simultaneous increases in muscle and fat are possible in adult Merino ewes, which we hypothesise as an ideal, all be it unexpected, outcome.

The amount and proportion of DXA fat varied between physiological states and was reflected in plasma leptin concentrations. This pattern of mobilising fat tissue in late pregnancy and early lactation and then rebuilding reserves in late lactation and post-weaning is consistent with expectations and other work (Lambe et al. 2003). Changes in DXA fat across the breeding cycle were reflected in changes in leptin concentration which is expected since the proportion of fat is usually well associated with plasma leptin concentration (Chilliard et al. 2001; Chilliard et al. 2005; Delavaud et al. 2007). In addition, leptin concentration was well associated with ewe condition score and ewe liveweight across all physiological states, demonstrating the close correlation between leptin concentration and ewe fatness. Furthermore, leptin concentration was associated with HFAT across the experiment which is consistent with the observation
that ewes with higher HFAT breeding values do indeed have a higher proportion of total body fat. Leptin concentrations aligned as expected with DXA fat, ewe condition score, ewe liveweight and HFAT breeding values.

The observed HWT and HEMD effects on ewe maternal performance could be partly controlled by differences in the growth hormone axis. Since the growth promoting effects of growth hormone are largely mediated by IGF-I, plasma concentrations of IGF-I may give an insight into any role of growth hormone. IGF-I concentration varied across physiological states and was lowest when physiological demands were low at conception and post-weaning. IGF-I concentration was positively associated with both ewe liveweight and condition score which is consistent with its known effects of increasing protein gain and glucose uptake in sheep (Oddy and Owens 1996). The result is also supported by a positive correlation between IGF-I and body size in sheep (Medrano and Bradford 1991; Oddy 1993). IGF-I concentration was also positively associated with HEMD but only during mid-pregnancy and the effect disappeared when condition score differences were taken into account. When condition score was included in the analysis there was a positive correlation between HWT and IGF-I concentration in mid- and late-pregnancy. The reason for this correlation occurring in two of the five states measured is again unclear, however the result is supported by the results of Oddy (1993) that showed that sheep that had been selected for higher growth to weaning had higher IGF-I concentrations. Furthermore, IGF-I concentrations were found to be positively associated with growth rate in sheep (Carter et al. 1989; Hegarty et al. 2006b). Some differences in IGF-I concentrations do suggest that growth hormone
may play a part in observed differences in genotype and our tentative hypothesis is supported. However IGF-I concentrations are quite variable as demonstrated here and strongly affected by nutrition (Breier et al. 1986). Further work is under conditions where nutrition is better controlled is required to confirm these findings.

Selection strategies that aim to concurrently improve growth and muscling are likely to have no net effect on lamb birthweight. Selection for higher growth will also result in higher ewe milk production and higher lamb weaning weight. High growth ewes also have more total body fat but the same proportion of fat than low growth equivalents. High muscled ewes produced less milk but this was not reflected in lower lamb growth to weaning. High muscled ewes also had a greater amount of fat in the body than low muscled ewes at some points throughout the year suggesting that simultaneous increases in muscling and fatness are possible in adult Merino ewes. The HFAT breeding value is associated with both an increase in the amount and proportion of whole body fat.
Chapter 6 Lamb birthweight is buffered by maternal reserves of genetically fat ewes during restricted nutrition.

6.1 Introduction

Selection for reduced fatness is advocated in sheep breeding programs in Australia to improve consumer acceptance of meat products. This trait is selected for using Australian sheep breeding values (ASBV) for subcutaneous fat depth (HFAT) measured using ultrasound (Kadim et al. 1989; Cameron and Bracken 1992) at the C-site (a point between the 12th and 13th ribs and 45mm from the dorsal midline; Pálsson 1939). The ASBVs are generated within the MERINOSELECT™ genetic evaluation system (Brown et al. 2007). HFAT is expected to be correlated with total body fatness since the breeding value for fatness at post-weaning age is correlated with the amount of whole body fat (Hopkins et al. 2007a). Therefore, selection of Merinos for lower HFAT values is expected to successfully lower the amount and proportion of fat in the animal. Currently very few producers select for lower fatness in Merinos since they are known to be leaner than other breeds, but as a result of the genetic association between higher fleece weight and reduced fatness (Fogarty et al. 2003) continued selection for higher fleece weights will result in a leaner genotype. The changes toward a leaner genotype are likely to result in fundamental changes in metabolism and be reflected in increases in appetite as a result of lower circulating levels of leptin (Chilliard et al. 2001) and up-regulation of the growth hormone axis resulting in higher circulating levels of IGF-I (Robinson and Oddy 2004). It is important to understand the impacts of these changes to the performance of breeding ewes.
Any selection that results in lower fatness may impact negatively on maternal performance since there is a minimum amount of fat in the body necessary for normal reproductive function in mammals (Hastings et al. 1991). Selection for lower ewe fatness is expected to reduce maternal performance by lowering lamb birthweight and survival, ewe milk production and quality and lamb growth to weaning. Lambe et al. (2005) demonstrated that sheep that had proportionately more internal fat produced lambs of higher birthweight. Furthermore ewes that were phenotypically fatter in early pregnancy and underfed in late pregnancy produced heavier lambs than ewes that were lean in early pregnancy (McNeill et al. 1999). These findings suggest that in feed restricted environments ewes with higher HFAT breeding values, and therefore more fat at the start of pregnancy, may be able to better buffer the effects of low nutrition on the developing fetus resulting in heavier lamb birthweights. This would have important implications for lamb survival since lamb birthweight is closely related to lamb survival (Fogarty et al. 1992; Fogarty and Hall 1995; Ferguson et al. 2004a). Ewe fatness may also be important for ewe milk production and lamb growth under feed restricted environments. Ewe milk production is known to be well correlated with lamb growth rate (Snowder and Glimp 1991), and increasing the dietary energy intake of ewes is known to increase ewe milk production and lamb growth (Langlands 1977; Jordan and Mayer 1989; Thompson et al. 2010). However, often in lactation nutritional demands are not met resulting in the mobilisation of fat tissue (Vernon and Finley 1985; Joseph and Foot 1990). Under these conditions ewe milk production and lamb growth is greatest in ewes that have the largest mobilisation of fat tissue (Brand and
Franck 2000). Lambe et al. (2005) demonstrated positive genetic correlations between pre-mating and pre-lambing fat proportions in ewes and the subsequent growth rate and weaning weight of offspring. These findings suggest that fatter ewes produce either more milk or better quality milk. Furthermore, ewes with higher HFAT breeding values are expected to have lambs of higher birthweight and improved growth, particularly when run under low nutrition. However there appears to be no studies that have looked at the combined effects of genetic (eg. HFAT) and phenotypic fatness (eg. condition score).

We tested the hypotheses that under restricted nutrition, ewes with higher HFAT breeding values would be phenotypically fatter, would have higher circulating leptin and IGF-I, produce lambs of higher birthweight, would produce more milk of higher fat percentage and wean heavier lambs.

6.2 Materials and Methods

Merino ewes with a wide divergence in HFAT breeding values were managed under either maintenance or sub-maintenance nutrition. The combined effects of ewe nutrition and ewe HFAT breeding value on ewe body composition, lamb birthweight and survival, ewe milk production and lamb growth were monitored for a breeding cycle. The experiment was approved and monitored by the CSIRO Floreat Animal Ethics Committee.
6.2.1 Experimental details

This experiment was conducted on “Yarrak” (33°56′15″ S; 116°56′25″ E) near Kojonup in Western Australia. A group of 100 Merino ewes were selected based on their HFAT from a group of 282 ewes from the Merinotech (WA) Ltd nucleus flock. The ewes were 1.5 years old and had not previously lambed. The 100 selected ewes were allocated to two different nutritional groups (low and high; n=50 in each). Allocation of ewes to the nutritional groups was completed to ensure the groups were balanced for average HFAT (low 0.5±0.1mm; high 0.6±0.1mm) as well as average hogget age ASBVs for eye muscle depth (HEMD; low 1.1±0.1mm; high 1.2±0.1mm) and weight (HWT; 5.0±0.3kg; 4.6±0.3kg).

The timing of ovulation was synchronised using progesterone intra-vaginal sponges (Chronogest®, Intervet Aust. Bendigo, Australia) and ewes were artificially inseminated with semen from two Merino rams on 13th February 2006 (day 0). On day 43 the ewes were allocated into two groups of 50 and different nutritional regimes were applied with one group being managed to be in an average condition score of 2.5 in late pregnancy (low) and the second group managed to be in an average condition score of 3 in late pregnancy (high). In order to achieve differences in condition different levels of pasture were made available as well as different levels of supplementary feeding of a pelletised sheep ration. Differential nutrition continued until day 198 when the groups were combined. On day 176 the nutrition supplied to ewes in the low group was improved to allow sufficient lamb weight at weaning.
Ewes were managed on cereal stubbles and annual pastures of subterranean clover with volunteer annual grasses throughout the experiment and they received supplementary feed in the form of oat grain or a commercial sheep pellet when required. Available pasture and supplementary feeding levels were adjusted regularly to achieve target condition scores. Ewes (and lambs once born) were weighed and condition scored (Jeffries 1961) regularly.

Pregnancy and fetus number were determined by ultrasound on day 88. All ewes that had conceived to artificial insemination remained in the experiment. At lambing, ewes were regularly monitored and lambs were weighed and tagged within 12 hours of birth, lambs were born over an 8 day period. Lambs were weighed at weaning on day 273. Single blood samples were collected from the ewes by jugular venipuncture into heparinised blood tubes at conception (day -14), during mid-pregnancy (day 86), pre-lambing (day 135), during lactation (day 194) and prior to weaning (day 246). Samples were centrifuged and the plasma was harvested and frozen at -20°C for later laboratory analysis.

Ewe milk output was measured on days 175 and 191 using the method developed by McCance (1959) and refined by Bencini et al. (1995). At each occasion the ewes were removed from their lambs and restrained in a head bail with access to lupin seed to keep them content while milking. Ewes were administered with an intra-muscular injection of 1IU of synthetic oxytocin (Syntocin, Troy Laboratories, Smithfield,
Australia) and then milked with a milking machine operated at a vacuum pressure of 40kPa and a pulsation rate of 100/min. Milk collected from the first milking was discarded. Ewes were kept separate from their lambs for 4h and the milking process was repeated. The total weight of milk collected at the second milking and the time between milkings was recorded. An estimate of milk production per day was calculated by scaling the milk weight recorded in approximately 4h to a 24h time period. A 10mL milk sample was kept from each ewe for later determination of composition. Milk composition was determined using a Milko Scan 133 (Foss Electric, Hillerød, Denmark) calibrated for sheep milk. The Milko Scan measures the infra-red absorption at wavelengths characteristic of the components to be analysed and provided the proportions of fat, protein, and lactose in the milk sample.

6.2.2 Plasma sample analysis

Plasma concentrations of insulin-like growth factor-1 (IGF-I) were measured on two sub-samples from each sample using the double antibody radioimmunoassay described by Breier et al. (1991). The samples were measured in two assays, the proportion of tracer bound without competitor (Bo) was 43.6% and the non-specific binding was 2.2%. The 50% maximal displacement on the standard curve was 14.7µg/L and the minimum detectable concentration was 1.7µg/L. Control samples included in the assay with average values of 4.7, 8.3, 15.0, 33.8, and 65.0 µg/L had intra-assay coefficient of variation of 22.3, 12.1, 12.1, 11.5, and 10.0% and inter-assay coefficient of variation of 24.0, 17.0, 11.4, 12.2, and 15.6%.
Plasma concentrations of leptin were measured on two sub-samples from each sample using the double antibody radioimmunoassay described by Blache et al. (2000). All samples were measured in a single assay with non-specific binding of 1.7% and B0 of 26.9%. The minimum detectable concentration was 0.07µg/L and 50% maximal displacement on the standard curve was at 1.1µg/L. Control samples included in the assay with average values of 0.51, 0.98 and 1.77µg/L had an intra-assay coefficient of variation of 6.9%, 5.3% and 6.1%.

Plasma urea nitrogen and plasma albumin were measured in duplicate on single samples collected using the Infinity™ Urea Liquid stable reagent (Thermo Electron Co., Melbourne, Australia) in a Cobas Mira Autoanalyser (Hoffman-La Roche Diagnostica, Basal, Switzerland). Plasma Albumin was measured using a DMA Albumin kit from Thermo Electron Co., Melbourne, Australia (Cat. No.: TR36026) with the Cobas Mira Autoanalyser.

6.2.3 Body composition measurement

Body composition was measured at conception (day -7) during late pregnancy (day 130), during lactation (day 197) and post-weaning (day 331) by Dual-energy x-ray absorptiometry (DXA) using a Norland XR-26 Fan Beam X-Ray Whole Body Densitometer (Inderlec Medical Systems Baulkham Hills, Australia). The DXA scanning was completed following the method of Pearce et al. (2009). The scan was
completed using the Whole Body Scan mode and a regional analysis was performed on
the image produced to provide total bone mass (DXA bone), total lean mass (DXA
lean) and total fat mass (DXA fat).

6.2.4 Statistical Analysis

All traits were analysed using linear mixed effects models in SAS software (SAS
version 9.1, SAS Institute, Cary, NC, USA). All analyses were completed with nutrition
treatment (low, high) and birth type (single, multiple) as fixed effects and HFAT, HWT
and HEMD as covariates. Animal was used as a random term. All first order
interactions were included in the starting model, and removed in a stepwise process if
non-significant ($P>0.05$). In addition, all models were run with and without the
inclusion of ewe liveweight and condition score except where these were the
dependent variable. The analysis of ewe liveweight and condition score included the
additional fixed effect of Time (-7, 43, 58, 70, 79, 86, 98, 109, 120, 135, 162, 171, 184, 190,
246, 273 or 336 days from artificial insemination). The analyses of DXA tissue weights
included an additional fixed effect of physiological state (conception, pre-lambing,
lactation, post-weaning). The analyses of lamb weights at birth and weaning included
an additional fixed effect of lamb sex with lamb sire included as an additional random
term. The analyses of ewe metabolites and hormones had an additional fixed effect of
physiological state (conception, mid-pregnancy, pre-lambing, lactation and weaning)
and the analyses of milk production, fat, protein, and lactose percentage were analysed
with the additional fixed effect of day of milking (175 or 191 days from artificial insemination).

6.3 Results

6.3.1 Ewe liveweight and condition score

Ewes in the high nutrition treatment were heavier ($P<0.001$) than those in the low nutrition treatment for the majority of the experiment (Figure 6.1). Due to poor seasonal conditions and despite heavy supplementary feeding it was not possible to maintain the high nutrition group at condition score 3 in very late pregnancy and lactation. Ewes that gave birth to twin lambs were 1.5kg heavier than those that gave birth to single lambs (50.7±0.7 v 49.2±0.5kg). For these and all results reported in this chapter the figures shown are means ±s.e. Ewe liveweight increased ($P<0.001$) with HWT (0.85±0.20kg liveweight per kg HWT) across both nutritional treatments and at all times (Table 6.1). There was a general negative association between HFAT and liveweight however the relationship was only significant ($P<0.05$) between day 120 and 336. The effect of HFAT on liveweight became more negative over time, reducing it by 0.95±0.66kg per mm HFAT on day 120 and 2.40±0.66kg per mm HFAT on day 336. There was also a negative relationship between HEMD and ewe liveweight across both treatments ($P<0.05$) between day -7 (1.39±0.56kg liveweight per mm HEMD) and day 135 (1.89±0.57kg liveweight per mm HEMD) with the HEMD effect relatively constant between these times.
Figure 6.1 Live weight a), and condition score b), of ewes fed high (black line filled in circles) or low (grey line open circles) nutrition across the experiment in relation to day 0 (13 February 2006).

Ewes in the high nutrition treatment were in a higher condition score for the majority of the experiment (Figure 6.1). The condition score of ewes with higher HWT tended (P=0.06) to be lower (0.04±0.02 condition score units per kg HWT). The effect was only significant in mid to late pregnancy on days 79, 86, 98, 109, 120, and 135. There was no effect of HFAT or HEMD breeding values on ewe condition score (Table 6.1).
Table 6.1 F values for the effect of ewe nutrition treatment, time, the number of
lambs born and reared (rear type), and breeding values for fat (HFAT), eye muscle
depth (HEMD) and weight (HWT) at hogget age and significant interactions
between terms on ewe liveweight and condition score.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Liveweight</th>
<th>Condition score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF, DDF</td>
<td>F value</td>
</tr>
<tr>
<td>Nutrition</td>
<td>1, 1213</td>
<td>19.5***</td>
</tr>
<tr>
<td>Time</td>
<td>16, 1213</td>
<td>80.8***</td>
</tr>
<tr>
<td>Nutrition x Time</td>
<td>16, 1213</td>
<td>12.8***</td>
</tr>
<tr>
<td>Rear type</td>
<td>1, 1213</td>
<td>3.5*</td>
</tr>
<tr>
<td>HFAT</td>
<td>1, 1213</td>
<td>2.1</td>
</tr>
<tr>
<td>HEMD</td>
<td>1, 1213</td>
<td>2.3</td>
</tr>
<tr>
<td>HWT</td>
<td>1, 1213</td>
<td>18.1***</td>
</tr>
<tr>
<td>HFAT x Time</td>
<td>16, 1213</td>
<td>2.7***</td>
</tr>
<tr>
<td>HEMD x Time</td>
<td>16, 1213</td>
<td>2.3***</td>
</tr>
<tr>
<td>HWT x Time</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom
Significant effect: ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001

6.3.2 Body composition

The amount of fat in the live animal measured by DXA varied throughout the
reproductive cycle and was lower (P<0.01) in the ewes in the low nutrition treatment
than those in the high nutrition treatment at the pre-lambing and post-weaning scans
(Table 6.2). DXA fat was lowest in lactation and highest at the post-weaning scans
(Table 6.2). Across both nutritional treatments DXA fat increased (P<0.01) with HFAT
at conception (1.46±0.42kg DXA fat per mm HFAT) and post-weaning (0.90±0.34kg
DXA fat per mm HFAT). There was also a positive trend between HFAT and DXA fat at pre-lambing and lactation measurements however the effect was not significant ($P>0.05$) at these times.

Table 6.2 Total mass of fat, lean, and bone tissue measured by dual-energy x-ray absorptiometry (DXA) of ewes from low and high nutritional treatments at conception, pre-lambing, lactation and weaning.

<table>
<thead>
<tr>
<th>State</th>
<th>Fat (kg)</th>
<th>Lean (kg)</th>
<th>Bone (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conception</td>
<td>4.4±0.3a</td>
<td>4.7±0.3a</td>
<td>ns</td>
</tr>
<tr>
<td>Pre-lambing</td>
<td>1.6±0.3b</td>
<td>2.9±0.3b</td>
<td>**</td>
</tr>
<tr>
<td>Lactation</td>
<td>1.1±0.3b</td>
<td>1.6±0.3c</td>
<td>ns</td>
</tr>
<tr>
<td>Weaning</td>
<td>5.3±0.3c</td>
<td>6.5±0.3d</td>
<td>**</td>
</tr>
</tbody>
</table>

abcd values with different superscripts within a column are different ($P<0.05$)

Nut. sig.: Difference between nutrition groups;
ns, not significant; * $P<0.05$; **, $P<0.01$; ***, $P<0.001$

Across physiological states increasing HEMD resulted in a reduction ($P<0.05$) in DXA fat in the high nutrition treatment (0.71±0.32kg DXA fat per mm HEMD), however there was no effect of HEMD on DXA fat at low nutrition. There was no effect of HWT on DXA fat (Table 6.3). Including liveweight in the analysis illustrated that increasing liveweight resulted in an increase ($F=29.8$; $P<0.001$) in DXA fat (0.12±0.02kg DXA fat per kg liveweight). When liveweight was included in the analysis, DXA fat increased ($P<0.05$) by 1.71±0.39, 0.53±0.42, 0.45±0.44, and 1.31±0.30kg per mm increase in HFAT at conception, pre-lambing, lactation and weaning measurements (Figure 6.2).
Figure 6.2 The effect of Australian sheep breeding value (ASBV) for ewe fatness (HFAT) on DXA fat when measured at conception (---), pre-lambing (----), mid-lactation (— ) and weaning (— ). Liveweight was included in the analysis. The relationship is significant (P<0.05) only at conception and post weaning measurements.

The effect of condition score was significant ($F=76.5; P<0.001$) when included in the analysis demonstrating that DXA fat increased with condition score (2.6±0.3kg DXA fat per unit condition score). There were no significant changes to the model associated with the inclusion of condition score.
Table 6.3 F values for the effect of nutrition treatment, physiological state, the number of lambs carried (birth type), Australian sheep breeding values for subcutaneous fat depth (HFAT), eye muscle depth (HEMD), and weight (HWT) at hogget age, and interactions of physiological state with nutrition, HWT, HFAT and HEMD on total mass of fat, lean, and bone tissue measured by dual-energy x-ray absorptiometry (DXA).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Fat</th>
<th>Lean</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF, DDF</td>
<td>F value</td>
<td>NDF, DDF</td>
</tr>
<tr>
<td>Nutrition</td>
<td>1, 210</td>
<td>11.5***</td>
<td>1, 207</td>
</tr>
<tr>
<td>State</td>
<td>3, 210</td>
<td>46.0***</td>
<td>3, 207</td>
</tr>
<tr>
<td>Nutrition x State</td>
<td>3, 210</td>
<td>1.7</td>
<td>3, 207</td>
</tr>
<tr>
<td>Birth type</td>
<td>ns</td>
<td>ns</td>
<td>1, 207</td>
</tr>
<tr>
<td>HFAT</td>
<td>1, 210</td>
<td>9.6**</td>
<td>1, 207</td>
</tr>
<tr>
<td>HEMD</td>
<td>1, 210</td>
<td>4.5*</td>
<td>1, 207</td>
</tr>
<tr>
<td>HWT</td>
<td>ns</td>
<td>ns</td>
<td>1, 207</td>
</tr>
<tr>
<td>HFAT x State</td>
<td>3, 210</td>
<td>3.8*</td>
<td>3, 207</td>
</tr>
<tr>
<td>HEMD x State</td>
<td>ns</td>
<td>ns</td>
<td>3, 207</td>
</tr>
<tr>
<td>HEMD x Nutrition</td>
<td>1, 210</td>
<td>3.7*</td>
<td>1, 207</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom
ns: not significant, * P<0.05; **, P<0.01; ***, P<0.001

DXA lean varied between physiological states and was lower (P<0.001) in the low nutrition treatment than the high nutrition treatment at the pre-lambing and lactation measurements but not at conception and post-weaning (Table 6.2). DXA lean was lowest at conception and highest at pre-lambing measurement. Across both nutritional treatments ewes with higher HFAT had lower (P<0.05) DXA lean at conception.
(2.7±0.6kg DXA lean per mm HFAT), pre-lambing (0.8±0.7kg DXA lean per mm HFAT), lactation (2.6±0.7kg DXA lean per mm HFAT) and post-weaning (3.4±0.7kg DXA lean per mm HFAT). DXA lean was lower (P<0.05) in ewes with higher HEMD at lambing (0.8±0.6kg DXA lean per mm HEMD) but not during any other state. The effect of HEMD on DXA lean also varied with nutritional level. At high nutrition DXA lean increased with ewe HEMD (1.0±0.8kg DXA lean per mm HEMD), however at low nutrition DXA lean decreased with an increase in ewe HEMD (1.1±0.7kg DXA lean per mm HEMD). Ewes with higher HWT had higher (P<0.001) DXA lean (0.7±0.2kg DXA lean per kg HWT) across time, state and nutritional treatment (Table 6.3). When liveweight was included in the analysis it had a significant effect on DXA lean (F=140.4; P<0.001). The inclusion of liveweight in the analysis resulted in the negative effect of HEMD on DXA lean at lambing being no longer significant (P>0.05) however the general effect of HEMD on DXA lean at low and high nutrition remained. In addition, the effect of HFAT was no longer different between physiological states and there was just a general negative effect of HFAT on DXA lean across all states (2.1±0.4kg DXA lean per mm HFAT). There were no other changes to the analysis as a result of including liveweight. Condition score was not significant (P>0.05) in the model.

DXA bone varied between physiological states and with nutritional treatment (Table 6.2). Ewes with higher HWT also had higher (P<0.001) DXA bone (0.02±0.01kg DXA bone per kg HWT) across states and nutritional treatments (Table 6.3). The effect of HEMD on DXA bone depended on nutritional level, at high nutrition DXA bone increased (P<0.05) with HEMD (0.04±0.03kg DXA bone per mm HEMD), however at
low nutrition DXA bone decreased with increases in HEMD (0.03±0.02kg DXA bone per mm HEMD). There was no effect of HFAT on DXA bone (Table 6.3). When liveweight was included in the analysis its effect was significant ($P<0.001$) and DXA bone increased by 0.01±0.001kg per kg liveweight increase. The inclusion of liveweight removed the significance of HWT, although there were no other changes to the model. When condition score was included in the analysis it had a significant effect ($P<0.001$) and DXA bone increased by 0.11±0.02kg per condition score unit increase.

6.3.3 Lamb birthweight

The birthweight of lambs from ewes in the low nutrition treatment (3.53±0.09kg) were 12% lighter ($P<0.001$) than those from ewes on high nutrition (4.07±0.09kg). In addition, the birthweight of single born lambs (4.30±0.08kg) was 1kg higher ($P<0.001$) than twin born lambs (3.30±0.10kg). Ewes with higher HEMD breeding values produced lambs of lower birthweight (0.29±0.10kg birthweight per mm HEMD) resulting in around 0.8kg difference in lamb birthweight across the range of HEMD values in this study. The effect of HFAT on lamb birthweight was dependent on ewe nutritional treatment (Table 6.4). For ewes on low nutrition, lamb birthweight increased (($P<0.05$) by 0.40±0.14kg for each 1 mm increase in HFAT, whereas for ewes on high nutrition there was no effect ($P>0.05$) of HFAT on lamb birthweight (Figure 6.3).
Figure 6.3 The effect of ewe fatness (HFAT) Australian sheep breeding value (ASBV) on lamb birthweight when ewes are managed on low or high nutrition during pregnancy.

When ewe liveweight at day 135 was included in the analysis it had a significant effect ($F=16.9; P<0.001$) on lamb birthweight. For each 1kg increase in liveweight at lambing, lamb birthweight increased by $0.05\pm0.01$kg. The inclusion of ewe liveweight in the analysis resulted in the effect of lamb sex on lamb birthweight being significant with male lambs ($3.91\pm0.07$kg) heavier than female lambs ($3.70\pm0.08$kg). There were no other major changes to the model as a result of including liveweight. The effect of condition score at lambing on lamb birthweight was not significant when it was included in the model.
Table 6.4 *F* values for the effect of ewe nutrition, lamb birth type, lamb sex, ewe breeding values for weight (HFAT), and eye muscle depth (HEMD) at hogget age, and the significant interactions between terms on lamb weights at birth and weaning.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Lamb birthweight</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NDF,</td>
<td><em>F</em></td>
<td>NDF,</td>
<td><em>F</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DDF</td>
<td>value</td>
<td>DDF</td>
<td>value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrition</td>
<td>1, 23</td>
<td>22.6</td>
<td>***</td>
<td>1, 18</td>
<td>9.0</td>
<td>**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth type</td>
<td>1, 23</td>
<td>63.0</td>
<td>***</td>
<td>1, 18</td>
<td>15.9</td>
<td>***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>1, 23</td>
<td>2.6</td>
<td></td>
<td>1, 18</td>
<td>9.8</td>
<td>**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HFAT</td>
<td>1, 23</td>
<td>2.1</td>
<td></td>
<td>1, 18</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEMD</td>
<td>1, 23</td>
<td>8.8</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HFAT x Nutrition</td>
<td>1, 23</td>
<td>5.2</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth type x Nutrition</td>
<td>ns</td>
<td></td>
<td></td>
<td>1, 18</td>
<td>6.9</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom
ns, non-significant; *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001

6.3.4 *Lamb weight at weaning*

The weight at weaning of male lambs (31.2±0.7kg) was about 10% heavier than female lambs (28.6±0.7 kg). The effect of lamb birth type on lamb weight at weaning differed (*P* < 0.01) with ewe nutrition treatment. For lambs raised in the low nutrition treatment the weight at weaning in single born lambs (29.0±0.8kg) were about 5% heavier (*P* < 0.05) than twin born lambs (27.6±1.3kg). However, for lambs raised in the high nutrition treatment, the lamb weights at weaning for single born lambs (35.0±1.0kg) were about 25% heavier (*P* < 0.01) than twin born lambs (28.0±1.1kg). There was no effect (*P* > 0.05) of ewe HFAT or other breeding values on lamb weight at weaning (Table 6.4).
6.3.5 Ewe milk production and quality

Across both milking times, milk production was 60% higher ($P<0.001$) in ewes in the high nutrition treatment (1.67±0.07 kg/day) than the low nutrition treatment (1.05±0.08kg/day). In the high nutrition group, milk production was not different ($P>0.05$) between the first (1.73±0.10kg/day) and second milkings (1.62±0.10kg/day). However, in the low nutrition treatment, ewe milk production almost doubled between the first (0.79±0.11kg/day) and second (1.32±0.10kg/day) milking. Milk production from ewes rearing twin lambs (1.54±0.09kg/day) was 30% higher than those rearing a single lamb (1.19±0.07kg/day) across both nutritional treatments. There was no effect ($P>0.05$) of HFAT on ewe milk production but there was an effect of HEMD on ewe milk production that varied with nutritional treatment (Table 6.5). In the high nutrition treatment as ewe HEMD increased ewe milk production decreased ($P<0.05$) by 0.16±0.15kg/day milk per mm HEMD. However, in the low nutrition treatment as ewe HEMD increased ewe milk production also increased by 0.16±0.12kg/day milk per mm HEMD. When liveweight was included it was positively correlated ($F=7.9; P<0.01$) with ewe milk production (0.03±0.01kg/day per kg liveweight) however there were no other changes to the model. Condition score had no effect ($P>0.05$) on milk production when it was included in the model.
Table 6.5 F values for the effect of nutrition treatment, milking occasion (time), the number of lambs carried (birth type), breeding values for sub-cutaneous fat depth (HFAT) and eye muscle depth (HEMD) at hogget age, and significant interactions between terms on ewe milk production, and the percentage of fat, protein and lactose in the milk.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Milk Production</th>
<th>Milk fat</th>
<th>Milk protein</th>
<th>Milk lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF, DDF F value</td>
<td>NDF, DDF F value</td>
<td>NDF, DDF F value</td>
<td>NDF, DDF F value</td>
</tr>
<tr>
<td>Nutrition</td>
<td>1, 60 23.9***</td>
<td>1, 61 0.7</td>
<td>1, 60 0.5</td>
<td>1, 60 1.3</td>
</tr>
<tr>
<td>Time</td>
<td>1, 60 5.6*</td>
<td>1, 61 3.3*</td>
<td>1, 60 2.5</td>
<td>1, 60 2.5</td>
</tr>
<tr>
<td>Nutrition x Time</td>
<td>1, 60 13.4***</td>
<td>ns</td>
<td>ns</td>
<td>1, 60 9.5**</td>
</tr>
<tr>
<td>Birth type</td>
<td>1, 60 9.9**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>HFAT</td>
<td>1, 60 0.12</td>
<td>1, 61 2.5</td>
<td>1, 60 0.1</td>
<td>1, 60 5.9*</td>
</tr>
<tr>
<td>HEMD</td>
<td>1, 60 0.0</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>HFAT x Nutrition</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>HEMD x Nutrition</td>
<td>1, 60 4.5*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom
ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001

The percentage of fat in the milk was not different (P>0.05) between the low (6.37±0.16%) and high (7.36±0.16%) ewe nutritional treatments (Table 6.5), however there was an effect of HFAT on milk fat percentage that differed between nutritional treatments. For ewes in the low nutrition treatment, milk fat percentage decreased by 0.42±0.25% for each 1mm increase in HFAT, however for ewes in the high nutrition treatment, milk fat percentage increased by 1.01±0.37% for each 1mm increase in HFAT. There were no effects of other ASBVs on the percentage of fat in the milk (Table 6.5). There was no effect (P>0.05) of ewe liveweight at milking when it was included in the model.
The percentage of protein in the milk was also not different ($P>0.05$) between the low (4.68±0.06%) and high (4.62±0.06%) nutritional treatments (Table 6.5). In the low nutrition treatment, milk protein percentage decreased between the first (4.73±0.08%) and second (4.63±0.07%) milkings. Conversely in the high nutrition treatment milk protein percentage increased between the first (4.47±0.08%) and second (4.77±0.07%). There was no effect ($P>0.05$) of ASBVs or liveweight on the percentage of protein in the milk (Table 6.5).

The percentage of lactose in the milk was again not different ($P>0.05$) between the low (5.25±0.03%) and high (5.30±0.03%) nutrition treatments. For ewes in the low nutrition treatment, milk lactose percentage was lower at the first milking (5.16±0.03%) than the second milking (5.35±0.03%), however, for ewes in the high nutrition treatment milk lactose percentage was higher at the first milking (5.36±0.03%) than the second milking (5.24±0.03%). Across nutritional treatments and milkings, milk lactose percentage was slightly lower in ewes with higher HFAT. Milk lactose percentage decreased ($P<0.05$) by 0.07±0.02% per mm of increase in HFAT. There was no effect ($P>0.05$) of the other ASBVs or ewe liveweight on milk lactose percentage (Table 6.5).

6.3.6 Hormones and metabolites

Plasma albumin concentrations were higher ($P<0.05$) in the high nutrition group but only at the mid-pregnancy sampling. Albumin concentrations were highest at weaning across both nutritional treatments (Table 6.6). Ewes with higher HFAT
breeding values had a higher ($P<0.05$) plasma concentration of albumin (0.66±0.34g/L albumin per mm HFAT) across nutritional treatments and states (Table 6.7). Including liveweight in the analysis showed that albumin concentration increased with liveweight (0.25±0.04g/L albumin per kg liveweight). Condition score was also positively correlated with the plasma concentration of albumin (2.96±0.48g/L albumin per unit condition score). Including condition score in the model resulted in the effect of HFAT being no longer significant ($P>0.05$).

The plasma concentration of insulin-like growth factor-I (IGF-I) varied between physiological state and between nutritional treatments during lactation (Table 6.6). Across both nutritional treatments ewes with higher HFAT had higher concentrations ($P<0.05$) of IGF-I at mid-pregnancy (12.7±4.0µg/L IGF-I per mm HFAT) and pre-lambing (11.4±4.2µg/L IGF-I per mm HFAT). There was no significant effect of HFAT on IGF-I concentration at conception, lactation or post-weaning states (Table 6.7). In addition, ewes with higher HEMD had higher ($P<0.05$) IGF-I concentrations during lactation (10.9±3.7µg/L IGF-I per mm HEMD) but not during any other physiological state. When liveweight was included in the analysis it was positively correlated ($F=10.9; P<0.01$) with IGF-I concentration (0.7±0.2µg/L IGF-I per kg liveweight). Its inclusion had no effect on the other significant terms in the analysis. Furthermore, the effect of condition score on IGF-I was significant ($F=21.1; P<0.001$) when it was included in the analysis. IGF-I concentration increased by 11.6±2.5µg/L per unit increase in condition score.
Table 6.6 Plasma concentration of leptin, insulin-like growth factor-I (IGF-I), urea nitrogen and albumin from ewes in low and high nutrition treatments at conception, mid-pregnancy, pre-lambing, lactation and at lamb weaning.

<table>
<thead>
<tr>
<th>State</th>
<th>Leptin (µg/L)</th>
<th>IGF-I (µg/L)</th>
<th>Urea nitrogen (mg/dL)</th>
<th>Albumin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conception</td>
<td>1.48±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.56±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ns</td>
<td>17.9±2.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mid-pregnancy</td>
<td>0.85±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.15±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>***</td>
<td>29.1±2.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pre-lambing</td>
<td>0.66±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.64±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ns</td>
<td>16.8±2.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactation</td>
<td>0.48±0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.55±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ns</td>
<td>47.1±2.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weaning</td>
<td>1.04±0.05&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.95±0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ns</td>
<td>21.0±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>abcd</sup> values with different superscripts within a column are different (P<0.05)

Nut. sig.: Difference between nutrition groups; ns: not significant, * P<0.05; **, P<0.01; ***, P<0.001
The concentration of leptin in plasma varied between physiological states and during mid-pregnancy leptin concentration was 35% higher ($P<0.001$) in the high nutrition ewes than the low nutrition ewes (Table 6.6). Nutrition treatment did not impact ($P>0.05$) on leptin concentration during the other physiological states. Generally across nutritional treatments and physiological states ewes with higher HFAT had higher ($P<0.001$) plasma concentrations of leptin, however the slope of the relationship differed with physiological state (Table 6.7). The slopes were $0.32\pm0.06$, $0.33\pm0.06$, $0.13\pm0.07$, $0.08\pm0.07$, and $0.23\pm0.06\mu g/L$ increase in leptin for each 1mm increase in HFAT at conception, mid-pregnancy, pre-lambing, lactation and weaning. There were no other ASBV effects ($P>0.05$) on plasma leptin concentration (Table 6.7), however the leptin concentration was lower ($P<0.05$) for ewes that gave birth to two lambs ($0.88\pm0.04\mu g/L$) than those that gave birth to a single lamb ($1.01\pm0.03\mu g/L$). When liveweight was included in the model it was significant ($F=34.0; P<0.001$) and as liveweight increased plasma leptin concentration also increased ($0.024\pm0.004\mu g/L$ leptin per kg liveweight). When liveweight was included an effect of HEMD on plasma leptin concentration became significant ($P<0.05$). The effect of HEMD on leptin varied with nutritional treatment, for ewes on low nutrition those with higher HEMD had higher leptin concentrations ($0.05\pm0.04\mu g/L$ leptin per mm HEMD), whereas for ewes on high nutrition, those with higher HEMD had lower leptin concentrations ($0.08\pm0.05\mu g/L$ leptin per mm HEMD). There were no other changes to the significant terms in response to including liveweight. Condition score was highly significant ($F=68.3; P<0.001$) when included in the analysis and leptin concentration increased by $0.36\pm0.04\mu g/L$ for each unit increase in condition score. The significant terms in the
analysis when condition score was included were the same as when liveweight was included.

The plasma concentration of urea nitrogen varied between physiological states and nutrition treatments (Table 6.6). There were no effects of HFAT, HEMD, HWT, liveweight or condition score on plasma urea concentrations (Table 6.7).

Table 6.7 $F$ values for the effect of nutrition treatment, physiological state, the number of lambs carried (birth type), breeding values for sub-cutaneous fat depth (HFAT) and eye muscle depth (HEMD) at hogget age, and significant interactions between terms on plasma concentrations of leptin, insulin-like growth factor-I (IGF-I), albumin and urea nitrogen.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Leptin $F$ value</th>
<th>IGF-I $F$ value</th>
<th>Urea nitrogen $F$ value</th>
<th>Albumin $F$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrition</td>
<td>2.5</td>
<td>0.9</td>
<td>1.8</td>
<td>2.5</td>
</tr>
<tr>
<td>State</td>
<td>87.6***</td>
<td>5.4***</td>
<td>373.0***</td>
<td>5.5***</td>
</tr>
<tr>
<td>Nutrition x State</td>
<td>7.3***</td>
<td>3.9**</td>
<td>9.4***</td>
<td>2.4*</td>
</tr>
<tr>
<td>Birth type</td>
<td>3.8*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>HFAT</td>
<td>40.8***</td>
<td>9.7**</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>HEMD</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>HFAT x State</td>
<td>6.0***</td>
<td>3.5**</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>HEMD x State</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom  
ns: not significant; *, $P<0.05$; **, $P<0.01$; *** $P<0.001$
6.4 Discussion

Lambs from ewes with higher HFAT breeding values were heavier at birth in the low nutrition treatment aligning with our initial hypothesis that ewes with higher HFAT would have lambs of higher birthweight when under restricted nutrition. There was no effect of HFAT on lamb birthweight in the high nutrition treatment. Both these results are contrary to the results of Morris et al. (1997) who showed lighter birthweights in lambs from a line selected for high fat compared to a line selected for lower fat and also unselected control ewes. However in that study, single trait selection for fatness also resulted in reduced liveweight in the fat line which could explain the reduction in lamb birthweight. In support of the result here, Vonnahme et al. (2006) reported that fetal weight was not impacted by restricted maternal nutrition in a desert adapted strain of sheep yet fetal weight was reduced by restricted maternal nutrition in a similar strain of sheep that were adapted to more favourable conditions. In that study the desert adapted strain was better able to maintain condition score with restricted nutrition than the non-adapted strain. In our study, there was a negative association between liveweight and HFAT however the effect of HFAT on lamb birthweight in low nutrition was evident with and without liveweight included in the analysis. When breeding Merino ewes are managed under restricted nutrition, those with higher genetic fatness have lambs that are heavier at birth and therefore more likely to survive. This finding has major implications for selection strategies that result in lower ewe fatness in environments where shortfalls in nutrition during pregnancy frequently occur.
It is likely that ewes with higher HFAT breeding values were better able to supply glucose to the growing fetus since glucose is the primary limiting nutrient for fetal growth (Wilson et al. 1983). In support of this notion, Vonnahme et al. (2006) demonstrated no impact of restricted maternal nutrition on fetal glucose in a desert-adapted strain of sheep, yet a reduction in fetal glucose when maternal nutrition was restricted in a non-adapted strain. A potential reason for this difference is a greater gluconeogenic capacity in high HFAT ewes when run under low nutrition. The rate of gluconeogenesis is known to be closely linked to substrate supply with glycerol being one of the key gluconeogenic substrates (Bergman et al. 1970; Lindsay 1970). Greater amounts of glycerol would have been released from the greater mobilisation of adipose tissue that occurred in high HFAT ewes since DXA fat and leptin concentrations show that high HFAT animals mobilised more fat during pregnancy than low HFAT ewes. Presumably in the high nutrition treatment, supply of propionate from the rumen, the preferred gluconeogenic substrate (Leng 1970), was sufficient to maintain a rate of gluconeogenesis necessary to maintain fetal growth and hence there was no difference in lamb birthweight. This is the first time genetic fatness has been shown to influence the mobilisation of reserves in times of nutritional stress to buffer the developing fetus. However, the result is supported by the work of McNeill et al. (1999) who showed that ewes that were phenotypically fatter as a result of improved nutrition prior to conception, produced heavier lambs than ewes that were leaner at conception when both groups were underfed in the last 40 days of pregnancy. In addition, Ferguson et al. (2004a) reported higher lamb birthweight in ewes that were in better condition at
joining when ewes were underfed in late pregnancy. These are clear examples of ewe fat storages being used to buffer lamb birthweight against the negative effects of under-nutrition in late pregnancy, even though the fat differences were nutritionally derived rather than genetic. While a potential mechanism is proposed, more detailed work is required to confirm the underlying physiology.

The effect of ewe nutrition during pregnancy on lamb birthweight found here is supported by previous work, which also shows that nutrition induced reductions in lamb birthweight often result in higher lamb mortality (Donnelly 1984; Holst et al. 1986; Jordan and Le Feuvre 1989; Jordan and Mayer 1989; Fogarty et al. 1992; Kelly et al. 1992; Everett-Hincks et al. 2004; Ferguson et al. 2004b). While the number of ewes in this experiment was insufficient to provide reliable information on lamb survival, it tended to be associated with lamb birthweight (data not shown). Many studies have shown a close relationship between lamb birthweight and lamb survival (eg. Holst et al. 1986) Therefore we can assume that lamb survival would have been higher in the high nutrition treatment and in lambs from high HFAT ewes. The differential impact of nutrition on lamb birthweight between different genotypes reported here and by Vonnahme et al. (2006) help to explain the variability of the effect of nutrition on lamb birthweight in the literature. In addition to the effect of ewe HFAT on lamb birthweight, there was also a general negative effect of ewe HEMD on lamb birthweight. A negative correlation between selection for muscling and lamb birthweight was also reported by Larsgard and Kolstad (2003) supporting our results. There was no effect of ewe HWT on lamb birthweight which is surprising considering
the general positive correlation between growth traits and lamb birthweight (see Safari et al. 2005). However, there was a positive effect of ewe liveweight at birth on lamb birthweight which is consistent with previous work (Fogarty et al. 1992; Greenwood and Thompson 2007). Also as expected from previous work, single lambs were heavier at birth than twin lambs (eg. Holst et al. 1986; Snowder and Glimp 1991; Hegarty et al. 2006c; Ferguson et al. 2004a). So we conclude that the nutritional restrictions imposed on the low nutrition group were sufficient to cause a reduction in lamb birthweight. In addition ewes with high HEMD had lambs of lower birthweight, while there was no effect of HWT on lamb birthweight.

There was no effect of HFAT breeding values on ewe milk production at either low or high nutrition, therefore rejecting our hypothesis that ewes with high HFAT breeding values would produce more milk when under restricted nutrition. The analysis of DXA fat showed that fat stores were depleted in late pregnancy and lactation and there was no difference in the amount and only slight differences in the proportion of fat associated with the HFAT ASBV at this time. It seems ewes had mobilised most available fat tissue prior to the onset of lactation resulting in no difference in milk production associated with HFAT. If nutritional treatments had have been imposed later in pregnancy and high HFAT ewes were able to display their genetic predisposition for higher fatness a different result may have occurred. While HFAT did not have an effect, there was an effect of HEMD on ewe milk production which differed with nutritional treatment. At high nutrition HEMD had a depressive effect on ewe milk production. However at low nutrition HEMD had a stimulatory effect on
ewe milk production, this result is difficult to explain and requires more detailed investigation. So in conclusion, ewe HFAT had no impact on ewe milk production at either level of nutrition, however there was an effect of HEMD on ewe milk production which varied with nutritional level.

There was no effect of HWT on milk production but liveweight was positively correlated with milk production. This result is supported by Thompson et al. (1987) who reported that milk production is proportional to ewe mature weight. As expected ewe nutrition had a large effect on ewe milk production. Milk production was 60% higher in the high nutrition group than the low nutrition group which is consistent with evidence that ewe milk production is directly affected by available nutrition (Langlands 1977; Jordan and Mayer 1989; Brand and Franck 2000). Furthermore, milk production increased between the first and second milkings in the low nutrition group, this was as a result of available pasture being increased between the two milkings which is again consistent with previous work. In addition, ewe milk production was 30% higher in ewes rearing twin lambs, a result that is consistent with that of Snowder and Glimp (1991), although they reported a 60% higher milk output in ewes rearing twins. Milk production responded to both liveweight of ewes and their available nutrition but the expected positive influence of HWT on milk production was not evident.
Generally there were only minor changes to ewe milk composition as a result of nutrition and ewe ASBVs. The percentage of fat in the milk was lower in ewes with high HFAT under low nutrition however, fat percentage was higher in ewes with high HFAT under high nutrition. Therefore, our hypothesis that ewes with higher HFAT breeding values would produce milk of greater milk fat percentage was only partially supported. The reason for this difference is unclear, but is potentially associated with differences in milk yield between animals. In lactating dairy cows, a reduction in milk production often results in the percentage of fat in the milk increasing (reviewed by Sutton 1989). This is a result of fatty acid supply remaining relatively constant but milk volume decreasing (reviewed by Sutton 1989). It is plausible that in the current study differences in milk production resulted in the change in fat percentage. The inherent error around measuring milk production in the field may have made it less likely to find this result in absolute milk yield. The measurement of milk fat percentage is considerably more precise. These results suggest that milk production was greater in high HFAT ewes under low nutrition but lower in high HFAT ewes under high nutrition. Experimental methods used here could not confirm this result but it should be further explored. There was no overall effect of nutrition treatment or ewe liveweight in lactation on the percentage of fat, protein or lactose in the milk. We conclude that the milk compositional changes were minor but differences in milk fat percentage are suggestive of differences in milk yield that were not identified by its direct measure.
There was no effect of HFAT on lamb weight at weaning therefore the hypothesis that ewes with higher HFAT breeding values would produce lambs of heavier weights at weaning when under restricted nutrition is rejected. Considering there were no effects of HFAT on milk production and only minor effects of HFAT on milk composition, it is not surprising that there was no effect of HFAT on lamb weaning weight. As with ewe milk production, the timing of nutritional restriction may be important for any positive effects of HFAT on lamb growth to be evident, since at the onset of lactation there were no differences in total body fat associated with ewe HFAT. There were also no effects of HEMD and HWT on lamb growth rate however ewe nutrition did have a large effect as expected. Lamb growth and therefore weight at weaning was higher in the higher nutrition treatment than the low nutrition treatment, a depression in lamb growth and weaning weight associated with reduced nutrition during lactation is consistent with previous reports (Langlands 1977; Arnold et al. 1977; Thompson et al. 2010). In addition, single born lambs were heavier at weaning than twin born lambs and male lambs heavier than females which is consistent with the literature eg. Snowder and Glimp (1991) and Hegarty et al. (2006; 2006c). Lamb weaning weight was not affected by ewe HFAT ASBV, this result agrees with the finding of there being no impact of ewe HFAT on ewe milk production. This finding may be a result of most fat being mobilised in late pregnancy and prior to the onset of lactation, and restriction of nutrition later in pregnancy than that imposed here may have caused a different result.

Ewe whole body fat as measured by DXA was positively correlated with ewe HFAT at conception and weaning, therefore our hypothesis that ewes with higher HFAT values
would be fatter is supported. However, when the fat stores were depleted in late pregnancy and lactation there was no effect of HFAT on DXA fat. DXA fat was positively associated with liveweight, this result is supported by that of Thompson et al. (1987) who showed mature weight was positively correlated with total body fat. Including liveweight in the analysis also demonstrated that DXA fat and HFAT were positively correlated in all physiological states when liveweight is taken into account. Therefore the proportion of fat in ewes was positively correlated with HFAT across all states. However, the differences in DXA fat associated with HFAT were much lower in late pregnancy and lactation compared with conception or weaning. It is normal for ewes to mobilise fat reserves in late pregnancy and early lactation to fuel the requirements of a growing fetus and lactation particularly when nutrition is limiting (Lambe et al. 2003). However the difference in slope shows that ewes with higher HFAT ASBVs had mobilised greater amounts of fat over pregnancy than low HFAT equivalents. In addition to the effect of HFAT there was also a negative influence of HEMD on DXA fat which only occurred in the high nutritional treatment. This result is supported by that of Hegarty et al. (2006a) who showed that in lambs grown under high nutrition there was a negative correlation between their eye muscle breeding value and fatness that was not evident when lambs were grown under low nutrition. However, other reports have shown that muscling and fatness are positively correlated in high nutrition environments (Kadim et al. 1989; Larsgard and Kolstad 2003). Therefore, the effect of HEMD on fatness is expected to vary with nutrition and further work is required to understand the conditions under which positive and negative correlations exist between muscling and fatness. Interestingly, this result aligns with
ewe milk production results with high muscled ewes having less fat and lower milk production when under high nutrition. Across all states there was a strong positive correlation between ewe condition score and DXA fat. This result is supported by those of Russel et al. (1969) who showed a close correlation between condition score and the proportion of fat in ewes. As expected DXA fat was positively correlated with the HFAT breeding value. Ewe fat depots were largely mobilised in late pregnancy and remained low in lactation and ewes with higher HFAT breeding values mobilised more fat during pregnancy which potentially explains the increase in lamb birthweight in high HFAT ewes run at low nutrition.

There was a strong positive correlation between HFAT breeding value and leptin concentration which is further confirmation that HFAT is associated with greater fatness in ewes. This result is expected since leptin is predominantly produced by adipocytes and the concentration of leptin is closely correlated with the proportion of fat in the body (Chilliard et al. 2001; Chilliard et al. 2005; Delavaud et al. 2007). In addition, there was a strong correlation between leptin concentration and ewe condition score which is further evidence of a strong link between ewe condition score and fatness as found by Russel et al. (1969) and shown in chapter 4. Leptin concentration decreased from conception through to lactation which is consistent with DXA fat results and confirms that fat was mobilised between conception and late pregnancy. The correlation between HFAT and leptin concentration is also similar to the correlation between DXA fat and HFAT. There was a much larger impact of HFAT on leptin concentration at conception, mid-pregnancy and weaning than in late
pregnancy and lactation. These leptin results confirm the DXA fat results which showed that fat storages were largely exhausted in late pregnancy and lactation and the difference in fatness associated with HFAT was diminished at these times. In addition to the HFAT effect on leptin concentration, ewe HEMD also had an effect which differed with nutrition treatment. Similar to the relationship between HEMD and DXA fat, at high nutrition, high HEMD was associated with a reduction in leptin concentration, while the opposite was true for ewes in the low nutrition treatment. The differential effect of HEMD on ewe fatness and the related leptin concentration only was significant when liveweight was included in the analysis and requires further investigation. Generally leptin concentrations agreed closely with other predictions of body fatness, therefore the use of leptin concentration as a predictor of total body fat could be considered for animal research and breeding purposes.

The total amount of lean tissue was closely correlated with ewe liveweight. In addition, ewe HWT was also positively correlated with DXA lean. As DXA lean is a measurement of total body lean tissue and includes muscle, organs and digestive tract this result is expected. The results for HFAT and HEMD on DXA lean essentially mirrored the results for DXA fat. Ewes with higher HFAT had lower amounts of DXA lean and ewes with higher HEMD had higher DXA lean at high nutrition but lower DXA lean at low nutrition. There was no effect of ewe condition score on DXA lean. As DXA lean is a measure of all protein and water in the body it is closely related to liveweight and HWT. The changes in DXA lean associated with ASBV’s are essentially as a result of the ASBV effects on total body fatness.
Ewe liveweight and condition score varied throughout the breeding cycle and nutritional treatments differed sufficiently to generate differences in liveweight and condition score for the majority of the experimental period. In addition to the large effect of nutrition treatment there were also other effects on ewe liveweight. Ewe liveweight was positively correlated with HWT which is expected as growth traits and mature weight are well correlated (see Safari et al. 2005). In addition, ewe liveweight was negatively correlated with HEMD, this finding is supported by Clarke et al. (2003) who reported a negative correlation between eye muscle depth and liveweight in the Australian Merino population. In addition, there is evidence of reduced lamb growth rate associated with selection for eye muscle depth in other breeds which would suggest reduced mature size (Larsgard and Kolstad 2003; Hopkins et al. 2007b). Furthermore, Cake et al. (2007) demonstrated that higher eye muscle depth breeding values are associated with reduced bone growth which again suggests reduced mature size associated with selection for muscling. Liveweight was also negatively correlated with ewe HFAT during most of the breeding cycle. This result is supported by that of McEwan et al. (2001) who showed a reduction in mature weight in lines selected for higher fatness compared with those selected for less fatness and unselected controls. Presumably, lambs that are fatter at measurement age are more mature and single trait selection for increased fatness results in selection of animals with lower mature weight. The results here demonstrate that selection for HEMD, HFAT and HWT will all change ewe mature weight and it is important to consider these changes when designing selection strategies.
Insulin-like growth factor I (IGF-I) is important for the regulation of both energy and protein metabolism. Both condition score and liveweight were positively correlated with IGF-I across states and treatments, these results are consistent with that of Oddy and Owens (1996) which showed that IGF-I increases protein gain and glucose uptake resulting in net tissue accretion. Furthermore, Medrano and Bradford (1991) reported a positive correlation between IGF-I concentration and body size in sheep. IGF-I concentration was also positively correlated with HFAT during mid and late pregnancy, a result that is supported by positive correlations between IGF-I concentrations and fatness in cattle and pigs (Robinson and Oddy 2004; Bunter et al. 2005). In addition, IGF-I and HEMD were positively correlated during lactation, this effect was not significant in the other physiological states and it is difficult to explain the short lived nature of this result. A positive correlation between IGF-I and HEMD is consistent with the known protein accretion effect of IGF-I. In summary, differences in energy and protein metabolism associated with sheep with differing ASBVs are associated with differences in IGF-I concentrations, further work is required to determine the relevance of these findings.

Plasma albumin is the largest pool of blood borne protein and represents an important store of labile protein and its concentration can reflect protein status in growing animals as demonstrated in young pigs (Lowrey et al. 1962). Plasma albumin was lower in the low nutrition treatment than the high nutrition in late pregnancy
suggesting protein deficiency in the low nutrition treatment which is feasible. In addition, both liveweight and condition score were positively correlated with plasma albumin concentration which supports the concept that albumin concentration reflects protein status. Interestingly, HFAT was positively correlated with plasma albumin across all nutritional treatments and states, suggesting ewes with lower HFAT are deficient in protein during a breeding cycle. This finding is supported by the results of Pond et al. (1980) who showed higher albumin concentration in genetically fat pigs compared with lean counterparts when fed a protein deficient diet. Furthermore, Davey and Morgan (1969) reported a greater requirement for protein in lean than obese pigs.

In addition to changes in albumin concentrations, another indicator of nitrogen status is plasma urea nitrogen concentration. Although concentrations were different between treatment groups, the difference was not consistent and since urea nitrogen can be affected by changes in short term nutrition, the differences may not reflect difference in nitrogen status. There were no effects of ASBVs on plasma urea nitrogen concentration. The finding that ewes of higher HFAT potentially have a lower requirement for protein and have improved protein status over low fat ewes may have significant consequences particularly in protein deficient times of the year such as summer and autumn.
When breeding Merino ewes are managed under restricted nutrition, those with higher genetic fatness have lambs that are heavier at birth and therefore more likely to survive. This finding has major implications for selection strategies that result in lower ewe fatness in environments where shortfalls in nutrition during pregnancy frequently occur. Ewes with higher genetic fatness mobilised more fat during pregnancy which potentially explains the increase in lamb birthweight from these ewes when managed under restricted nutrition.
Chapter 7 Merino ewes selected for rapid lean growth have higher circulating growth hormone.

7.1 Introduction

Growth hormone has a key role in the partitioning of nutrients from storage tissues and toward the mammary gland during lactation and its actions direct the flow of nutrients toward tissues and processes of the highest priority (Bauman 2000). Its effects on energy and protein metabolism are partly mediated through the insulin-like growth factor system. Growth hormone concentration is known to increase in late pregnancy and further increase in lactation resulting in the mobilisation of stored fat leading to elevated plasma non-esterified fatty acid (NEFA) concentrations (Hart et al. 1978; Vernon et al. 1981). While the associations between changes in physiological state and growth hormone are well known, the impact of animal breeding strategies on the growth hormone axis is less understood. The known effects of higher growth hormone are similar to the selection goals of many sheep breeding programs and it has been suggested that selection for larger, leaner sheep results in enhanced growth hormone activity (Adams et al. 2006b). Furthermore, increasing growth hormone supply by exogenous treatment resulted in increased growth and nitrogen accretion and reduced accumulation of fat in lambs (Wagner and Veenhuizen 1978; Pell et al. 1990). These changes were also associated with increased circulating IGF-I, insulin and glucose. (Pell et al. 1990) Similarly, increasing endogenous growth hormone secretion by the addition of an extra copy of the ovine growth hormone gene resulted in increased growth, organ size and skin weight, as well as reduced fatness (Adams et al. 2002b).
Furthermore, reducing endogenous growth hormone secretion by immunisation against growth-hormone releasing hormone resulted in an increase in body fatness and a decrease in circulating Insulin and IGF-I in Merino ewes (Adams et al. 1996a). Based on the effects of altering growth hormone concentration on animal growth and body composition in sheep, we hypothesise that selection for fast growing, lean sheep would therefore select animals with higher circulating growth hormone.

In the Australian Merino industry, increased growth and reduced fatness can be selected for using a combination of Australian Sheep Breeding Values (ASBVs) for hogget-age weight (HWT), subcutaneous fat depth (HFAT), and eye muscle depth (HEMD). Evidence suggests that selection for these ASBVs may be associated with differences within the growth hormone axis or other endocrine systems. Selection for HWT is expected to result in increased growth hormone concentrations as rams selected for feed efficiency and growth had greater levels of plasma growth hormone than unselected controls (Dodson et al. 1983). Selection for lower HFAT breeding values is likely to result in elevated growth hormone concentrations since lambs from lines selected for lower back fat depth had higher growth hormone concentrations than those selected for higher fat (Francis et al. 1997). Lastly, selection for higher HEMD breeding values is likely to reduce growth hormone secretion since selection for muscling has been associated with reduced rate of growth to weaning (Hegarty et al. 2006a), reduced bone growth, and increased muscle to bone ratio (Cake et al. 2006), all indicative of reduced growth hormone activity. This is supported by results from growth hormone transgenic sheep where a two fold increase in endogenous growth
hormone was associated with a 20% decrease in eye muscle depth (Adams et al. 2002b).

Considering the concentration of circulating growth hormone is elevated during lactation, it is likely that any differences between HWT, HEMD or HFAT genotypes will be most evident when ewes are lactating.

The hypotheses tested were that: growth hormone, IGF-I and insulin concentrations will be increased with higher HWT and decreased with higher HFAT and higher HEMD ASBVs in Merino ewes across the breeding cycle; and that these differences will be more pronounced in lactation.

7.2 Materials and Methods

Merino ewes were monitored across a full breeding cycle. Serial blood samples were collected and measured for hormones and metabolites in late pregnancy, early lactation, and after weaning when ewes were non-breeding. Single blood samples were also collected at conception and mid-pregnancy for measurement of plasma concentrations of albumin, leptin and urea nitrogen. The experiments was approved and monitored by the CSIRO Floreat Animal Ethics Committee.

7.2.1 Animals

109 Merino ewes that were 1.5 years old and had not previously lambed were sourced from the Merinotech (WA) nucleus flock based at “Yarrak” near Kojonup, Western
Australia. All ewes had pedigree and performance information in the form of Australian Sheep Breeding Values (ASBVs). Ewes were selected to maximise the spread of ASBVs for the depth of eye muscle (\textit{M. longissimus lumborum}; HEMD) and subcutaneous fat (HFAT) measured by ultrasound on the 12\textsuperscript{th} rib, 45mm from the midline at hogget age. Selected ewes also had a wide range of ASBVs for weight at hogget age (HWT). The ewes were transported from “Yarrak” to Yalanbee Research Station near Bakers Hill, Western Australia on day -22. Ewes were maintained on dry summer pasture (subterranean clover and annual grasses) with oat and lupin grain supplementation as required.

7.2.1.1 Pregnancy and Lambing

The timing of ovulation for all ewes was synchronised using progesterone intravaginal sponges (Chronogest\textsuperscript{®}, Intervet Aust. Bendigo, Australia). Ewes were randomly allocated into two groups and naturally mated with three Poll Dorset rams in each group over a three day period around day 0 (7 March 2007). Rams were fitted with harness crayons and were removed from ewes on day 3 after all ewes had been marked. The actual ram each ewe was mated to was later determined by DNA testing of the resultant lambs (Pfizer Animal Genetics, Albion, Queensland). The ewes were pregnancy scanned by ultrasound on day 63 and only ewes that were pregnant with a single fetus remained in the experiment (n=66). This number was further reduced to 55 after 11 ewes were removed from the experiment over lambing and lactation. Only ewes that had contributed data throughout the entire study (n=55) were included in the analyses. Those remaining had an average HWT ASBV of 5.5kg (range 1.7 to 8.6kg), an
average HFAT ASBV of 0.5mm (range -0.6 to 1.7mm), and an average HEMD ASBV of 1.3mm (range 0.0 to 2.6mm; Figure 7.1).

Figure 7.1 Australian Sheep Breeding Values (ASBV) at hogget age for subcutaneous fat depth (HFAT) and eye muscle depth (HEMD) of single bearing ewes used in the experiment (n=55).

Between day 0 and day 109 ewes were managed to maintain conceptus free liveweight (Wheeler et al. 1971) by weekly assessments of liveweight and condition score and adjustment of supplementary feeding and pasture feed on offer. From day 96 to day 108 ewes were introduced to a pelleted feed to assist with the transition to pellet feeding in the animal house. On day 110 ewes were transported to an animal house.
and housed in individual pens. Upon arrival ewes were drenched with an ivermectin based anthelmintic (Ivomec®, Merial Aust. Parramatta, Australia), injected with clostridial disease vaccine and selenium (Glanvac™ 6S Vaccine, Pfizer Aust. West Ryde Australia), injected with vitamin B1 (Thiamine Hydrochloride, Nature Vet, Glenorie, Australia) and with vitamins A, D and E (Vitamin ADE, Animal Health Products, Dandenong, Australia). Ewes were individually fed a pelleted ration (11.3MJ metabolisable energy/kg dry matter and 13% crude protein) at energy maintenance as calculated using Grazfeed® (Horizon Technologies Ltd, Armidale, Australia) based on animal house entry weight and including an allowance for pregnancy status. Ewes remained in individual pens until lambing and lambed between days 145 and 154. Ewes and lambs were removed from the animal house approximately 2 days after lambing.

7.2.1.2 Lactation

Between lambing (days 145-154) and day 168, ewes were grazed on a small pasture paddock and were supplemented with 1kg/head/day of a pelleted ration (11.8MJ metabolisable energy/kg dry matter and 15.5% crude protein). On day 169 ewes (n=55) and their single lambs were returned to individual pens in the animal house. On re-entry to the animal house ewes were again drenched with a broad spectrum anthelmintic (Triton®, Merial Aust. Parramatta, Australia) and lambs were injected with a clostridial disease vaccine which also contained selenium (Glanvac™ 6S Vaccine, Pfizer Aust. West Ryde Australia). Ewes were fed a pelleted ration (11.8MJ
metabolisable energy/kg dry matter and 15.5% crude protein) sufficient for energy maintenance as calculated using Grazfeed® (Horizon Technologies Ltd, Armidale, Australia) based on individual weight and including an allowance for the energy requirements of lactation. Ewes and lambs were removed from individual pens on day 196 and transported back to Yalanbee Research Station on day 197 where they grazed subterranean clover and annual grass pastures. Lambs were weaned from the ewes on day 244 of the experiment.

7.2.1.3 Non-breeding

Ewes were managed on senescing pasture from day 244 to 256. On day 257, ewes (n=55) were transported to the CSIRO Floreat Animal house and penned individually. Ewes were again drenched with a broad spectrum anthelmintic (Triton®, Merial Aust. Parramatta, Australia) on entry to the animal house. They were fed a pelleted ration (10.9MJ metabolisable energy/kg dry matter and 13.5% crude protein) sufficient for energy maintenance as calculated using Grazfeed® (Horizon Technologies Ltd, Armidale, Australia) based on individual weight.

7.2.2 Plasma sample collection

Blood samples were collected on days 35 and 91 via venipuncture into heparinised tubes and plasma was harvested and frozen at -20°C for later processing. In addition
to this plasma samples were also collected each time the ewes were in the animal house (pregnant, lactating and non-breeding) to determine fasting plasma concentration of a range of hormones and metabolites. The animal house sample collections occurred in conjunction with metabolic studies involving adrenaline and insulin infusions in a subset of 24 ewes (described in chapters 9 and 10). To facilitate the range of metabolic studies that were being undertaken the ewes were divided into three groups (two groups of 12 and a group with the remaining ewes) and samples were collected on different days for each group. Samples were collected on days 130, 135 and 139 during pregnancy; 181, 186 and 189 during lactation; and 272, 277 and 284 during the non-breeding state. All sheep were fitted with jugular catheters at least 18h prior to sample collection. Blood samples were collected prior to sheep receiving their ration for the day and 24h after receiving their previous ration. Blood samples (6mL) were collected into heparinised tubes every 20min for 3h In addition, for the 60, 80, 100 and 120min samples additional aliquots were also collected into EDTA (3mL) for NEFA analysis and fluoride oxalate tubes (1mL) for glucose analysis. Plasma was separated by centrifugation and stored at either -20°C (heparin and fluoride oxalate) or -80°C (EDTA).

7.2.3 Measurements

Ewes were weighed and condition scored according to the method of Jefferies (1961), this being done weekly while ewes were in the animal house and approximately fortnightly while ewes were at Yalanbee. On day 230 ewes were shorn and the fleece
weight was recorded. Mid-side samples were collected prior to shearing. A single staple was randomly selected from the sample and was scoured in a 80:20 mixture of Hexane:Isopropanol and allowed to condition at 20°C and 65% humidity for 24 hours. The mean fibre diameter was then measured using an OFDA2000 (BSC Electronics, Ardross, Western Australia).

Plasma concentrations of IGF-I were measured on two sub-samples from each sample collected at day 35, 91, 130-139, 181-189 and 272-284 using the double antibody radioimmunoassay described by Breier et al. (1991). The samples were measured in a single assay, the proportion of tracer bound without competitor (B₀) was 27.4% and the non-specific binding (NSB) was 2.5%. Control samples were included in the assay with average values of 8.5, 24.7 and 43.8µg/L and had intra-assay coefficients of variation (CVs) of 14.5, 13.7, and 12.8%. The 50% maximal displacement on the standard curve was 32.0µg/L and the minimum detectable concentration was 7.8µg/L.

Insulin was measured on 4 samples taken from each sheep on each sampling day, collected on days 130-139, 181-189 and 272-284. Concentrations of insulin in plasma were measured in duplicate using a double anti-body radioimmunoassay (Tindal et al. 1978). The samples were measured in a single assay and three control samples averaging 2.4, 3.7 and 8.6µU/mL were included in the assay to determine the intra-assay CVs of 6.5%, 7.2% and 8.8%. The minimum detectable concentration was 0.78µU/mL and 50% maximal displacement on the standard curve was 4.8µU/mL. The B₀ was 20.5% and the NSB was 1.2%.
Growth hormone was measured on 10 samples taken from each sheep collected across the three hour sampling time on days 130-139, 181-189 and 272-284. Growth hormone concentration in plasma was measured in duplicate using a double antibody homologous radioimmunoassay described by Adams et al. (1996a). The intra-assay CVs for three control samples averaging 2.25, 2.82 and 5.91 µg/L were 8.1%, 7.9% and 4.8%. The corresponding inter-assay CVs were 7.8%, 4.8% and 8.1%. The NSB for the assay was 2.1% and B₀ was 35.5%. The minimum detectable concentration was 0.48 µg/L and 50% displacement on the standard curve occurred at 3.4 µg/L.

Plasma concentrations of Leptin were measured on duplicates from single samples collected at day 35, 91, 130-139, 181-189 and 272-284 using the double antibody radioimmunoassay described by Blache et al. (2000). All samples were measured in a single assay with NSB of 1.7% and B₀ of 26.9%. The minimum detectable concentration was 0.07 µg/L and 50% maximal displacement on the standard curve was at 1.1 µg/L. Control samples included in the assay with average values of 0.51, 0.98 and 1.77 µg/L had an intra-assay CV of 6.9%, 5.3% and 6.1%.

Plasma Glucose was measured on 4 samples taken from each sheep on each sampling day, collected on days 130-139, 181-189 and 272-284, using fluoride-oxalate as the anticoagulant. Concentrations of glucose in plasma were measured in a Cobas Mira Autoanalyser (Hoffman-La Roche Diagnostica, Basal, Switzerland) with an Infinity™ Glucose hexokinase kit (Cat. No. TR15421, Thermo Electron Co., Melbourne, Australia).

Plasma urea nitrogen and plasma albumin were measured in duplicate on single samples collected on days 35, 91, 130-139, 181-189 and 272-284. Plasma urea nitrogen was measured using the Infinity™ Urea Liquid stable reagent (Thermo Electron Co.,
Melbourne, Australia) in a Cobas Mira Autoanalyser (Hoffman-La Roche Diagnostica, Basal, Switzerland). Plasma Albumin was measured using a DMA Albumin kit from Thermo Electron Co., Melbourne, Australia (Cat. No.: TR36026) with the Cobas Mira Autoanalyser.

The concentration of L-lactate was measured in duplicate in a Cobas Mira Autoanalyser with a Randox L-lactate reagent kit (LC 2389; Randox Laboratories Ltd, Antrim, UK). Plasma non-esterified fatty acids (NEFA) were measured on 4 samples taken from each sheep on each sampling day, collected on days 130-139, 181-189 and 272-284. Concentrations of NEFA in plasma were measured in duplicate on samples collected in EDTA blood tubes and stored at -80°C using a Wako NEFA C Kit (Wako Pure Chemical Ind., Osaka, Japan) and a plate reader with the Soft-max® PRO program.

For all hormones measured the variation between duplicate samples was less than 10% of the mean. Where the variation was greater than this the sample was re-measured in duplicate.

7.2.4 Body composition measurement

Body composition was estimated on day -23 by Dual-energy X-ray Absorptiometry (DXA) using a Norland XR-26 Fan Beam X-Ray Whole Body Densitometer (Inderlec Medical Systems Baulkham Hills, Australia following the method described by Pearce et al. (2009). Briefly, prior to DXA scanning sheep were anaesthetised by inhalation of
nitrous oxide to induce and isoflurane to maintain anaesthesia. The sheep were then lifted on to the scanning bed and positioned in sternal recumbency. The scan was completed using the Whole Body Scan mode and a regional analysis was performed on the image produced to provide a total mass of fat (DXA fat). Sheep had not had access to feed and water for between 18 and 24 h at the time of scanning.

7.2.5 Statistical Analysis

All traits were analysed using linear mixed effects models in GENSTAT 11.1 (VSN International 2008). The analysis included the fixed effect of physiological state (conception, mid-pregnancy, late-pregnancy, lactating, non-breeding) for each of liveweight, condition score, lactate concentration, urea nitrogen concentration, albumin concentration, IGF-I concentration, and leptin concentration. The covariates included were HFAT, HWT, HEMD. Animal tag and the ram the ewe conceived to were used as random terms. Models were run with and without the inclusion of condition score. All first and second order interactions were included in the starting model, and removed in a stepwise process if non-significant (P>0.05). For the analysis for insulin, growth hormone, glucose and NEFA concentrations, a fixed effect of sampling time within state was also included to account for the serial samples taken from the same animal.
7.3 Results

7.3.1 Liveweight, fatness and wool measurements

Liveweight (including the weight of the gravid uterus and wool weight) varied by about 12kg across the experimental period (Figure 7.2). Liveweights at blood sampling times were similar across physiological states (Table 7.2). Ewe condition scores increased by about 20% from when ewes were pregnant and lactating to when ewes were non-breeding (Table 7.2).

![Liveweight profile and experimental time line from day 0 (7 March 2007), arrows under the line represent blood sampling time points.]

Across the entire experimental period, liveweights and condition scores were greater ($P<0.01$) with an increase in the HWT ASBV. For each 1kg increase in HWT, liveweight increased by 1.1±0.2kg and condition score increased by 0.04±0.02units. For these and all results reported in this chapter the figures shown are means ±s.e. There were no
effects ($P>0.05$) of HEMD or HFAT ASBVs on liveweight or condition score (Table 7.1).

Mean wool fibre diameter of the ewes was 19.3±1.3µm and greasy fleece weight was 4.36±0.68kg. There were no effects ($P>0.05$) of HEMD, HFAT or HWT ASBVs on either of these fleece parameters.

Table 7.1 $F$ values for the effect of day of experiment, breeding values for weight (HWT), sub-cutaneous fat depth (HFAT), eye muscle depth (HEMD) at hogget age and condition score on ewe liveweight (LW), condition score (CS) and fat amount measured by Dual-energy x-ray absorptiometry (DXA fat).

<table>
<thead>
<tr>
<th>Effect</th>
<th>LW NDF, DDF</th>
<th>F value</th>
<th>CS NDF, DDF</th>
<th>F value</th>
<th>DXA fat NDF, DDF</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>28,1509</td>
<td>117.0***</td>
<td>25,1347</td>
<td>35.8***</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>HWT</td>
<td>1,51</td>
<td>26.7***</td>
<td>1,51</td>
<td>5.7**</td>
<td>1,51</td>
<td>3.9</td>
</tr>
<tr>
<td>HFAT</td>
<td>1,51</td>
<td>0.1</td>
<td>1,51</td>
<td>1.7</td>
<td>1,51</td>
<td>0.2</td>
</tr>
<tr>
<td>HEMD</td>
<td>1,51</td>
<td>0.1</td>
<td>1,51</td>
<td>0.3</td>
<td>1,51</td>
<td>4.4*</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

na: not applicable

The amount of fat in the body measured by dual energy x-ray absorptiometry (DXA fat) averaged 3.73±0.22kg when measured prior to mating. DXAfat increased ($P<0.05$) by 1.20±0.57kg with each 1mm increase in HFAT but there was no relationship ($P>0.05$) between HWT or HEMD with DXA fat (Table 7.1). When liveweight was included in the model it was not related to DXA fat and its inclusion had no effect on the association between HFAT and DXA fat. Condition score was also related to DXA fat.
when it was included in the model (\(P<0.01\)) and DXA fat increased by 2.20±0.76kg for each one unit increase in condition score.

Table 7.2 Ewe liveweight (LW), condition score (CS), and plasma concentrations of albumin, urea nitrogen (Urea N), glucose, non-esterified fatty acids (NEFA) and lactate across the breeding cycle.

<table>
<thead>
<tr>
<th>State</th>
<th>Day</th>
<th>LW (kg)</th>
<th>CS</th>
<th>Albumin (g/L)</th>
<th>Urea N (mM)</th>
<th>Glucose (mg/dL)</th>
<th>NEFA (mM)</th>
<th>Lactate (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conception</td>
<td>35</td>
<td>49.3±0.5a</td>
<td>3.3±0.04a</td>
<td>36.5±0.39ab</td>
<td>14.8±0.42a</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Mid-pregnant</td>
<td>91</td>
<td>49.9±0.5bc</td>
<td>3.0±0.04b</td>
<td>36.2±0.39b</td>
<td>22.6±0.42b</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Late pregnant</td>
<td>140</td>
<td>51.8±0.5b</td>
<td>2.7±0.04b</td>
<td>35.5±0.39b</td>
<td>9.7±0.42b</td>
<td>2.6±0.06a</td>
<td>0.37±0.02a</td>
<td>3.5±0.11a</td>
</tr>
<tr>
<td>Lactating</td>
<td>182</td>
<td>50.2±0.5c</td>
<td>2.7±0.04c</td>
<td>39.6±0.39c</td>
<td>25.2±0.42c</td>
<td>3.3±0.06d</td>
<td>0.37±0.02d</td>
<td>3.5±0.13c</td>
</tr>
<tr>
<td>Non-breeding</td>
<td>272</td>
<td>51.1±0.5d</td>
<td>3.1±0.04d</td>
<td>38.3±0.39d</td>
<td>19.2±0.42d</td>
<td>3.1±0.06d</td>
<td>0.12±0.02d</td>
<td>2.7±0.11d</td>
</tr>
</tbody>
</table>

\(a,b,c,d\) values with different superscripts within columns are significantly different (\(P<0.05\))
n: not applicable

Table 7.3 \(F\) values for the effect of physiological state, its interaction with time of sample, breeding values for weight (HWT), subcutaneous fat depth (HFAT) and eye muscle depth (HEMD) at hogget age on ewe plasma concentrations of albumin, urea nitrogen (urea N), glucose, non-esterified fatty acids (NEFA) and lactate.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Albumin</th>
<th>Urea N</th>
<th>Glucose</th>
<th>NEFA</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>State x Time</td>
<td>NDF, DDF</td>
<td>(F) value</td>
<td>NDF, DDF</td>
<td>(F) value</td>
<td>NDF, DDF</td>
</tr>
<tr>
<td>State</td>
<td>4,216</td>
<td>22.9***</td>
<td>4,216</td>
<td>250.5***</td>
<td>2,592</td>
</tr>
<tr>
<td>State x HEMD</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>9,592</td>
</tr>
<tr>
<td>HWT</td>
<td>1,51</td>
<td>0.9</td>
<td>1,51</td>
<td>1.6</td>
<td>1,48</td>
</tr>
<tr>
<td>HEMD</td>
<td>1,51</td>
<td>4.5*</td>
<td>1,51</td>
<td>0.1</td>
<td>1,48</td>
</tr>
<tr>
<td>HFAT</td>
<td>1,51</td>
<td>0.1</td>
<td>1,51</td>
<td>0.5</td>
<td>1,48</td>
</tr>
<tr>
<td>State x HEMD</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>2,592</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom
* \(P<0.05\); **, \(P<0.01\); *** \(P<0.001\)
na: not applicable
7.3.2 Plasma albumin, urea nitrogen, and non-esterified fatty acid

Plasma concentration of urea nitrogen differed ($P<0.001$) between all physiological states (Table 7.2) however there were no effects of HWT, HEMD, HFAT on urea nitrogen at any stage (Table 7.3). When included in the model there was no effect of condition score on urea nitrogen. Plasma albumin concentration differed ($P<0.001$) between states and was lowest at conception and late pregnancy and highest when the ewes were lactating (Table 7.2). Plasma albumin was positively related to HEMD ($P<0.05$), for each 1mm increase in HEMD albumin concentration increased by 1.04±0.49g/L. There were no effects ($P>0.05$) of HFAT, HWT or condition score (when included in the model) on plasma albumin (Table 7.3). Plasma non-esterified fatty-acid (NEFA) concentrations were three times higher ($P<0.001$) when ewes were pregnant and lactating than when ewes were non-breeding (Table 7.2). There were no effects of HWT, HEMD, HFAT or condition score (when included) on plasma NEFA concentration (Table 7.3).

7.3.3 Plasma glucose and lactate

Plasma concentrations of glucose were lower ($P<0.001$) in pregnancy and higher ($P<0.001$) in lactation than when non-breeding (Table 7.2). Plasma glucose increased ($P<0.05$) with HEMD both when the ewes were non-breeding and pregnant (0.06±0.03mM and 0.04±0.03mM with each 1mm increase in HEMD). However, HEMD and glucose concentration were negatively related ($P<0.05$) when the ewes were
lactating (-0.04±0.03 mM per mm HEMD; Table 7.3). When condition score was
included in the model plasma glucose concentration and condition score were
negatively associated \((P<0.05)\) when ewes were pregnant and non-breeding (-0.13±0.07
and -0.10±0.06 mM per condition score unit) and positively related when ewes were
lactating (0.14±0.08 mM per condition score unit).

Plasma lactate concentration was 25% higher \((P<0.001)\) when ewes were pregnant and
lactating compared to when ewes were non-breeding (Table 7.2). There were no effects
of HWT, HEMD, HFAT or condition score (when included), on fasting lactate
concentrations (Table 7.3).

7.3.4 Growth hormone

Mean growth hormone concentrations were twice as high in pregnancy as when non-
breeding and almost three times higher than non-breeding values during lactation
(Table 7.4). Growth hormone concentration was negatively associated \((P<0.05)\) with
HEMD both when ewes were pregnant and lactating (-0.88±0.53 and -1.80±0.53 \(\mu\)g/L per
mm HEMD). However there was no association between growth hormone and HEMD
\((P>0.05)\) when ewes were non-breeding (Table 7.5). Growth hormone generally
decayed as HFAT increased except in low HWT ewes when pregnant and high HWT
ewes when non-breeding (Table 7.3). This association was most consistent when the
ewes were lactating (-1.90±0.36 \(\mu\)g/L per mm of HFAT). Growth hormone was higher
in high HWT ewes in lactation, but there was no relationship between the two in
pregnancy and growth hormone was lower in high HWT ewes when non-breeding
(Figure 7.3). Again the association between growth hormone and HWT was most consistent when the ewes were lactating (0.70±0.14µg/L per kg of HWT).

Figure 7.3 The relationship between plasma growth hormone concentration and the Australian sheep breeding value (ASBV) fat depth at hogget age (HFAT) at a hogget-weight ASBV (HWT) of 2, 5 or 8 when ewes were pregnant (a), lactating (b) and non-breeding (c).
When condition score was added to the model, growth hormone concentration decreased (P<0.05) as condition score increased when ewes were pregnant (-2.6±0.86µg/L per unit of condition score), lactating (-4.1±0.93µg/L per unit of condition score) and when non-breeding (-1.5±0.83µg/L per unit of condition score; Table 7.5). There were no other significant changes to the model when condition score was included.

7.3.5 Insulin-like Growth Factor 1

IGF-I concentrations were 50% higher (P<0.001) in pregnancy than when non-breeding and a further 70% higher in lactation than in pregnancy (Table 7.4). IGF-I concentration decreased (P<0.05) by 9.5±3.69µg/L for each 1mm increase in HEMD when the ewes were lactating, however there was no correlation between HEMD and IGF-I when ewes were either pregnant or non-breeding (Table 7.5). In addition, IGF-I concentration increased (P<0.05) by 6.0±1.41µg/L for each 1kg increase in HWT when the ewes were lactating, however there was no relationship between HWT and IGF-I when ewes were either pregnant or non-breeding (Table 7.5). The relationship between HFAT and IGF-I differed with HWT such that for each 1kg increase in HWT the slope between HFAT and IGF-I increased by 3.9±1.23µg/L per mm HFAT. For example, the influence on IGF-I of higher HFAT was -12.0, -0.3 or 11.5µg/L per mm HFAT at HWT values of 2, 5 or 8kg respectively. When condition score was included in the model, IGF-I concentration increased (P<0.001) by 30.7±7.7µg/L for each unit increase in condition score when the ewes were lactating. However, there was no relationship between IGF-I concentration and condition score when ewes were pregnant or non-breeding (Table 7.5). When condition score was included in the
model the impact of HWT became significant ($P<0.05$) across all states such that, each 1kg increase in HWT resulted in an increase in IGF-I concentration of $2.4\pm0.8\mu g/L$.

Table 7.4 Mean ± s.e. of plasma concentrations of growth hormone, insulin like growth-factor-1 (IGF-I), insulin and leptin across a physiological states.

<table>
<thead>
<tr>
<th>State</th>
<th>Growth Hormone</th>
<th>IGF-I</th>
<th>Insulin</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/L</td>
<td>µg/L</td>
<td>mU/L</td>
<td>µg/L</td>
</tr>
<tr>
<td>Conception</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>0.89±0.03a</td>
</tr>
<tr>
<td>Mid-pregnant</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>0.74±0.03b</td>
</tr>
<tr>
<td>Late-pregnant</td>
<td>6.7±0.58a</td>
<td>24.0±2.38a</td>
<td>3.6±0.29a</td>
<td>0.81±0.03b</td>
</tr>
<tr>
<td>Lactation</td>
<td>9.4±0.59b</td>
<td>57.2±2.40b</td>
<td>4.3±0.29b</td>
<td>0.63±0.03b</td>
</tr>
<tr>
<td>Non-breeding</td>
<td>2.9±0.59c</td>
<td>37.3±2.38c</td>
<td>3.6±0.29a</td>
<td>1.2±0.03c</td>
</tr>
</tbody>
</table>

abc values with different superscripts are significantly different ($P<0.01$)
na: not applicable

Table 7.5 $F$ values for the effect of physiological state, its interaction with time of sample, breeding values for weight (HWT), subcutaneous fat depth (HFAT), eye muscle depth (HEMD) at hogget age, on ewe plasma concentrations of growth hormone, insulin-like growth factor-1 (IGF-I), insulin, and leptin.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Growth hormone</th>
<th>IGF-I</th>
<th>Insulin</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF, DDF</td>
<td>NDF, DDF</td>
<td>NDF, DDF</td>
<td>NDF, DDF</td>
</tr>
<tr>
<td>State</td>
<td>2,1546</td>
<td>343.3***</td>
<td>2,103</td>
<td>94.7***</td>
</tr>
<tr>
<td>State x Time</td>
<td>27,1546</td>
<td>3.0***</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>HWT</td>
<td>1.48</td>
<td>0.0</td>
<td>1.48</td>
<td>15.8***</td>
</tr>
<tr>
<td>HEMD</td>
<td>1.48</td>
<td>8.2**</td>
<td>1.48</td>
<td>0.4</td>
</tr>
<tr>
<td>HFAT</td>
<td>1.48</td>
<td>1.7</td>
<td>1.48</td>
<td>0.4</td>
</tr>
<tr>
<td>State x HWT</td>
<td>2,1546</td>
<td>10.0***</td>
<td>2,103</td>
<td>5.2**</td>
</tr>
<tr>
<td>State x HEMD</td>
<td>2,1546</td>
<td>6.6**</td>
<td>2,103</td>
<td>5.5**</td>
</tr>
<tr>
<td>State x HFAT</td>
<td>2,1546</td>
<td>16.4***</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>HWT x HFAT</td>
<td>1.48</td>
<td>0.0</td>
<td>1.48</td>
<td>10.1**</td>
</tr>
<tr>
<td>State x HWT x HFAT</td>
<td>2,1546</td>
<td>8.1***</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom
ns, not significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$
7.3.6 Insulin

Mean insulin concentration was 17% higher ($P<0.001$) in lactation than when ewes were pregnant or non-breeding (Table 7.4). Insulin concentration was only positively correlated with HWT ASBV when the ewes were lactating, the concentration increasing by $0.4\pm0.08\text{mU/L}$ for each 1kg increase in HWT. There were no correlations between HWT and insulin concentration when ewes were pregnant or non-breeding (Table 7.5). When ewes were lactating the insulin concentration decreased ($P<0.05$) by $-0.7\pm0.22\text{mU/L}$ with each 1mm increase in HEMD, however there was no association between HEMD and insulin when ewes were pregnant or non-breeding. When condition score was included in the model it was negatively related ($P<0.001$) to when ewes were non-breeding ($-1.0\pm0.46\text{mU/L}$ per unit of condition score) but positively correlated with insulin concentration when ewes were lactating ($0.9\pm0.49\text{mU/L}$ per unit of condition score). There was no correlation between condition score and insulin when the ewes were pregnant. Including condition score in the model did not affect the other significant traits.

7.3.7 Leptin

Plasma leptin concentration was lowest during mid-pregnancy and lactation and highest when the ewes were non-breeding (Table 7.4). Plasma leptin increased with HFAT ($0.15\pm0.06\text{µg/L}$ per mm HFAT) across all physiological states (Table 7.5). There was also a positive association between HWT and leptin concentration ($0.03\pm0.01\text{µg/L}$
per kg HWT) but no association between HEMD and plasma leptin (Table 7.5). When condition score was included in the model, plasma leptin also increased ($P<0.001$) with condition score (0.46±0.06µg/L per unit of condition score) across all physiological states. When condition score was included in the model the effect of HFAT remained significant but HWT was no longer significant. Similarly inclusion of liveweight in the model resulted in the effect of HWT being no longer evident but the HFAT effect remained.

### 7.4 Discussion

Ewes with higher HFAT breeding values generally had lower mean growth hormone concentration when the ewes were pregnant, lactating, and non-breeding. The association between HFAT and growth hormone differed between states and with the HWT breeding value of the ewe therefore our hypothesis that growth hormone concentrations would be lower in ewes with high HFAT is partially supported. The negative correlation between growth hormone and HFAT was most consistent when the ewes were lactating and growth hormone concentrations and NEFA were elevated. However, the difference in growth hormone concentration was not reflected in lower NEFA concentrations in high HFAT ewes as would be expected from the findings of Cameron (1992b) and Bremmers et al. (1988). The correlation between HFAT and growth hormone is supported by Carter et al. (1989) and Francis et al. (1997) who showed that sheep selected for low subcutaneous fat depth had higher growth hormone concentrations than high fat equivalents. Elevated growth hormone would
result in lower muscle to bone ratios and an increase in organ weights as demonstrated in sheep selected for reduced fatness (Abdullah et al. 1998), and transgenic sheep with an additional copy of the ovine growth hormone gene (Adams et al. 2006b). Furthermore, treatment of lambs with exogenous growth hormone results in lower total fatness than in un-treated controls (Pell et al. 1990). As growth hormone stimulates IGF-I release, IGF-I concentrations also provide evidence for differences in growth hormone. In sheep selected for leanness or fatness, the lean lines had higher concentrations of IGF-I than the fat equivalents (Cameron 1992b; Francis et al. 1997). Similarly, the breeding value for sub-cutaneous fat depth was negatively correlated with plasma IGF-I in Merino ewes (Adams et al. 2007). These results provide further evidence of increased growth hormone activity in genetically lean sheep. The association between HFAT and IGF-I in our experiment was consistent across states but differed considerably with HWT values such that low growth sheep showed a negative association whereas high growth sheep showed a positive relationship the reason for which is not immediately obvious. The results here, combined with those from the literature demonstrate that selection for higher fatness in the Australian Merino population is likely to inadvertently result in sheep with lower circulating levels of growth hormone. At present the long term implications of such a shift are unclear and require further investigation.

The concentration of growth hormone increased with HWT but only during lactation and therefore our hypothesis is only partially supported. There was no association of HWT with growth hormone concentration when the ewes were pregnant and notably,
growth hormone concentration was lower in high HWT ewes when they were non-breeding. The reason for the varied response depending on the physiological state is not known, however the correlation between HWT and growth hormone concentration was seen when growth hormone was elevated as part of the normal physiological adaptation to lactation (Hatfield et al. 1999). In support of this finding, lactating ewes that had been selected for lifetime performance of total weight of lamb weaned were heavier, produced more milk, weaned heavier lambs and had higher circulating levels of growth hormone (Head et al. 1996). Similarly, rams selected for improved rate and efficiency of gain, had higher concentration of growth hormone than unselected controls (Dodson et al. 1983) and growing lambs had higher bone weight and lower fat depth in response to higher HWT which is suggestive of elevated levels of growth hormone (Gardner et al. 2010). Furthermore, treatment of lambs with exogenous growth hormone results in an increase in growth rate, and similarly lowering circulating growth hormone concentrations results in slower growth, higher fat accumulation and lower organ weights (Wagner and Veenhuizen 1978; Pell et al. 1990; Adams et al. 1996b). In our study, the increase in growth hormone in ewes with higher HWT in lactation was also reflected in higher IGF-I and insulin concentrations which are also known effects of administering exogenous growth hormone (Pell et al. 1990). Higher IGF-I concentration has also been demonstrated in lambs selected for higher growth (Oddy 1993; Hegarty et al. 2006b). Based on the available literature a positive relationship between HWT and growth hormone was expected across all physiological states since the anabolic effects of growth hormone are well established. However, most studies report a positive relationship between growth genotype and growth
hormone concentrations in either growing animals or during lactation when the growth hormone axis is most active. Our results suggest that this relationship is not evident when the axis is less active in pregnancy or in mature animals in the non-breeding state. The result here of a negative relationship between HWT and growth hormone in non-breeding ewes may be partly explained by the finding of higher condition score in high HWT sheep and the finding of a negative relationship between condition score and growth hormone concentration. The results, while not definitive, suggest an up-regulation of the growth hormone axis in ewes selected for high growth, resulting in a greater elevation of growth hormone during lactation in high HWT ewes.

The mean concentration of growth hormone was lower in ewes with higher HEMD when the ewes were both pregnant and lactating, but was not correlated with HEMD when the ewes were non-breeding. Therefore, the hypothesis that ewes with high HEMD will have lower growth hormone concentrations is partially supported. The negative correlation during lactation is supported by a range of information in the literature that intimates reduced growth hormone activity in sheep with high HEMD. For example, lambs from sires selected for high muscling ASBVs had shorter bones and reduced bone mineral content (Cake et al. 2006, 2007; Ponnampalam et al. 2007b) and also slower growth to weaning (Hopkins et al. 2007b). Furthermore, selection for increased loin muscle depth resulted in depressed growth to weaning and increased subcutaneous fat depth in Norwegian sheep (Larsgard and Kolstad 2003). In addition, transgenic sheep with an extra growth hormone gene had lower eye muscle depths than normal controls (Adams et al. 2002b). These combined findings suggest reduced
activity of growth hormone in high muscled sheep. However this was only found
during pregnancy and lactation in our study. It is not clear why the same association
was not found when the ewes were non-breeding. It is possible that in mature ewes,
receiving adequate nutrition and under no physiological stress any genotypic
differences in growth hormone secretion are not expressed. Importantly, IGF-I
concentrations also decreased as ewe HEMD increased when ewes were lactating, a
result that supports the growth hormone result considering the known positive
correlation between circulating levels of growth hormone and IGF-I (Pell et al. 1990;
Adams et al. 1996b). These combined results suggest that selection for higher muscling
is likely to lower the activity of the growth hormone axis in breeding Merino ewes.

Ewe HFAT was positively correlated with plasma leptin across all physiological states.
The assay used (Blache et al. 2000) is known to be highly sensitive but produces lower
values than other published assays (Chilliard et al. 2005). Leptin is produced by
adipose tissue and is positively correlated with the proportion of fat in sheep and is
also higher when short term nutrition is improved (Chilliard et al. 2001; Chillard et al.
2005; Hegarty et al. 2006b; Delavaud et al. 2007). HFAT breeding values are expected to
be well correlated with fat proportion as selection for subcutaneous fat depth is
effective at changing the proportion of fat in sheep (Kadim et al. 1989; Cameron 1992a;
Cameron and Bracken 1992). The positive correlation between HFAT and leptin
concentration demonstrated in this experiment strongly suggests a greater proportion
of fat in high HFAT animal, this result is supported by a positive association between
HFAT and fat measured by DXA. Furthermore, the positive association between DXA
fat and HFAT was evident both with and without liveweight included in the model suggest that higher HFAT is associated with both an increase in the proportion and amount of whole body fat. The positive correlation between HFAT and leptin is supported by the results of Kumar et al. (1998) who reported a higher level of leptin mRNA in adipose tissue from sheep from a high fat line than a low fat selection line. Ewe HWT was also positively correlated with plasma leptin concentration suggesting greater fat in high HWT animals, but this effect was not significant if either liveweight or condition score was included in the model suggesting there was an increase in the total amount of fat but not proportion of fat in high HWT ewes. This finding is supported by results of McFadin et al. (2002) who reported positive correlations between leptin, body condition score and liveweight. In addition to changes associated with total body fat and nutrition, leptin concentrations also change with altered physiological state. This study showed an increase in leptin concentration from mid- to late-pregnancy and then a decline in lactation, a pattern that is normal for sheep (Ehrhardt et al. 2001). Leptin concentration was highest when sheep were non-breeding, but the sheep were also in a higher condition score and more mature at that point than the previous states. Fatness differences associated with higher HFAT were sufficient to increase the amount of total body fat, the proportion of fat and plasma leptin concentrations in breeding Merino ewes.

Plasma glucose concentrations were highest during lactation and lowest during pregnancy. Plasma insulin concentration also showed the same pattern. The finding of higher glucose concentration during lactation than when non-breeding is unusual.
For example, Hatfield et al. (1999) reported an approximate 40% lower glucose in lactating compared with non-breeding ewes and showed that the reduction of glucose concentration was independent of the level of feeding. In a different study there were no changes in either insulin or glucose concentration between pregnancy and lactation (Ingvartsen and Boisclair 2001). This discrepancy between our results and other work is possibly associated with differences in milk production potential of the ewes in each study. The Merino ewes in this study may have produced less milk and therefore placed a reduced demand on glucose than the sheep studied by Hatfield et al. (1999). Along with differences between states there were some genotype differences. There was an increase in glucose concentration in high HEMD ewes when they were non-breeding and pregnant but the relationship was opposite when the ewes were lactating. Although these correlations have not been previously reported the higher glucose concentration in high HEMD ewes when non-breeding was reflected in higher insulin concentrations in high HEMD ewes at this time. An increase in glucose concentration in non-breeding animals offers a possible explanation for an increase in fecundity in high muscled animals (Ferguson et al. 2007). A higher glucose concentration in lactation than non-breeding is an unusual result and may be confounded by feeding or energy balance differences or associated with low milk production in the ewes studied here.

Plasma albumin and urea nitrogen concentrations were assayed to determine differences in protein status between different genotypes. Plasma albumin concentration increased as HEMD increased in all physiological states. Plasma
albumin is an indicator of labile protein stores (Young et al. 1990) and a positive correlation with HEMD suggests ewes with higher HEMD have greater labile protein reserves than low HEMD equivalents throughout the breeding cycle. However all ewes were fed proportionally to their body weight so the mechanism by which different albumin levels arose is unclear but this finding suggests that there are differences in protein metabolism between HEMD genotypes. In this experiment there were no effects of ewe genotype on urea nitrogen status which varied considerably across the breeding cycle. This finding is contrary to expectations since sheep which are genetically leaner have a lower concentration of serum urea than those that were genetically fatter in sheep across a range of selection lines (Bremmers et al. 1988; Carter et al. 1989; Cameron 1992b; Francis et al. 1994). However, it is likely that the achieved differences in fatness were greater in these selection lines than in the range of HFAT breeding values achieved in our experiment. Generally, small differences existed in protein status of animals with high HEMD ewes having higher albumin concentrations throughout the year suggesting higher levels of labile protein reserves.

Selecting Merino ewes based on HWT, HEMD and HFAT results in changes to the concentration of circulating hormones and metabolites throughout a breeding cycle. Ewes with lower HFAT breeding values have higher mean concentrations of growth hormone. Our results, while not definitive, also suggest greater activity of the growth hormone axis in ewes selected for high growth, resulting in a greater elevation of growth hormone in lactation in high HWT ewes. While fatness differences associated with ewe HFAT were not large enough to be differentiated by DXA or condition score,
the correlation of HFAT with leptin concentration suggests an increased fat proportion in ewes with higher HFAT values as would be expected. The increased glucose and albumin concentrations associated with high HEMD in the non-breeding state provide possible mechanisms by which high HEMD ewes are more likely to produce multiple offspring. This work provides a new understanding into the hormonal axes that are likely to change in response to selection of Merino sheep based on ASBVs. The combination of selection for high growth and reduced fatness is highly likely to result in elevated concentrations of growth hormone
Chapter 8 Lamb energy metabolism at birth is altered by maternal genotype and lamb gestation length.

8.1 Introduction

Lamb survival is closely linked to lamb birthweight which is under both genetic and nutritional control (Huisman and Brown 2008; Ferguson et al. 2004a). However lamb birthweight does not explain all of the variation in lamb survival (Ferguson et al. 2004a). At birth, rapid maturation of metabolic processes occurs resulting in the activation of glycogenolysis, gluconeogenesis, lipolysis and ketogenesis (Sperling et al. 1984; Mellor 1988 Girard et al. 1992). There is evidence this metabolic adaptation differs between individuals and that this variation is linked to newborn survival in piglets and lambs (Leenhouwers et al. 2002; Greenwood et al. 2002; Thompson et al. 2006). The link between metabolic maturity and neonatal survival is likely to be a consequence of a reduced ability to maintain glycaemia and body temperature in the period prior to successfully suckling (Bassett 1989; Leenhouwers et al. 2002; Dwyer and Morgan 2006). Selection strategies that increase metabolic maturity at birth and therefore improve lamb survival would be of considerable interest to industry.

Selection strategies that are of interest and may alter the metabolic maturity of newborn lambs are breeding for higher muscling and high growth. The depth of the eye muscle (m. longissimus lumborum) is negatively genetically correlated with lamb birthweight whereas growth traits tend to be positively genetically correlated with lamb birthweight (Huisman and Brown 2008). Yet neither of these changes in lamb
birthweight was associated with changes in survival in our earlier study (Ferguson et al. 2007, chapter 3). The reason for this apparent uncoupling of the relationship between lamb birthweight and survival may be associated with differences in metabolic maturity at birth. In support of this notion higher eye muscle depth and higher lamb survival were unintended outcomes of selecting for higher wool staple strength in Merinos (Greeff et al. 1997). This improvement in survival was associated with a more mature metabolism at birth rather than differences in storages of glycogen, fat or brown fat (Thompson et al. 2006). Quantification of differences in the metabolic maturity of the neonate is provided by the concentration in blood of three key metabolites prior to suckling. Firstly, a higher concentration of glucose reflects a greater capacity for gluconeogenesis which must quickly begin following birth (Townsend et al. 1989; Thompson et al. 2006; Miller et al. 2010). Secondly, a lower blood concentration of urea nitrogen, may reflect the lower level of amino acid oxidation characteristic of the switch from fetus to lamb (Greenwood et al. 2002; Thompson et al. 2006). Thirdly a higher concentration of NEFA reflects a greater capacity to mobilise fat stores (Thompson et al. 2006). It is anticipated that the levels of these metabolites will reflect a greater metabolic maturity in lambs born from ewes with higher muscling.

The survival and subsequent growth of lambs following the perinatal period depends predominantly on the quantity and quality of ewe milk supply. Ewe milk production is dependent on ewe body reserves and nutrition and the partitioning of glucose toward the mammary gland. Lamb growth is closely correlated with ewe milk
production in the first 4 weeks of lactation but the correlation weakens as lactation
continues (Snowder and Glimp 1991). In general, ewes of higher liveweight produce
more milk (Owen 1957; Pattie 1965a), and feeding in pregnancy to increase condition
results in greater milk supply in early lactation (Treacher 1970). In addition, selection
of ewes for higher Australian Sheep Breeding Values (ASBVs) for growth to hogget age
(HWT) results in higher mature size and may therefore result in higher milk
production. Lactation results in loss of liveweight across a number of sheep breeds
(Snowder and Glimp 1991). The majority of this loss is due to mobilisation of fat tissue,
with rapid loss in body condition score in early lactation observed in dairy cows
(Friggens and Badsberg 2007). Ewes that have greater reserves at parturition are likely
to be able to support increased milk production, therefore ewes with high ASBVs for
fat depth on the 12th rib, 45mm from the midline (C site) at hogget age (HFAT) should
have greater reserves to partition toward lactation.

The hypotheses tested were: i) Lambs from ewes with high hogget age ASBVs for eye
muscle depth at the C site (HEMD) will have lower lamb birthweight, lower urea
nitrogen, higher glucose and higher NEFA at birth; and ii) Ewe HWT, HFAT and
liveweight will be positively correlated with lamb birthweight and growth and ewe
milk production.
8.2 Materials and Methods

Merino ewes with known ASBVs for HFAT, HEMD and HWT breeding values were mated to Poll Dorset sires of known ASBVs. Lambs were weighed at birth, at peak lactation, at weaning and post-weaning and ewe milk production was measured during peak lactation. Blood samples were collected from lambs at 1 and 24h post birth and analysed for metabolite concentrations. The experiment was approved and monitored by the CSIRO Floreat Animal Ethics Committee.

8.2.1 Animals

The ewes used in this experiment and their management have been previously described (Chapter 7). Briefly, 109 Merino ewes that were 1.5 years old were sourced from the Merinotech WA nucleus. The ewes had full Australian Sheep Breeding Values (ASBVs) and had not previously lambed. The ewes were selected to achieve a wide range in hogget-age ASBVs for weight (HWT; range 1.7 to 8.0kg), eye muscle depth (HEMD; range -0.6 to 2.6mm), and subcutaneous fat depth (HFAT; range -0.6 to 1.7mm). Ewe oestrus was synchronised using progesterone intravaginal sponges (Chronogest®, Intervet Aust. Bendigo, Australia) and ewes were randomly allocated to two groups of three Poll Dorset rams for mating on day 0 (7 March 2007). The rams had ASBVs for post-weaning weight (PWWT; range 11.0 to 14.2kg), eye muscle depth (PEMD; 0.2 to 2.6mm) and subcutaneous fat depth (PFAT; -1.2 to -0.4mm). Six was thought to be the minimum number of rams that could be used to cover the number of
ewes in oestrus at the same time. The two groups of rams were balanced for the ASBVs and ewes were randomly allocated to sire group. The actual sire of lambs was later identified by a commercial laboratory by DNA profiling of blood samples collected from sires and lambs. The ewes were pregnancy scanned using real-time ultrasound on day 63 and ewes that had conceived on the first oestrus cycle and were pregnant with a single fetus remained in the experiment (n=66).

8.2.2 Experimental procedures

On day 110 ewes were transferred from pasture to single pens in an animal house and were fed a pelleted feed to maintain conceptus-free liveweight based on predictions from Grazfeed® (Horizon Technologies Ltd, Armidale, Australia, Australia). Ewes lambed between days 145 and 154 and remained in the animal house until approximately 2 days following lambing. Between lambing and day 168 ewes ran as a group in a small paddock and had access to pasture as well as pelleted feed. Between days 169 and 196 ewes and lambs were held in single pens in the animal house and fed a pelleted feed at a level to maintain liveweight. Between days 197 until weaning on day 244 ewes and lambs were managed as a group on pasture. Ewes were weighed and condition scored (Jefferies 1961) regularly.

Ewes were monitored near parturition and immediately after birth lambs were placed in a box in the pen with the ewe, allowing the ewe access to the lamb but not allowing the lambs to suckle. One hour following birth, the lambs were weighed and a 5mL
blood sample was collected from the jugular via venipuncture and equal aliquots were placed in tubes with either fluoride/oxalate or EDTA as the anti-coagulate. The blood samples were centrifuged and the plasma harvested and frozen at -20°C (fluoride/oxalate) or at -80°C (EDTA) for later laboratory analysis. Lambs were also weighed at peak lactation (day 176), at weaning (day 244) and two months post weaning (day 313). Gestation length was determined as the number of days between mating and birth.

Ewe milk output was measured according to the method developed by McCance (1959) and refined by Bencini et al. (1995). Milk output was measured on two occasions in early lactation on days 162 and 169. At each occasion ewes were removed from their lambs and restrained in a head bail with access to lupin seed to keep them content while milking. Ewes were administered with an intra-muscular injection of 1IU of synthetic oxytocin (Syntocin, Troy Laboratories, Smithfield, Australia) and then milked with a milking machine operated at a vacuum pressure of 40kPa and a pulsation rate of 100/min. Milk collected from the first milking was discarded. Ewes were kept separate from their lambs for 4h and the milking process was repeated. The total weight of milk collected at the second milking and the time between milkings was recorded. An estimate of milk production per day was calculated by scaling the milk weight recorded in approximately 4h to a 24h time period. A 10mL milk sample was kept from each ewe and frozen at -20°C for later determination of composition.
8.2.3 Sample analysis

Glucose, urea nitrogen, albumin, and lactate were measured on plasma samples collected at 1 and 24h post birth using Fluoride/oxalate as the anticoagulant and were all measured in duplicated in a Cobas Mira Autoanalyser (Hoffman-La Roche Diagnostica, Basal, Switzerland). Glucose concentration in plasma was with an Infinity™ Glucose hexokinase kit (Cat. No. TR15421, Thermo Electron Co., Melbourne, Australia). Plasma urea nitrogen was measured in using the Infinity™ Urea Liquid stable reagent (Thermo Electron Co., Melbourne, Australia). Plasma Albumin was measured using a DMA Albumin kit from Thermo Electron Co., Melbourne, Australia (Cat. No.: TR36026). Concentrations of L-lactate were measured using a Randox L-lactate reagent kit (LC 2389; Randox Laboratories Ltd, Antrim, United Kingdom). Concentrations of non-esterified fatty acids (NEFA) in plasma samples collected at 1 and 24h post birth using EDTA as the anticoagulant and stored at -80°C and were measured in duplicate using a Wako NEFA C Kit (Wako Pure Chemical Ind., Osaka, Japan).

Frozen milk samples were slowly thawed and the proportion of fat, protein, lactose and total solids was determined using a Milko Scan 133 (Foss Electric, Hillerød, Denmark) calibrated for sheep milk. The Milko Scan measures the infra red absorption at wavelengths characteristic of the components to be analysed.
8.2.4 Statistical Analysis

All traits were analysed using linear mixed effects models in SAS software (SAS version 9.1, SAS Institute, Cary, NC, USA). Lamb birthweight, weight at peak lactation, weaning weight, post weaning weight and gestation length were analysed with the fixed effect of sex (male, female). The covariates included were gestation length (when not the dependent variable), ewe HFAT, ewe HWT and ewe HEMD. The sire of the lamb was included as a random term. All first order interactions were included in the starting model, and removed in a stepwise process if non-significant ($P>0.05$). Models were run with and without the inclusion of ewe liveweight at lambing. For analysis of lamb plasma concentrations of glucose, albumin, NEFA, lactate and urea nitrogen, the above process was followed with the addition of the fixed effect of time post-birth (1hour, 24hours) and the addition of lamb tag as a random term. Furthermore, models were fitted with and without the inclusion of lamb birthweight. Milk production, fat, protein, and lactose were analysed with the fixed effect of day of milking (162, 169), and covariates of HFAT, HWT, and HEMD. The sire of the lamb and ewe tag were both included as random terms. All first order interactions were included in the starting model and removed in a stepwise process if not significant ($P>0.05$).
8.3 Results

8.3.1 Lamb metabolites at birth

All plasma metabolites studied had different ($P<0.001$) concentrations at 1 hour post birth compared to 24 hours post birth (Table 8.1). Plasma glucose concentration increased two-fold between 1h and 24h post birth. Mean plasma glucose concentration was lower ($P<0.05$) in lambs from higher muscled ewes (-0.48±0.20mM per mm HEMD). For this and all results reported in this chapter the figures shown are means ±s.e. There was no effect of either ewe HFAT or HWT on lamb plasma glucose concentration. There was a positive association between mean glucose concentration and gestation length (range 146 to 153 days), such that, glucose increased ($P<0.05$) by 0.28±0.10mM for each extra day of gestation. However there was no correlation between lamb birthweight and plasma glucose.

Plasma albumin decreased ($P<0.001$) by 6% between 1h and 24h post birth. Plasma albumin increased ($P<0.05$) with lamb birthweight (0.61±0.28g/L per kg of lamb birthweight). However there was no association ($P>0.05$) between plasma albumin and gestation length or HWT, HEMD or HFAT ASBVs.

Plasma lactate decreased by 34% between 1h and 24h post birth. There were no effects of lamb birthweight, gestation length, HWT, HEMD or HFAT on lactate concentration. Plasma NEFA concentration decreased by 30% between 1 and 24h post birth but there were no other effects ($P>0.05$) on plasma NEFA concentrations.
Table 8.1 Concentrations of glucose, albumin, urea nitrogen, non-esterified fatty acids (NEFA) and lactate in lamb plasma collected 1 hour and 24 hours post birth.

<table>
<thead>
<tr>
<th>Time post birth</th>
<th>Glucose mM</th>
<th>Albumin g/L</th>
<th>Urea nitrogen mg/L</th>
<th>NEFA mM</th>
<th>Lactate mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour</td>
<td>3.12±0.19\textsuperscript{a}</td>
<td>30.29±0.23\textsuperscript{a}</td>
<td>6.44±0.53\textsuperscript{a}</td>
<td>0.57±0.02\textsuperscript{a}</td>
<td>4.56±0.22\textsuperscript{a}</td>
</tr>
<tr>
<td>24 hours</td>
<td>6.45±0.19\textsuperscript{b}</td>
<td>28.60±0.23\textsuperscript{b}</td>
<td>11.66±0.53\textsuperscript{b}</td>
<td>0.40±0.02\textsuperscript{b}</td>
<td>2.99±0.22\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b}Values with different superscripts within a column are different (\(P<0.001\)).

Plasma urea nitrogen rose by 82% between 1 and 24 hours post birth. Urea nitrogen increased (\(P<0.05\)) with an increase in gestation length (0.89±0.25 mg/L per day of gestation) but decreased as lamb birthweight increased (1.14±0.55 mg/L lower for each 1 kg increase in lamb birthweight). There were no effects (\(P>0.05\)) of any of the ewe ASBVs on urea nitrogen in the newborn lamb.

8.3.2 Ewe milk production and quality

Ewe milk production was 32% higher (\(P<0.001\)) on day 162 (1258±58 g/day) than on day 169 (955±60 g/day). Milk production on both days was higher in ewes with higher liveweight (25±10 g/day per kg liveweight). There was also a positive association (\(P<0.05\)) between ewe HEMD and milk production across both days (124±60 g/day higher per mm HEMD). There were no effects of HWT or HFAT on ewe milk production. The percentage of milk fat was higher at day 162 (4.00±0.16%) than day 169 (3.50±0.17%). There were no effects of liveweight, HWT, HEMD, or HFAT on milk fat. The percentage of protein in the milk was also higher at day 162 (4.78±0.05%) than
day 169 (4.55±0.05%). Protein percentage increased ($P<0.05$) with HWT across both milking times, and for each 1kg increase in HWT, milk protein increased by $0.04±0.02\%$. HEMD, HFAT, and ewe liveweight did not have an effect ($P>0.05$) on the percentage of protein in milk. The percentage of lactose in milk did not alter ($P<0.05$) between day 162 and 169 or with HWT, HEMD, HFAT or liveweight.

8.3.3 Lamb weights and gestation length

The mean gestation length was 149.5±0.7 days and ewe liveweight or ASBVs did not affect ($P>0.05$) gestation length. Male lambs tended to be heavier ($P<0.1$) than female lambs at birth (4.38±0.12kg male, 4.12±0.11kg female), at peak lactation (12.2±0.4kg male, 11.6±0.4kg female), at weaning (32.4±0.7kg male, 30.5±0.7kg female), and two months post-weaning (34.8±0.8kg male, 33.1±0.9kg female). There were no effects ($P>0.1$) on weight at any age of ewe ASBVs for HWT, HEMD or HFAT.

8.4 Discussion

Mean plasma glucose concentration was lower in lambs from ewes with higher muscle which was opposite to our hypothesis. Furthermore there was no effect of either ewe HFAT or HWT on lamb plasma glucose concentration. We expected lambs from high muscled ewes to have higher glucose based on the findings of Thompson et al. (2006) of higher glucose in newborn lambs from ewes selected for high staple strength (Greeff et al. 1997) and the generally positive genetic correlations between staple strength and
muscling (Huisman and Brown 2009). A potential explanation for this difference is that in the study of Thompson et al. (2006) the lambs were sired by rams of the same genotype as the ewe, whereas in our study common sires of very different genotype were used across all ewes. Considering glucose concentration is likely to be a trait of the lamb rather than a trait of the dam the different sires used in our study may have masked any effects of ewe genotype on the lamb. Lamb glucose concentration doubled between 1 hour and 24 hours post birth, this increase in glucose concentration in the first day after birth and following suckling is consistent with previous results (Greenwood et al. 2002; Thompson et al. 2006). There was no effect of lamb birthweight on glucose concentration; similarly Greenwood et al. (2002) also reported no association between birthweight and newborn glucose concentration. However, there was a positive association between mean glucose concentration and gestation length in our study, which again differs from the study of Thompson et al. (2006) where lambs from the high staple strength ewes had both a shorter gestation and higher glucose.

There were no associations between ewe ASBVs and lamb birthweight or growth, this result was unexpected and is contrary to our hypothesis. There are generally positive genetic correlations between lamb birthweight and growth traits and also between growth traits in the sheep population (Huisman and Brown 2008). Furthermore the existence of these correlations has been confirmed in a field study in the population where the ewes used in this study were sourced from. In that study ewe HWT was positively correlated with lamb birthweight and lamb growth (Ferguson et al. 2007; Chapter 3). That study involved large numbers of ewes and lambs and it is possible
that the restricted numbers in this study and the experimental design which maximised variation within the ewe population for other traits limited our ability to find a significant correlation between HWT and lamb birthweight and growth. In support of our finding here, Hopkins et al. (2007a) reported no correlation between yearling weight ASBV and lamb birthweight. The lack of negative relationships between HEMD and lamb birthweight and growth is also surprising. Huisman and Brown (2008) reported a negative genetic correlation between HEMD and lamb birthweight in the Australian sheep population. In addition, we have previously shown (Ferguson et al. 2007; Chapter 3), a negative correlation between ewe HEMD breeding value and lamb birthweight in the same flock the ewes used in this study were sourced from. Furthermore, Larsgard and Kolstad (2003) have also shown lower birthweights and lower growth to weaning in sheep selected for increased muscling. There were very few relationships with lamb birthweight and lamb growth in this study, the reasons for limited results are difficult to explain but are likely to be associated with the limited numbers reported here and should be viewed with a degree of caution.

Ewe milk production was positively related to liveweight but not correlated with HWT or HFAT, our hypothesis is therefore only partially supported. Ewe milk production decreased between the two milking times which suggests the ewes reached peak lactation within the first 10 to 14 days of lactation. The positive association between ewe liveweight and ewe milk production is supported by the results of Pattie (1965a) who showed that ewes selected for weaning weight were both heavier and produced
more milk. It is also supported by the general across-species correlation of milk production with mature size (Taylor 1973). The lack of an association between HWT and ewe milk production was unexpected because the ewes with high HWT breeding values had higher circulating levels of growth hormone (Chapter 7) which was expected to result in an increase in milk production. Furthermore, previous studies have shown that sheep selected for increased growth were heavier, had higher growth hormone concentrations and produced more milk than unselected controls (Head et al. 1996). Considering these correlations it is not clear why HWT was not associated with milk production in this study. However there was positive association between ewe milk production and HEMD, but the mechanism driving this relationship is unknown. Ewe milk production was higher in ewes with higher liveweight and also in those ewes with higher muscling breeding values.

The concentration of urea nitrogen almost doubled between 1h and 24h post birth. This large increase in urea nitrogen concentration was also seen in the first 1 to 2 days after birth by Greenwood et al. (2002) and urea nitrogen levels remained elevated for up to 9 hours after birth in the experiment of Thompson et al. (2006). A high concentration of urea nitrogen is likely to indicate a high rate of amino acid catabolism which is required to support the high rate of gluconeogenesis in the neonate (Warnes et al. 1977; Hodgson et al. 1982). Plasma urea nitrogen was also higher in lambs with longer gestation times suggesting that gluconeogenesis from oxidation of amino acids was greater in these lambs. The increase in plasma urea nitrogen with gestation length is supported by the results of Thompson et al. (2006) who found that lambs from ewes.
selected for higher wool staple strength had both shorter gestation lengths and a lower concentration of urea nitrogen than lambs from unselected controls. Furthermore, lambs with lower birthweights had higher urea nitrogen concentrations, a result which was also found by Greenwood et al. (2002). These combined results are suggestive of reliance on amino acids as gluconeogenic precursors in the neonatal lamb for over 24 hours after birth. Lambs with longer gestation and lower birthweights appear to rely on the oxidation of amino acids to fuel gluconeogenesis (i.e. maintain a fetal-like metabolism; Greenwood et al. 2002) longer than those with a short gestation or higher birthweights and therefore have a less mature metabolism.

Plasma concentrations of lactate and NEFA were lower at 24h than 1h after birth, these results are supported by those of Thompson et al. (2006) who found that lactate and NEFA concentrations reduced over the first 9h after birth. Plasma concentrations of albumin decreased slightly between 1 and 24h after birth, possibly as a result of improved hydration following suckling over this period. There was a positive association between lamb birthweight and plasma albumin which is opposite to results in newborn piglets (Mersmann et al. 1984).
Chapter 9  
Ewes selected for high muscling mobilise less muscle glycogen and ewes selected for leanness release less glucose in response to adrenaline.

9.1 Introduction

Genetic selection for higher muscling and lower fatness is commonly practiced in sheep breeding programs to increase the relative proportion of muscle to fat in sheep carcasses (Kadim et al. 1989; Hegarty et al. 2006a). This results in improved processing efficiency and more appealing meat for consumers. This selection strategy has also resulted in changes in the response to either physiological or environmental stressors in pigs, sheep and cattle (Standal and Vold 1973; Mersmann 1985; Gardner et al. 2005; McGilchrist et al. 2011). Part of the normal response to stress is an increase in circulating levels of adrenaline resulting in the rapid mobilisation of energy stores. This mobilisation results in the release of glucose, lactate, and non-esterified fatty acids (NEFA) from liver, muscle, and adipose tissue (Leenanuruksa and McDowell 1985). Animals that are more sensitive or responsive to stress will mobilise more energy stores in response to small stressors and may therefore be less efficient at converting available feed into storage tissues (Knott et al. 2008). In addition, maternal tissue sensitivity and responsiveness to adrenaline is increased in the catabolic states of late pregnancy and lactation, particularly in adipose tissue (Vernon and Finley 1985; Guesnet et al. 1987; McNamara 1988). It is expected that these adaptations due to changes in physiological state will over-ride any impacts of selection for muscling or fatness on response to adrenaline. It is important to determine the impact of selection for higher muscling and lower fatness on responses to stress in breeding Merino ewes.
as their ability to build energy stores in nutritionally marginal environments is paramount for reproductive success.

The impact of selection for higher muscling and lower fatness on the response to adrenaline is expected to differ between liver, muscle, and adipose tissue. Adrenaline stimulation of the liver results in a rapid increase in the output of glucose as a result of higher rates of mobilisation of glycogen and synthesis of glucose (Brockman 1991). The rate of gluconeogenesis in response to adrenaline may be higher in genetically fat sheep as both the basal and adrenaline-stimulated rates of glucose synthesis are higher in genetically fat rats (Rohner-Jeanrenaud et al. 1986; Sánchez-Gutiérrez et al. 2000). Furthermore, obese humans have higher rates of gluconeogenesis and higher concentrations of hepatic glycogen than their lean counterparts, both of which would favour greater glucose release in response to adrenaline (Müller et al. 1997). To the best of my knowledge changes in muscularity are not associated with liver metabolism therefore the muscling genotype is not expected to be correlated with glucose output. However selection for muscling is expected to reduce the glycogenolytic response to adrenaline in muscle tissue because high muscled sheep and cattle have been shown to have a lower glycogenolytic response to adrenaline in muscle tissue than their low muscled counterparts (Gardner et al. 2005; Martin et al. 2011). In summary, selection of sheep for less fat is expected to reduce liver glucose output in response to adrenaline while selection for muscling will not change glucose output from the liver but will reduce the glycogenolytic response to adrenaline in muscle.
Selection for muscling is not expected to change the response to adrenaline within adipose tissue in adult ewes. While high muscled lambs had a greater lipolytic response to exogenous adrenaline when they were four months old, this difference was no longer evident at 14 months of age (Martin et al. 2011). Alternatively, the effect of selection for lower fatness is expected to lower the lipolytic response to adrenaline in adult ewes. As sheep become fatter the size of adipocytes is increased (see Allen 1976) resulting in a greater responsiveness of adipocytes to adrenaline (Vernon and Finley 1985; Gilson et al. 1996). In addition, response to adrenaline has been shown to be correlated with the weight of fat tissue (Gilson et al. 1996). It is therefore expected that in adult ewes the lipolytic responsiveness to adrenaline will be positively correlated with body fatness, as is found in dairy cows (Theilgaard et al. 2002).

My hypotheses are that: i) glucose output following adrenaline challenge will be decreased as the breeding value for fatness of ewes is decreased; ii) the glycogenolytic response to adrenaline in muscle will decrease as the muscling breeding value of ewes is increased and the fatness breeding values are decreased; iii) the lipolytic response to adrenaline will be higher in ewes with higher fatness breeding values; iv) any breeding value effects on response to adrenaline across all tissues will not be evident in late pregnancy and lactation.
9.2 Materials and Methods

9.2.1 Experimental Design

A range of doses of exogenous adrenaline were administered to ewes of known muscling and fatness breeding values and the resultant change in plasma glucose, lactate and NEFA concentrations were measured. Adrenaline was administered to the same ewes during late pregnancy, at peak lactation, and when ewes were non-breeding (non-pregnant, non-lactating). Experiments were approved and monitored by the CSIRO Floreat Animal Ethics Committee.

9.2.2 Animals

The ewes used in this experiment were part of a larger experiment, the details of this experiment and the source and overall management of the animals has been described in chapter 7. Briefly, 109 Merino ewes were selected based on divergence in their Australian Sheep Breeding Values (ASBVs) for eye muscle depth (HEMD) and subcutaneous fat depth (HFAT) at hogget age. On day zero (7 March 2007) the ewes were naturally mated over a three day period after the timing of ovulation had been synchronised, and 24 ewes of those pregnant with a single fetus (determined by ultrasound) were selected for use in this experiment. The ewes were maintained on pasture until day 110 when they were individually penned indoors in an animal house. Ewes lambed between days 145 and 154 and remained in individual pens until
approximately 2 days after lambing. Ewes were individually penned between days 168 and 197 and between days 257 and 287 and the lambs were weaned on day 244. When animals were not in individual pens they were managed as a group on pasture.

Selection of the 24 ewes was based on their individual ASBVs for HEMD and HFAT. These two ASBVs are positively correlated within the Australian Merino population (Clarke et al. 2003), so to determine their independent effects on energy metabolism it was important to select sheep such that the HEMD/HFAT correlation was minimised within the experimental population. To achieve this we selected ewes within three muscling groups (high, medium and low) based on their HEMD ASBVs. Within each muscling group we selected ewes across the widest range of HFAT possible. The ASBVs used in selection of animals for this experiment were calculated by MERINOSELECT on 21 March 2007, however those used in the analysis of this experiment were those current on 21 March 2008 (Figure 9.1).
Figure 9.1 Subcutaneous fat depth (HFAT) and eye-muscle depth (HMD)

Australian Sheep Breeding Value (ASBV), calculated 21 March 2008, for 8 ewes in each of the high, medium and low muscling groups.

Ewes were individually fed at maintenance for each of the three occasions they were in the animal house, and maintenance requirements were calculated using Grazfeed® (Horizon Technologies Ltd, Armidale, Australia). They were fed a pelleted ration containing 11.3, 11.8 or 10.9MJ metabolisable energy/kg drymatter and 13%, 15.5% or 13.5% crude protein while in the animal house during each of the physiological states of late pregnancy, lactation and non-breeding. Ewes were weighed and condition scored (Jefferies 1961) weekly while in the animal house and the ration was adjusted weekly according to liveweight change and to account for the increasing energy requirements of pregnancy or lactation when applicable.
9.2.3 Preparation of animals

Three days prior to adrenaline challenges, animals were fitted with a polyvinyl catheter in each external jugular vein. Catheters were kept patent by filling with $2.5 \times 10^4$ U/L of heparinised sterile saline (Heparin Sodium, Pharamacia Australia, Bentley, WA in NaCl 9g/L, Baxter Healthcare, Old Toongabbie, Australia) when not in use. The catheter used for sample collection was flushed with 12.5g/L EDTA (Product code: ED2P, Sigma-Aldrich Pty. Ltd., Castle Hill, Australia) in sterile saline between sample collections. On the day of the procedure the daily allocated feed ration was divided into four equal portions and a portion was fed at 0700, 1015, 1200 and 1600h.

9.2.4 Experimental Procedure

At each physiological state, adrenaline challenge experiments were carried out in two groups of 12 ewes, with each group of 12 ewes involved in experiments for three consecutive days. The experiments were performed between days 130-132, and 135-137 during pregnancy, 187-189 and 194-196 during lactation and days 278-280 and 285-287 when non-breeding. Five experiments were completed on each ewe and each experiment involved administering a different bolus dose of adrenaline (0.2, 0.6, 1.2, 2.0 and 3.0µg/kg liveweight adrenaline acid tartrate; Adrenaline Injection BP, AstraZeneca Pty Ltd, North Ryde, Australia) in each physiological state. All bolus doses were diluted in sterile saline to a total volume of 5mL. Experiments on each ewe were completed in the morning (0800h) and afternoon (1300h) for two consecutive days and
in the morning of the third day. Within groups of 12 ewes, the level of adrenaline was administered according to a randomised block design across the five experiments such that only 2 or 3 ewes were receiving the same adrenaline dose at any one time.

The adrenaline challenges used during this study were administered on a per kilogram liveweight basis, with liveweights corrected for the estimated weights of the gravid uterus (during pregnancy) and wool weight such that animal response to adrenaline between physiological states could be directly compared. The estimated weight of the gravid uterus was based on the equations of Wheeler et al. (1971). The wool weight correction had to take account of the time since last shearing. The ewes were shorn 5 months prior to the commencement of the experiment (ie day -150) and on day 230 between the lactation and non-breeding insulin challenges. The small amount of wool present during the non-breeding challenges did not warrant a correction. The wool weight used to calculate corrected liveweight during pregnancy and lactation was determined by using the ewes’ day-150 fleece weight and days since shearing to calculate estimated wool weight.

In each experiment, 6mL blood samples were collected from a jugular catheter at -30, -15, -10, -5, 0, 2.5, 5, 10, 15, 20, 30, 45, 60, 120 and 130min relative to the bolus dose of adrenaline. Immediately after the collection of the blood at time 0, the bolus dose of adrenaline was administered via a jugular catheter and the catheter was flushed with 12.5g/L EDTA. Blood samples were collected by syringe and an aliquot was immediately transferred into both fluoride-oxalate and EDTA blood tubes and placed
on ice. Blood samples were centrifuged and the plasma harvested from fluoride/oxalate tubes was frozen at -20°C for later determination of lactate and glucose concentrations, and plasma from EDTA tubes was frozen at -80°C for later measurement of non-esterified fatty acid concentration.

9.2.5 Chemical Analysis

Concentrations of glucose in plasma were measured in duplicate in a Cobas Mira Autoanalyser (Hoffman-La Roche Diagnostica, Basal, Switzerland) with an Infinity™ Glucose hexokinase kit (Cat. No. TR15421, Thermo Electron Co., Melbourne, Australia). Plasma L-lactate concentration was measured in duplicate in a Cobas Mira Autoanalyser with a Randox L-lactate reagent kit (LC 2389; Randox Laboratories Ltd, Antrim, UK). Plasma concentrations of NEFA were measured on samples collected in EDTA, plasma samples were measured in duplicate using a Wako NEFA C Kit (Wako Pure Chemical Ind., Osaka, Japan).

9.2.6 Calculation of the response to adrenaline

Basal concentrations of glucose, lactate and NEFA were calculated as the mean of samples collected at -30, -15, -10, -5, 0min relative to the adrenaline challenge. The concentrations of each substrate were plotted against time for each experiment on each ewe and a derived function with multiple exponential components was fitted to the
raw data following the method described by McGilchrist et al. (2011). The function was then used to determine the time to maximum substrate concentration, the maximum substrate concentration and the area under the response curve between 0 and 10 min (AUC10) relative to administering the adrenaline challenge. The cut-off of 10 min for area under the response curve calculations was chosen because it best reflected the time to maximum concentration of the three substrates studied.

9.2.7 Statistical Analysis

The basal substrate concentration, time to reach maximum concentration, maximum concentration, and AUC10 for each of glucose, lactate and NEFA were analysed using linear mixed effects models in SAS software (SAS version 9.1, SAS Institute, Cary, NC, USA). For each term analysed the fixed effects included physiological state (pregnant, lactating, non-breeding) and muscling level (low, medium, high). Covariates included were adrenaline dose, the squared term of adrenaline dose, HFAT, and HWT. Animal tag and ewe birth type were used as random terms. All first and second order interactions were included in the starting model, and removed in a stepwise process if non-significant ($P>0.05$). All models were run with and without the inclusion of ewe condition score (and condition score within state). For the analysis of time to reach maximum concentration, maximum substrate concentration, and AUC10, the models were run with and without the inclusion of basal substrate concentration.
9.3 Results

9.3.1 Liveweight and condition score

Liveweight (50.4±2.5kg) did not differ (p>0.05) between physiological states. For this and all results reported in this chapter the figures shown are means ±s.e. Liveweight did differ between muscle groups with ewes in the high muscled group (48.4±1.9kg) being about 6% lighter (P<0.05) than the medium (51.2±1.9kg) and low (51.6±1.9kg) muscled groups. Liveweight increased with HWT (1.3±0.3kg per kg HWT) but it was not affected by HFAT. Condition score of ewes when non-breeding (3.1±0.1units) was higher (P<0.001) than when ewes were either pregnant (2.7±0.1units) or lactating (2.8±0.1units). Condition score did not differ between muscling groups or with HFAT level but was higher (P<0.05) in ewes with higher HWT (0.06±0.03 condition score units per kg HWT).

9.3.2 Basal substrate concentrations

Basal glucose concentration was higher (P<0.001) when the ewes were lactating (4.18±0.06mM) than when ewes were pregnant (3.21±0.08mM) or non-breeding (3.22±0.06mM). There was no overall difference (P>0.05) in basal glucose concentration between muscling groups however the effect of HFAT on basal glucose differed between states and muscle groups (Table 9.1). In the non-breeding state, basal glucose decreased (P<0.05) with increasing HFAT (0.30±0.21mM per mm HFAT) but only in the
low muscled group. In pregnancy there was only an effect within the medium muscled group where basal glucose was negatively correlated with HFAT (0.37±0.12 mM per mm HFAT). During lactation there was no effect of HFAT or muscle group on basal glucose. Similarly the effect of HWT on basal glucose differed between states and muscle groups (Table 9.1). When non-breeding, basal glucose increased in the low muscled group only (0.11±0.05 mM per kg HWT) and during pregnancy basal glucose increased with HWT but only in the medium muscled group (0.16±0.04 mM per kg HWT). However, during lactation, basal glucose decreased with an increase in HWT but only in the high muscled group (0.07±0.05 mM per kg HWT). When condition score was included in the model its effect on basal glucose concentration differed between states ($F=5.2; P<0.01$). Basal glucose concentration increased with each unit increase in condition score, but only when ewes were pregnant (by $0.45±0.10$ mM) or lactating (by $0.27±0.09$ mM).

Basal lactate concentration was higher ($P<0.01$; Table 9.2) when ewes were lactating (0.75±0.03 mg/L) than when the ewes were pregnant (0.55±0.03 mg/L) or non-breeding (0.60±0.03 mg/L). It was lower ($P<0.05$) in the low muscled group (0.55±0.03 mg/L) than the medium (0.67±0.03 mg/L) or high (0.68±0.03 mg/L) muscled groups, but there was no correlation between basal lactate concentrate and HFAT or HWT (Table 9.2). When condition score was included in the analysis basal lactate concentration increased with each unit increase in ewe condition score by $0.14±0.06$ mg/L in all physiological states ($F=5.15; P<0.05$).
Basal NEFA concentration differed \( (P<0.001) \) between all states. Compared with when ewes were non-breeding \((0.03\pm0.01\text{mM})\), values were twice as high when lactating \((0.06\pm0.03\text{mM})\) and three times as high when the ewes were pregnant \((0.09\pm0.03\text{mM})\).

There was no overall effect of ewe muscling group on basal NEFA concentration, however in pregnancy and in the low muscled group only, basal NEFA concentration reduced as HFAT increased \((0.05\pm0.03\text{mM per mm HFAT})\). There were no other effects of HFAT or muscle group or HWT on basal NEFA concentration. In addition, there was no effect of condition score on basal NEFA concentration when it was included in the model.

9.3.3 Glucose response to adrenaline

When averaged across adrenaline doses the maximum concentration of glucose reached in response to adrenaline was 18% higher \( (P<0.001) \) during lactation \((5.25\pm0.06\text{mM})\) than non-breeding \((4.44\pm0.06\text{mM})\), and was lowest \( (P<0.001) \) when ewes were pregnant \((3.94\pm0.06\text{mM})\). These differences appeared to be explained by basal glucose concentration which, when included in the model (within state), removed the effect of physiological state. For each 1mM increase in basal glucose, the maximum concentration of glucose in response to adrenaline increased \( (P<0.001) \) by 0.96\( \pm 0.09 \), 0.98\( \pm 0.09 \), and 0.72\( \pm 0.09 \)mM when ewes were pregnant, lactating and non-breeding.

The maximum concentration of glucose was reached in a longer time when ewes were lactating \((9.5\pm0.6\text{min})\) than when ewes were pregnant \((8.1\pm0.6\text{min})\) or non-breeding \((8.4\pm0.6\text{min}; \text{Table 9.1})\). Across all physiological states the time taken to reach
maximum concentration of glucose was 0.7±0.3 minutes longer ($P<0.05$) with each 1kg increase in HWT (Table 9.1). The time to maximum concentration reduced as the level of adrenaline challenge administered increased, however this effect was only evident in ewes with a HWT value higher than 4kg (Table 9.1). There was no effect of either muscle group or HFAT on the time to maximum concentration (Table 9.1).

**Table 9.1 F values for the effect of physiological state, muscle group, ewe Australian sheep breeding values (ASBVs) for sub-cutaneous fat depth (HFAT) and weight (HWT), adrenaline challenge and condition score and significant interactions on basal glucose concentration, maximum glucose concentration, time to maximum glucose concentration and area under curve of glucose response between 0 and 10 minutes relative to administering adrenaline (AUC10).**

<table>
<thead>
<tr>
<th>Effect</th>
<th>NDF, DDF</th>
<th>$F$ value</th>
<th>NDF, DDF</th>
<th>$F$ value</th>
<th>NDF, DDF</th>
<th>$F$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>State</td>
<td>2,304</td>
<td>36.8***</td>
<td>2,288</td>
<td>3.3*</td>
<td>2,311</td>
<td>3.1*</td>
</tr>
<tr>
<td>Muscle Group</td>
<td>2,304</td>
<td>0.2</td>
<td>2,288</td>
<td>0.5</td>
<td>2,311</td>
<td>2.2</td>
</tr>
<tr>
<td>HFAT</td>
<td>1,304</td>
<td>0.1</td>
<td>1,288</td>
<td>0.3</td>
<td>1,311</td>
<td>0.2</td>
</tr>
<tr>
<td>HWT</td>
<td>1,304</td>
<td>4.1*</td>
<td>1,288</td>
<td>4.9*</td>
<td>1,311</td>
<td>0.2</td>
</tr>
<tr>
<td>State x Muscle Group</td>
<td>4,304</td>
<td>6.1***</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>State x HFAT</td>
<td>2,304</td>
<td>2.6</td>
<td>ns</td>
<td>ns</td>
<td>2,311</td>
<td>2.5</td>
</tr>
<tr>
<td>State x HWT</td>
<td>2,304</td>
<td>4.7**</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Muscle Group x HFAT</td>
<td>2,304</td>
<td>1.5</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Muscle Group x HWT</td>
<td>2,304</td>
<td>0.1</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>State x Muscle Group x HFAT</td>
<td>4,304</td>
<td>13.1***</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>State x Muscle Group x HWT</td>
<td>4,304</td>
<td>6.7***</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Challenge</td>
<td>ns</td>
<td>ns</td>
<td>1,288</td>
<td>0.36</td>
<td>1,311</td>
<td>135.5***</td>
</tr>
<tr>
<td>Challenge x Challenge</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>1,311</td>
<td>31.7***</td>
</tr>
<tr>
<td>State x Challenge</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>2,311</td>
<td>3.49*</td>
</tr>
<tr>
<td>Challenge x HFAT</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>1,311</td>
<td>2.82</td>
</tr>
<tr>
<td>Challenge x HWT</td>
<td>ns</td>
<td>ns</td>
<td>1,288</td>
<td>4.73*</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Challenge x state x HFAT</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>2,311</td>
<td>4.54**</td>
</tr>
</tbody>
</table>

NDF, DDF; numerator and denominator degrees of freedom

ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$
Given the time to reach maximum concentration of about 10 min, AUC was calculated for the first 10 min of the response (AUC10). When glucose AUC10 was averaged across all dose levels it was around 20% lower ($P<0.05$) when ewes were pregnant (6.52±0.53mM/10min) than when lactating (8.20±0.53mM/10min) or non-breeding (8.64±0.53mM/10min). Glucose AUC10 increased ($P<0.001$) by around 500% across the range of adrenaline doses administered and was greater ($P<0.05$) in ewes with higher HFAT ASBVs but only when they were pregnant or non-breeding (Figure 9.2). During pregnancy this HFAT effect was evident across all levels of adrenaline challenge with this difference as large as 4mM/10min between the HFAT extremes used in this study. When the ewes were non-breeding the HFAT effect was only evident at adrenaline challenges greater than 2ug/kg liveweight, and was similar in magnitude to that seen at these challenge levels during pregnancy.
Figure 9.2 Glucose concentration area under curve between 0 and 10 minutes (AUC10) relative to adrenaline dose in: a) pregnancy; b) lactation; and c) non-breeding ewes with breeding values for subcutaneous fat over the loin at hogget age (HFAT) of -0.5, 0.5 and 1.5mm.

There was no effect of either muscle group or HWT on the glucose AUC10 in response to adrenaline (Table 9.1). There was no change to the glucose AUC10 model when basal glucose was included. For each 1mM increase in basal glucose the AUC10 was reduced ($P<0.001$) by 0.98±0.66, 1.04±0.59, and 3.61±0.79mM/10 min when ewes were pregnant, lactating and non-breeding. When condition score was included in the
model its effect differed between states ($F=4.1; P<0.05$). There was no effect of ewe condition score on glucose AUC10 when ewes were pregnant or non-breeding but during lactation for each 1 unit increase in condition score, glucose AUC10 decreased by $2.16\pm1.35\text{mM}/10\text{min}$).

9.3.4 Lactate response to adrenaline

The time taken to reach maximum lactate concentration did not differ between pregnant, lactating and non-breeding ewes (12.1±0.84, 10.5±0.85, and 11.9±0.83 min). There were no effects of muscle group, HFAT or HWT on time to reach maximum lactate concentration (Table 9.2). There was no effect of condition score when it was included in the model. The maximum concentration of lactate in response to adrenaline was on average 50% higher ($P<0.01$) when ewes were lactating (1.54±0.06 mg/L) and 30% higher when ewes were non-breeding (1.30±0.06 mg/L) than when the ewes were pregnant (1.03±0.06 mg/L). There was no change to the maximum lactate concentration model when basal lactate was included. For each 1 mg/L increase in basal lactate concentration the maximum lactate concentration increased ($P<0.001$) by 1.02±0.07 mg/L across all physiological states. The impact of adrenaline dose, muscling and other genotype effects within the maximum lactate concentration analysis were similar to the results for lactate AUC10 (Table 9.2).
Table 9.2 F values for the effect of physiological state, muscle group, ewe Australian sheep breeding values (ASBVs) for subcutaneous fat depth (HFAT) and weight (HWT), adrenaline challenge and condition score and significant interactions on basal lactate concentration, maximum lactate concentration, time to maximum lactate concentration and area under curve of lactate response between 0 and 10 minutes relative to administering adrenaline (AUC10).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Basal lactate</th>
<th>Time to lactate maximum conc.</th>
<th>Lactate AUC10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF, DDF</td>
<td>F value</td>
<td>NDF, DDF</td>
</tr>
<tr>
<td>State</td>
<td>2,42</td>
<td>12.6***</td>
<td>2,317</td>
</tr>
<tr>
<td>Muscle group</td>
<td>2,280</td>
<td>2.9*</td>
<td>2,317</td>
</tr>
<tr>
<td>HFAT</td>
<td>1,280</td>
<td>0.2</td>
<td>1,317</td>
</tr>
<tr>
<td>HWT</td>
<td>1,280</td>
<td>1.5*</td>
<td>1,317</td>
</tr>
<tr>
<td>Challenge</td>
<td>-</td>
<td>-</td>
<td>1,317</td>
</tr>
<tr>
<td>Challenge x Challenge</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>State x Challenge</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>State x Muscle group</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>State x HFAT</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Muscle group x HFAT</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Muscle group x Challenge</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>State x Muscle group x Challenge</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>State x Muscle group x HFAT</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom
* P<0.1; **, P<0.05; ***, P<0.01

When lactate AUC10 in response to adrenaline was averaged across all challenges it was 40% lower (P<0.001; Table 9.2) when ewes were pregnant (3.23±0.30mgL⁻¹/10min) than when lactating (5.45±0.29mgL⁻¹/10min) or non-breeding (5.74±0.28mgL⁻¹/10min).

The effect of muscling group on lactate AUC10 differed (P<0.01) between physiological states (Table 9.2). When pregnant, low muscled ewes had lactate AUC10 around half of the value of medium and high muscled ewes at the highest level of adrenaline...
challenge (Figure 9.3). However, when ewes were non-breeding, low muscled ewes had lactate values around 50% higher than medium and high muscled ewes at the highest levels of adrenaline challenge (Figure 9.3). There was no effect ($P>0.05$) of muscling group on lactate AUC10 when ewes were lactating. When condition score was included in the model there was no impact on the fitted terms. Across all physiological states, for each 1 unit increase in condition score, lactate AUC10 increased ($P<0.05$) by $1.04\pm0.48\text{mgL}^{-1}\text{/10min}$.

Figure 9.3 Lactate concentration area under curve between 0 and 10 minutes (AUC10) relative to an adrenaline dose in: a) pregnancy; b) lactation; and c) non-breeding ewes from high, medium and low muscling groups.
The effect of HFAT on lactate AUC10 differed between physiological states and muscle groups within state (Table 9.2). The only consistent effect of HFAT on lactate AUC10 was a general positive correlation that existed within all muscle groups when the ewes were non-breeding (Figure 9.4). The effect of HFAT on lactate AUC10 when the ewes were pregnant or lactating was inconsistent and varied with muscle group (Figure 9.4).

Condition score was positively correlated with lactate AUC10, but did not affect the significance or effect of other terms when it was included in the model. Across all physiological states for each 1 unit increase in condition score, lactate AUC10 increased ($P<0.05$) by 1.04±0.48mgL⁻¹/10min. There was no change to the lactate AUC10 model when basal lactate was included in the analysis. When ewes were pregnant and lactating there was no effect of basal lactate concentration on AUC10, however during the non-breeding state there was a decrease of 3.17±1.59mgL⁻¹/10min for each 1mg/L increase in basal lactate concentration. There was no effect of HWT on lactate AUC10 at any stage.
Figure 9.4 Area under curve (AUC) of lactate concentration between 0 and 10 minutes relative to adrenaline challenges in: a) pregnant; b) lactating; and c) non-breeding ewes from high, medium and low muscling groups and with a range in hogget-age subcutaneous fat depth (HFAT) Australian sheep breeding value (ASBV). Values presented are averages across all levels of adrenaline challenge.
9.3.5 NEFA response to adrenaline

The time to reach the maximum concentration following an adrenaline challenge was lower ($P<0.05$) when ewes were non-breeding (5.0±0.28min) than when ewes were lactating (5.9±0.28min) and highest when ewes were pregnant (7.8±0.28min; Table 9.4). The effect of muscle group on the time to reach maximum NEFA concentration was only evident in the non-breeding ewes, where the medium muscle group took about 50% longer to reach maximum concentration (Table 9.3).

Table 9.3 Time to reach maximum NEFA concentration (minutes) following an adrenaline challenge in Merino ewes of high, medium and low muscling during pregnancy, lactation or non-breeding.

<table>
<thead>
<tr>
<th>Muscle Group</th>
<th>Pregnancy</th>
<th>Lactation</th>
<th>Non-breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>7.4±0.56abc</td>
<td>6.8±0.58bc</td>
<td>4.3±0.56d</td>
</tr>
<tr>
<td>Medium</td>
<td>7.7±0.48ab</td>
<td>6.1±0.49bc</td>
<td>6.2±0.49c</td>
</tr>
<tr>
<td>High</td>
<td>8.4±0.56a</td>
<td>4.8±0.56cd</td>
<td>4.4±0.55d</td>
</tr>
</tbody>
</table>

abc values with a different superscript are different ($P<0.05$)

There were no effects of HFAT, HWT or condition score (when included) on the time to reach maximum NEFA concentration (Table 9.4). The maximum concentration of NEFA in response to adrenaline averaged almost three times higher ($P<0.001$) when the ewes were pregnant (0.29±0.02mM) and twice as high ($P<0.001$) when lactating (0.22±0.25mM) than when ewes were non-breeding (0.11±0.02mM).
Table 9.4 F values for the effect of physiological state, muscle group, ewe Australian sheep breeding values (ASBVs) for subcutaneous fat depth (HFAT) and weight (HWT), adrenaline challenge and condition score and significant interactions on basal non-esterified fatty acid (NEFA) concentration, maximum NEFA concentration, time to maximum NEFA concentration and area under curve of NEFA response between 0 and 10 minutes relative to administering adrenaline (AUC10).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Basal NEFA F value</th>
<th>Time to NEFA maximum conc. F value</th>
<th>NEFA AUC10 F value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF, DDF</td>
<td>NDF, DDF</td>
<td>NDF, DDF</td>
</tr>
<tr>
<td>State</td>
<td>2,306  21.09***</td>
<td>2,307  5.38***</td>
<td>2,312  17.07***</td>
</tr>
<tr>
<td>Muscle group</td>
<td>2,306  1.15</td>
<td>2,307  1.65</td>
<td>2,312  1.04</td>
</tr>
<tr>
<td>HFAT</td>
<td>1,306  0.62</td>
<td>1,307  0.76</td>
<td>1,312  0.90</td>
</tr>
<tr>
<td>HWT</td>
<td>1,306  0.00</td>
<td>1,307  0.39</td>
<td>1,312  0.01</td>
</tr>
<tr>
<td>Challenge</td>
<td>-</td>
<td>1,307  0.07</td>
<td>1,312  26.93***</td>
</tr>
<tr>
<td>Challenge x Challenge</td>
<td>-</td>
<td>-</td>
<td>1,312  7.84***</td>
</tr>
<tr>
<td>State x Challenge</td>
<td>-</td>
<td>2,307  3.01*</td>
<td>2,312  9.26***</td>
</tr>
<tr>
<td>State x Muscle group</td>
<td>4,306  3.81**</td>
<td>4,307  3.47*</td>
<td>-</td>
</tr>
<tr>
<td>State x HFAT</td>
<td>2,306  1.34</td>
<td>2,307  3.37*</td>
<td>-</td>
</tr>
<tr>
<td>Muscle x HFAT</td>
<td>2,306  1.32</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>State x Muscle group x</td>
<td>4,306  4.82**</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HFAT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NDF, DDF, numerator and denominator degrees of freedom
* P<0.05; **, P<0.01; ***, P<0.001

The AUC10 for NEFA concentration increased (P<0.01) with increasing levels of adrenaline administered (Figure 9.5; Table 9.4). The average NEFA AUC10 in response to adrenaline was twice as high (P<0.001) when ewes were lactating as when non-breeding and was a further 20% higher (P<0.01) when ewes were pregnant compared with lactating ewes (Figure 9.5).
Figure 9.5 NEFA concentration area under curve between 0 and 10 minutes (AUC10) relative to adrenaline doses in pregnant, lactating and non-breeding ewes.

There was no effect of muscle group, HFAT, HWT, or condition score (when included) on NEFA AUC10 (Table 9.4). Basal NEFA impacted on AUC10 but this varied between states. There was no effect ($P>0.05$) of basal NEFA on AUC10 in lactation, however for each 1mM increase in basal NEFA, AUC10 was increased ($P<0.001$) in pregnant ewes ($3.53\pm0.55$mM/10 min) and reduced when ewes were non-breeding ($-6.19\pm3.49$mM/10 min). The inclusion of basal NEFA in the model accounted for the difference in NEFA AUC10 between pregnant and lactating states which was no longer evident ($P>0.05$).
9.4 Discussion

Glucose release in response to adrenaline decreased as the ewe HFAT breeding value decreased when ewes were non-breeding. This finding supports our hypothesis and could be explained by three possible mechanisms. The increase in blood glucose in response to a rise in circulating adrenaline levels is the net result of the stimulation of glycogenolysis in the liver and gluconeogenesis in both liver and kidney (Bassett 1971; Brockman 1991; Meyer et al. 2003). Therefore, the measured difference associated with HFAT breeding values may be a result of physiological changes in animals bred for low fat that alters one, or a combination of these three mechanisms. While the methods used in this study do not allow us to differentiate between these three sources of endogenous glucose production, it is possible that reduced hepatic glucose release in response to adrenaline may be associated with the reduced genetic propensity to fatten in the low HFAT ewes.

Understanding the relative contributions of liver glycogenolysis and liver and kidney gluconeogenesis to glucose release in response to adrenaline in sheep will assist in identifying the probable cause of a reduction in glucose release in low HFAT ewes. However there are no comparable studies which have defined the relative contributions of glycogenolysis and gluconeogenesis where bolus doses of adrenaline were administered. The immediate increase in glucose production in response to adrenaline is attributed to a direct stimulation of liver glycogenolysis (Bassett 1970). The glycogenolytic effect is short-lived and with time the glucose output from
glycogenolysis is replaced by that from gluconeogenesis. The increase in hepatic gluconeogenesis in response to adrenaline is mainly an indirect effect as the result of increased gluconeogenic substrate supply (Chu et al. 1997).

A direct stimulatory effect of adrenaline on gluconeogenesis has been demonstrated in isolated rat hepatocytes (Sánchez-Gutiérrez et al. 2000). Together these two processes result in increased uptake of gluconeogenic precursors by the liver and increased gluconeogenic efficiency occurring within minutes of adrenaline being administered (Chu et al. 1997). While increasing the supply of gluconeogenic substrate results in an increase in gluconeogenesis in humans, total hepatic glucose output is not increased due to an associated suppression of glycogenolysis (Jenssen et al. 1990; Müller et al. 1997; Gustavson et al. 2003). Chu et al. (1997) demonstrated a 60-80% reduction in the effect of adrenaline on liver glycogenolysis when gluconeogenic substrates are present. The mechanism for an inhibitory role of gluconeogenesis is unclear, but could be a result of increased glucose-6-phosphate and glucose concentrations deactivating glycogen phosphorylase (Müller et al. 1997). Elevated gluconeogenic substrate levels in response to adrenaline also result in an increase in kidney glucose output (Stumvoll et al. 1997; Meyer et al. 2003). All glucose released from the kidneys is produced by gluconeogenesis and they contribute around 40% of glucose released from gluconeogenesis in response to adrenaline, and around 25% of all glucose appearance in humans and around 30% in pregnant and lactating sheep (van der Walt 1983; Stumvoll et al. 1997; Meyer et al. 2003). While it is difficult to determine the relative contributions of glycogenolysis and gluconeogenesis to total glucose release in
response to adrenaline, it is clear that both processes will contribute. We conclude that even though glycogenolysis is more rapidly activated, a combination of gluconeogenesis in both liver and kidney and its depressive effects on glycogenolysis will result in both gluconeogenesis and glycogenolysis contributing to the rise in glucose concentration in response to administering adrenaline challenges.

The reduction in glucose output in response to adrenaline in sheep with lower HFAT breeding values is likely to be a result of reduced gluconeogenesis in these animals. Humans that are obese have two fold higher rates of gluconeogenesis and lower rates of glycogenolysis compared to lean humans (Müller et al. 1997; Buijs et al. 2004). The link between fatness and gluconeogenesis is possibly explained by elevated levels of circulating leptin in fatter individuals. Plasma leptin concentrations are generally positively correlated with the proportion of fat in the body in sheep (reviewed by Chilliard et al. 2005), and the ewes with lower HFAT breeding values in this study did have lower concentrations of plasma leptin (Chapter 7). Leptin stimulates gluconeogenesis but inhibits glycogenolysis in the liver of rats, therefore as fatness increases, the liver's capacity for gluconeogenesis is also increased (Nemecz et al. 1999; Frühbeck and Salvador 2000). Since the rate of gluconeogenesis is higher and the rate of glycogenolysis in the liver is lower in obese compared with lean humans, obese humans have higher concentrations of hepatic glycogen (Müller et al. 1997) which may also contribute to the increased release of glucose from high HFAT ewes in our study. While the experimental methods used in this study do not enable a complete definition of the physiological changes associated with high HFAT that result in greater glucose
produced in response to adrenaline, we suggest that the up-regulation of gluconeogenesis by leptin is likely to be an important contributor. While the mechanism is not well understood the reduced glucose output in genetically lean ewes is of some concern as glucose production rate in late pregnancy and lactation are well correlated with lamb birthweight and ewe milk production (Wilson et al. 1983). In a different experiment with ewes of the same genotype, ewes with lower HFAT produced lambs of lighter birthweight when nutrition during pregnancy was restricted (Chapter 6). The results from this and previous experiments suggest that selection for reduced fatness in maternal sheep breeds may have adverse effects on their maternal performance.

The lactate released from muscle as a result of adrenaline-stimulated glycogenolysis was greater in low muscled ewes when they were non-breeding. This results support my hypothesis that the glycogenolytic response to adrenaline in muscle will decrease as muscling breeding values of ewes increase. This result aligns with those in similar studies that showed that high muscled sheep and cattle have a lower glycogenolytic response to adrenaline in muscle tissue than low muscled equivalents (Gardner et al. 2005; Martin et al. 2011; McGilchrist et al. 2011). Fernandez et al. (1995) showed a greater glycogenolytic response to adrenaline and greater lactate concentration in a predominantly oxidative muscle compared with a mostly glycolytic muscle in anaesthetised pigs. This result provides further support for the findings of this study as selection for higher muscling and lower fatness increases the proportion of glycolytic to oxidative metabolism in muscle fibres in sheep (Greenwood et al. 2006).
Higher proportions of glycolytic muscle are likely to reduce the density of β-adrenergic receptors in muscle as has been found in rats (Martin et al. 1989). Therefore a potential mechanism for the decrease in glycogenolysis in muscle tissue of high muscled ewes is a decrease in the density of β-adrenergic receptors and therefore reduced response to exogenous adrenaline. Lower adrenaline-stimulated glycogenolysis in muscle is likely to be an important energy saving mechanism and may partly explain previous evidence that suggests animals with increased muscling are more fertile (Chapter 3; Safari et al. 2008; Huisman and Brown 2009).

Lactate release in response to adrenaline was higher in ewes with high HFAT but only during the non-breeding state. This result supports our hypothesis that the glycogenolytic response to adrenaline in muscle would be lower in ewes with higher fatness breeding values. It also supports our hypothesis that breeding value effects on the response to adrenaline would not be evident in late pregnancy or lactation. During pregnancy and lactation the correlation between HFAT and lactate release differed between muscle groups but was not consistent. There are two potential mechanisms by which high HFAT sheep could release a greater amount of lactate when stimulated by adrenaline. Firstly, there is some evidence that fatter animals could have a higher amount of stored glycogen because circulating leptin is higher in fat sheep (Chilliard et al. 2005) and the presence of leptin stimulated glycogen synthesis in in vitro studies with rat muscles (Ceddia et al. 1998). Furthermore Kadim et al. (1993) found a slightly lower ultimate muscle pH in lambs selected for fatness compared with lambs selected for leanness and it is known that ultimate pH and the
amount of stored glycogen are negatively correlated (Gardner et al. 1999). The second potential mechanism is that high HFAT sheep had an increased muscle response to adrenaline as a result of higher density of β-adrenergic receptors. This concept is supported by the fact that genetically fatter sheep have a higher proportion of oxidative to glycolytic metabolism in muscle fibres (Kadim et al. 1993; Greenwood et al. 2006) and a higher proportion of oxidative metabolism in muscle is associated with higher density of β-adrenergic receptors (Martin et al. 1989). Ewes with higher HFAT release higher amounts of lactate in response to adrenaline stimulation. With the methods used it is not possible to provide a definitive mechanism but two possibilities are proposed.

Lactation but not pregnancy suppressed the genotype effects on glucose and lactate release in response to adrenaline, thus partially supporting our fourth hypothesis. The increased requirement for glucose in late pregnancy resulted in an increase in glucose production rate up to 50% in ewes carrying twin foetuses (Wilson et al. 1983). In addition, ewes develop a degree of insulin resistance in muscle and fat tissue in late pregnancy to spare glucose for preferential use by the central nervous system and the growing fetus (Petterson 1993). These expected changes in glucose metabolism in late pregnancy did not result in any changes to the impact of the HFAT breeding value on glucose release in response to adrenaline compared to the non-breeding state. However, the effect of muscle group on adrenaline-stimulated lactate release was the opposite when the ewes were pregnant than when they were non-breeding. In pregnant ewes, those with higher muscling breeding values had greater adrenaline-
stimulated lactate release rather than less when non-breeding. The reason for this dramatic change is not known and to our knowledge this differential response has not been previously reported. Smiley and Finster (1996) did report a reduction in density or functionality in muscle β-adrenergic receptors in human pregnancy. If the same is true in sheep pregnancy, the low muscled ewes with a higher proportion of oxidative fibres and therefore greater β-adrenergic receptors density would be expected to show a greater reduction in muscle glycogenolytic response to adrenaline. This may have contributed to the observed result but it is likely that other factors also have an impact. Pregnancy does not change the impact of HFAT breeding value on glucose output but interestingly results in a large reduction in adrenaline stimulated lactate output in low muscled ewes.

Lactate release in response to adrenaline was higher in ewes with high HFAT but only during the non-breeding state, thus supporting our second and fourth hypotheses. During pregnancy and lactation the correlation between HFAT and lactate release differed between muscle groups but was not consistent. There are two potential mechanisms by which high HFAT sheep could release a greater amount of lactate when stimulated by adrenaline, a higher amount of stored glycogen or an increased muscle response to adrenaline as a result of higher density of β-adrenergic receptors. There is some evidence that fatter animals could have a higher amount of stored glycogen because circulating leptin is higher in fat sheep (Chilliard et al. 2005) and the presence of leptin stimulated glycogen synthesis in in vitro studies with rat muscles (Ceddia et al. 1998). Furthermore Kadim et al. (1993) found a slightly lower ultimate
muscle pH in lambs selected for fatness compared with lambs selected for leanness and it is known that ultimate muscle pH the and amount of stored glycogen are negatively correlated Gardner et al. (1999). The second potential mechanism is that high HFAT sheep had a greater density of β-adrenergic receptors on muscle tissue. This concept is supported by the fact that genetically fatter sheep have a higher proportion of oxidative to glycolytic metabolism in muscle fibres (Kadim et al. 1993; Greenwood et al. 2006) and a higher proportion of oxidative metabolism in muscle is associated with higher density of β-adrenergic receptors (Martin et al. 1989).

Despite the increased demand for glucose by the mammary gland, the ewes had higher basal glucose during lactation than in pregnancy. van der Walt et al. (1983) also found that ewes had higher basal glucose in lactation than pregnancy even though they were fed to energy requirements in both circumstances. During lactation the effects of muscling on lactate release and of HFAT on glucose release were not present as was expected. The large demand for glucose in lactation results in large changes in energy metabolism. It is unlikely that the sensitivity of the liver to adrenaline increases in lactation, because there are no changes in the number or affinity of β-adrenergic receptors on liver cells between lactating and non-lactating sheep (Dunphy et al. 1992). However, there is a large change in the capacity for gluconeogenesis which is amplified by 125% in lactating ewes rearing a single lamb compared to non-breeding ewes (Wilson et al. 1983). This large demand for glucose and gluconeogenic substrates is likely to overwhelm any genetic differences in glucose or lactate metabolism and thus genetic differences apparent in the non-breeding state are no longer evident.
There was no effect of either HFAT ASBVs or muscling group on non-esterified fatty acid (NEFA) release in response to adrenaline, in contradiction to our third hypothesis. These results are supported by those of Carter et al. (1989) who also found no differences in lipolytic response to adrenaline in lines of sheep selected for or against subcutaneous fat depth. Other results tend to be contradictory, the lipolytic sensitivity to adrenaline was found to be lower in subcutaneous fat collected from pigs selected for increased fatness compared to those selected for low fatness (Standal and Vold 1973; Mersmann 1985). This was likely to be associated with a greater density of β-adrenergic receptors in adipose tissue of the lean genotypes (Böcklen et al. 1986). In contrast, the sensitivity to adrenaline was shown to be higher in adipocytes from a fat breed compared with a leaner breed of sheep (Gilson et al. 1996). Gilson et al. (1996) also showed that while adipocytes from the high fat breed were more responsive to adrenaline, this was due to a positive correlation between adipocyte volume and lipolytic responsiveness to adrenaline as has also been demonstrated by Vernon and Finley (1985). Therefore when compared on a per gram of fat tissue basis, there were no differences in adipose tissue responsiveness to adrenaline (Gilson et al. 1996). As ewes with high HFAT would be expected to be fatter with an associated greater adipocyte volume (Allen 1976; Kadim et al. 1989), a greater lipolytic response to adrenaline was expected, as is found in dairy cows (Theilgaard et al. 2002). It is not understood why this relationship was not found in this experiment. However, since there were no differences in fatness associated with the HFAT ASBV when non-breeding ewes were scanned by dual-energy X-ray absorptiometry (DXA), it is possible
that phenotypic differences in fatness were not significant enough to provide this predicted result. It is also possible that the opposing effects of the affinity and density of β-adrenergic receptors and the post-receptor responsiveness may have counteracted each other to result in no detectable net outcome. Across the breeding cycle there are no differences in adrenaline-stimulated lipolysis associated with selection of ewes for either more muscle or less fat.

The lipolytic response to adrenaline was higher when ewes were pregnant and lactating compared to non-breeding as expected (Metz and van den Bergh 1977; Guesnet et al. 1987). However, the finding of a slightly greater response to adrenaline in pregnancy than lactation is unexpected and contrary to other published information in ruminants (Vernon and Finley 1985; Guesnet et al. 1987; McNamara 1988; Vernon et al. 1995). During lactation the cellular density of β-adrenergic receptors in adipose tissue is higher (Jaster and Wegner 1981; Vernon et al. 1995) thus responsiveness was expected to be greater. However, an alternative explanation may be found in energy balance differences between the two states in our study. Condition score was lower during pregnancy than during lactation and although feeding was designed to maintain maternal weight the condition score differences suggest that energy requirements were slightly under estimated in pregnancy or over-estimated in lactation. As adipose tissue is more responsive to adrenaline when nutrition is reduced or under fasting (Blum et al. 1982; Ferlay et al. 2001), this may have contributed to both the higher basal NEFA and higher NEFA response to adrenaline infusion during pregnancy. Thus, when the analysis of NEFA response to adrenaline was corrected for
basal NEFA, which may have reflected this difference, it accounted for the difference between lactation and pregnancy. The net catabolism of fat depots during pregnant and lactating states was reflected in both higher basal NEFA and increased response to adrenaline compared with the non-breeding state.

The key findings of this study are the reduced breakdown of muscle glycogen stores in response to adrenaline in high muscled ewes and the reduced glucose output in response to stress in genetically lean ewes. While the mechanisms behind these changes remain unclear both of these findings may have implications for on-farm performance of Merino ewes. These findings suggest that high muscled ewes may retain energy by mobilising less muscle glycogen in response to stress. They also suggest that selection of ewes for leanness may reduce the amount of glucose mobilised in response to stress. It is hypothesised that this is in part due to a reduced gluconeogenic capacity in lean ewes and if proven correct, selection for reduced fatness is likely to have adverse effects on maternal performance.
Chapter 10 Breeding for increased muscling and reduced fatness decreases the response to insulin in reproducing Merino ewes.

10.1 Introduction

Insulin is the only hormone capable of lowering blood glucose levels and its actions result in the uptake and storage of glucose by insulin-responsive tissues. Muscle and fat are the major insulin-responsive tissues in all mammals including ruminant species (Weekes 1991). Given that muscle uses more glucose than fat, a greater proportion of muscle to fat will increase the uptake of glucose per kilogram liveweight in response to stimulation by insulin (Prior and Smith 1982). This concept has been demonstrated in sheep where the response to insulin was higher in animals that have less fat as a result of either lower nutrition or selection against glucose clearance rate (Bergman et al. 1989; Francis and Bickerstaffe 1996). A number of studies have shown that the proportion of fat to muscle in sheep can be lowered by up to 10% by selecting animals for either lower fat or higher muscle (Kadim et al. 1989; Hegarty et al. 2006a). Small changes in the relative proportions of these tissues could have a large impact on glucose use in response to insulin considering insulin-stimulated use of glucose by fat is less than 10% of that by muscle on a weight basis (Kraegen et al. 1985). As well as differences due to body composition, glucose uptake can also be affected by differences in the metabolic properties of muscle tissue due to variation in muscle fibre type. Muscle fibres that predominantly convert glucose to lactate anaerobically (by glycolysis) use more glucose than those that oxidise glucose aerobically (Hocquette et al. 1995). These metabolic properties of muscle are affected by selection for muscling and leanness. Greenwood et al. (2006) demonstrated that sheep genotypes with either higher
muscling or less fat had a higher ratio of glycolytic to oxidative muscle fibre types. These changes in muscle metabolism are likely to increase the glucose response to insulin in response to either selection for more muscle or less fat. We expect that changes in body composition and muscle metabolic properties associated with animals with higher muscling or lower fatness will result in greater glucose uptake in response to insulin stimulation in these animals. It is likely that these genetic differences will be evident in non-breeding animals, but the large changes to glucose use associated with pregnancy and lactation will overwhelm these small changes.

Pregnancy and lactation require large amounts of glucose and accordingly the insulin-stimulated uptake of glucose by muscle and fat changes significantly with changes in physiological state. Glucose is the primary limiting nutrient for both fetal growth and milk synthesis with around 40% of total body glucose use being absorbed by the mammary gland or uterus (Pethick and Lindsay 1982; Oddy et al. 1985). In order to meet the increased demand for glucose there is a systematic reduction of glucose uptake in fat and muscle tissue and an increase in the synthesis of glucose in the liver (Bergman et al. 1970; Bauman and Currie 1980). These changes to glucose uptake and synthesis are achieved by lowering the response to insulin, in muscle, fat, and liver tissue in pregnancy and both muscle and adipose tissue during lactation (Leturque et al. 1987; Vernon and Taylor 1988; Vernon et al. 1990; Petterson et al. 1994). The reduction in the response to insulin results in decreased glucose use by peripheral tissues, sparing glucose for preferential use by the uterus and the mammary gland which do not require insulin to stimulate glucose uptake (Vernon et al. 1990). These
changes to metabolism over-ride the normal modes of homeostasis and make glucose available for either the uterus or mammary gland. The altered priority for glucose use in pregnancy and lactation is likely to mask the more subtle differences that are likely to be present between genotypes during the non-breeding state.

This study tested the hypotheses that the uptake of glucose in response to insulin: i) is higher in ewes bred for high muscling; ii) is lower in ewes bred for high fat; and, iii) that these breeding differences will no longer be present during pregnancy and lactation.

10.2 Materials and Methods

10.2.1 Experimental Design

In this experiment we measured the uptake of glucose in response to infusion of insulin in Merino ewes of known muscle and fat genotype. The measurement was repeated on the same ewes during late pregnancy, at peak lactation and when ewes were non-pregnant and non-lactating. Experiments were approved and monitored by the CSIRO Floreat Animal Ethics Committee.
10.2.2 *Animals*

The ewes used in this experiment were part of a larger experiment, and the source and overall management of animals has been described elsewhere (Chapter 7). Briefly, 109 Merino ewes were selected based on divergence in their Australian Sheep Breeding Values (ASBVs) for eye muscle depth (HEMD) and subcutaneous fat depth (HFAT) at hogget age. The ewes were 19 months old at the start of the experiment and were naturally mated after the timing of ovulation had been synchronised over a three day period around day 0 (7 March 2007), and 24 ewes of those pregnant with a single fetus (determined by ultrasound) were selected for use in this experiment. The 24 ewes used in these experiments were the same as those described in chapter 9 and their selection and management is detailed there. The uptake of glucose in response to insulin infusion was repeated on the ewes during late pregnancy, peak lactation and when the ewes were non-breeding.

At each physiological state, insulin infusion experiments were carried out on 6 ewes per day for four days on days 128, 129, 133 and 134 during pregnancy, 183, 184, 190 and 191 during lactation and days 274, 275, 281 and 282 when non-breeding. Insulin was infused on a corrected per kilogram liveweight basis, with liveweights corrected for the weights of the gravid uterus (during pregnancy) and wool weight such that animal response to insulin between physiological states could be directly compared. The weight of the gravid uterus was based on the equations of Wheeler *et al.* (1971). The ewes were shorn 5 months prior to the commencement of the experiment (ie day -
150) and on day 230 between the lactation and non-breeding insulin challenges. The small amount of wool present during the non-breeding challenges did not warrant a correction. The wool weight used to calculate corrected liveweight during pregnancy and lactation was determined by using their day -150 fleece weight and days since shearing to calculate estimated wool weight.

10.2.3 Preparation of animals

On the day prior to the infusion, animals were fitted with a polyvinyl catheter in each external jugular vein, one catheter for blood sampling and the other for infusion. Catheters were kept patent by filling with $2.5 \times 10^4$ U/L of heparinised sterile saline (Heparin Sodium, Pharamacia Australia, Bentley, WA in NaCl 9g/L, Baxter Healthcare, Old Toongabbie, Australia) when not in use. The catheter used for sample collection was flushed with 12.5g/L EDTA (Product code: ED2P, Sigma-Aldrich Pty. Ltd., Castle Hill, Australia) in sterile saline between sample collections. On the day of the procedure the daily allocated feed ration was divided into four portions and a portion was fed at 0700, 1000, 1300 and 1600h.

10.2.4 Experimental Procedure

The hyper-insulinaemic, euglycaemic clamp technique as described by DeFronzo et al. (1979) for use in humans and adapted for use in sheep by Bergman et al. (1989) was
used to determine whole body glucose use in response to insulin. Starting at 0800h and prior to starting infusions, basal blood glucose level was determined by successive samples taken every 15 min for a total of 60 min. A 5 mL sample was collected into Fluoride/Oxalate blood tubes, centrifuged and the harvested plasma frozen at -20°C for later laboratory determination of glucose. In addition a drop from each sample was measured for blood glucose using a Medisense Optium Xceed blood glucose meter (Abbott Australasia, Doncaster, Australia). The five glucose meter readings were used to estimate basal glucose, the exact value of which was later confirmed by laboratory analysis of the full samples taken at the same time.

The infusion was commenced with a single priming dose of 6 mU/kg liveweight of insulin (Actrapid®, Novo Nordisk Pharmaceuticals Pty. Ltd., Baulkham Hills, Australia) administered through the infusion catheter. A continuous infusion of insulin was then commenced at a rate of 0.6 mU/kg liveweight per minute using a dual channel infusion pump (LIFECARE® 5000 Plum™ Infusion system, Abbott Laboratories, USA.). Glucose (50% w/v; Baxter Healthcare, Old Toongabbie, Australia) was infused concurrently utilising the same infusion pump, at an initial infusion rate of 10 ml/h. Every 5 minutes 1 mL blood samples were collected and rapidly assayed using the blood glucose meter. Depending on the blood glucose result, the infusion rate of glucose was adjusted with the aim of establishing a constant level of blood glucose at the same level as the pre-infusion level (basal blood glucose). Once basal blood glucose level had been reached, the infusion rate of glucose was held constant for an hour and defined as the steady state glucose infusion rate (SSGIR). Blood samples
were collected every 15 min into 5mL Fluoride/Oxalate blood tubes, with one drop used to confirm that basal levels were being maintained and a further 5mL centrifuged and the harvested plasma frozen at -20°C for later laboratory determination of glucose. Once complete the rate of insulin infusion was then increased to 6mU/kg liveweight per minute and the above process repeated to establish the SSGIR of the higher insulin infusion rate. Insulin infusion was then ceased and glucose infusion was continued until blood glucose was above basal level and stable or rising.

10.2.5 Body composition measurement

Body composition was estimated on day 296 by Dual-energy X-ray Absorptiometry (DXA) using a Norland XR-26 Fan Beam X-Ray Whole Body Densitometer (Inderlec Medical Systems Baulkham Hills, Australia) following the method described by Pearce et al. (2009). Briefly, sheep were anaesthetised and then lifted on to the scanning bed and positioned in sternal recumbency, with forelimbs flexed toward the sternum and hind limbs extended caudally. The scan was completed using the Whole Body Scan mode and a regional analysis was performed on the image produced in the DXA software to provide total bone mineral mass, total lean mass, and total fat mass. The percentage of fat was subsequently determined by dividing DXA fat by the total weight of tissue (bone + lean + fat).
10.2.6 Chemical Methods

Plasma glucose concentrations were measured on samples collected prior to glucose infusion and those collected while maintaining blood glucose at basal level when insulin was infused at both 0.6 and 6.0mU/kg liveweight per minute. Concentrations of glucose in plasma were measured in a Cobas Mira Autoanalyser (Hoffman-La Roche Diagnostica, Basal, Switzerland) with an Infinity™ Glucose hexokinase kit (Cat. No. TR15421, Thermo Electron Co., Melbourne, Australia)

10.2.7 Statistical Analysis

The SSGIR was analysed using a linear mixed effects model in SAS software (SAS version 9.1, SAS Institute, Cary, NC, USA). Fixed effects included physiological state (pregnant, lactating, non-breeding), insulin infusion rate (0.6 and 6.0mU/kg liveweight per minute) and muscling level (low, medium, high). Covariates included were HFAT, HWT and condition score. Animal tag was used as a random term. All first and second order interactions were included in the starting model, and removed in a stepwise process if non-significant \((P>0.05)\). The model was run with and without the inclusion of basal glucose within state as a covariate, as well as with and without the inclusion of liveweight and adjusted liveweight. As basal glucose differed between states and there was very little overlap of data between states, it was only included in the model as a “within state“ covariate. This same rationale was applied for liveweight. Basal glucose was analysed using the same method with physiological
state and muscling level used as fixed effects. Covariates included were HFAT, HWT, liveweight and condition score. Animal tag was again used as a random term.

10.3 Results

10.3.1 Liveweight, condition score and whole body fat percentage

Liveweight was lower ($P<0.01$) by around 1kg when the ewes were pregnant compared with when lactating or non-breeding (Table 10.1). Adjusted liveweight differed ($P<0.001$) between all states by a maximum of 8kg when comparing the non-breeding and pregnant ewes (Table 10.1). Condition score was higher ($P<0.05$) by about 10% when the ewes were non-breeding compared to other states, and was positively correlated ($P<0.05$) with HWT (0.06±0.03 condition score units per kg HWT) across all physiological states. For this and all results reported in this chapter the figures shown are means ±s.e. There was no effect of either HFAT or muscle group on condition score. The percentage of whole body fat measured by DXA averaged 13.2±7.6% when the ewes were non-breeding. The percentage of fat increased ($P<0.01$) by 17.0±2.4% for each unit increase in condition score. There were no relationships ($P>0.05$) between whole body fat percentage and muscle group, HWT or HFAT.
Table 10.1 Predicted means of steady state glucose infusion rate (SSGIR) at insulin infusion rates (IIR) of 0.6 and 6.0mU/kg.min, basal blood glucose, liveweight, adjusted liveweight (adjusted for conceptus and wool weights where appropriate) and condition score across three physiological states. Average standard error of means across states are presented.

<table>
<thead>
<tr>
<th>State</th>
<th>SSGIR at IIR= 0.6 (mL/h)</th>
<th>SSGIR at IIR = 6.0 (mL/h)</th>
<th>Basal Glucose (mmol/L)</th>
<th>Liveweight (kg)</th>
<th>Adjusted Liveweight (kg)</th>
<th>Condition Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant</td>
<td>7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactating</td>
<td>9.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Non-breeding</td>
<td>9.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Av. s.e.m.</td>
<td>1.14</td>
<td>1.14</td>
<td>0.06</td>
<td>1.72</td>
<td>1.73</td>
<td>0.07</td>
</tr>
</tbody>
</table>

<sup>abc</sup> Values with different superscripts across a row are significantly different (P<0.05)

10.3.2 Effect of physiological state, liveweight and condition score on basal glucose

Basal glucose differed between all three metabolic states (P<0.05), with the lowest seen during pregnancy and the highest during lactation (Table 10.1). Muscle group, HWT and HFAT ASBVs had no effect. There was a positive relationship (P<0.001) between condition score and basal glucose, although this differed between states (Table 10.2). Basal glucose in non-breeding ewes increased by 0.26±0.23mM for each unit increase in condition score, with pregnant ewes increasing by 0.48±0.17mM, and lactating ewes increasing by 0.96±0.27mM. The effect of liveweight on basal glucose also differed between states (P<0.05), with each kg increase in liveweight decreasing it by 0.02±0.02mM in both the pregnant and non-breeding ewes, and by 0.08±0.02mM in lactating ewes (Table 10.2).
Table 10.2  F values and regression coefficients (±s.e.) for the effect of physiological state, muscle group, insulin infusion rate, hogget weight (HWT) and fat depth (HFAT), Australian sheep breeding values (ASBV) and condition score (CS) and liveweight (LW) on steady state glucose infusion rate (SSGIR; 50% glucose solution) and basal glucose.

<table>
<thead>
<tr>
<th></th>
<th>SSGIR</th>
<th>Basal Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDF, DDF(^A)</td>
<td></td>
</tr>
<tr>
<td>State</td>
<td>2,104</td>
<td>7.28*</td>
</tr>
<tr>
<td>Muscle</td>
<td>2,104</td>
<td>3.53*</td>
</tr>
<tr>
<td>Insulin</td>
<td>1,104</td>
<td>6.26*</td>
</tr>
<tr>
<td>Insulin x Muscle</td>
<td>2,104</td>
<td>4.92*</td>
</tr>
<tr>
<td>HFAT ASBV</td>
<td>1,104</td>
<td>1.52</td>
</tr>
<tr>
<td>HWT ASBV</td>
<td>1,104</td>
<td>0.26</td>
</tr>
<tr>
<td>Condition Score</td>
<td>1,104</td>
<td>6.74*</td>
</tr>
<tr>
<td>State x HWT ASBV</td>
<td>2,104</td>
<td>7.41*</td>
</tr>
<tr>
<td>State x HFAT ASBV</td>
<td>2,104</td>
<td>3.50*</td>
</tr>
<tr>
<td>Insulin x HFAT ASBV</td>
<td>1,104</td>
<td>4.89*</td>
</tr>
<tr>
<td>Insulin x HWT ASBV</td>
<td>1,104</td>
<td>7.04*</td>
</tr>
<tr>
<td>LW x State</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>CS x State</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

ns, not significant; *, P<0.05
\(^A\) NDF, DDF, numerator and denominator degrees of freedom

10.3.3 The effect of muscling group on SSGIR

There was no effect of muscle group (P>0.05) on SSGIR at the low rate of insulin infusion (IIR). However at the high IIR high muscling was associated with lower SSGIR, with this effect evident across all physiological states. Differences were found between all 3 muscling groups (P<0.05), with the largest evident between the high and low with the high having a SSGIR 24% less than the low (Figure 10.1).
Figure 10.1 The effect of ewe muscle group on steady state glucose infusion rate (SSGIR; 50% glucose solution) at insulin infusion rates of 0.6 and 6.0mU/kg.min. Values are predicted means ± s.e. and are averaged across pregnant, lactating and non-breeding states. Muscle groups are not significantly different (P>0.05) at the 0.6 infusion rate but are all significantly different (P<0.05) at the 6.0 rate.

10.3.4 The effect of HFAT on SSGIR

The effect of HFAT on SSGIR was only evident at the high IIR, showing a positive association (P<0.05) between the two but only for the pregnant and lactating states (Figure 10.2). The effect during lactation was twice that of the pregnant state, with SSGIR increasing by 65% across the range of HFAT ASBVs during lactation compared to only 29% during pregnancy.
Figure 10.2 The effect of ewe fat breeding value (HFAT ASBV) on steady state glucose infusion rate (SSGIR; 50% glucose solution). Predicted means ± se at insulin infusion rates of 0.6 (grey line, open squares) and 6.0mU/kg.min (black line, closed squares) during (a) pregnant, (b) lactating, and (c) non-breeding. Symbols are adjusted raw data and each represent an experiment on a single sheep.
10.3.5 The effect of HWT on SSGIR

The effect of the HWT ASBV differed between states and insulin infusion rates (Figure 10.3). At the low IIR the only effect of HWT was seen during lactation (P<0.05), where SSGIR decreased by about 57% across the range of HWT used in this study. At the high IIR HWT tended to increase SSGIR by about 38% in pregnant (P<0.1) and 53% in non-breeding (P<0.05) ewes across the HWT range, with no effect evident during lactation.
Figure 10.3 The effect of ewe weight breeding value (HWT ASBV) on steady state glucose infusion rate (SSGIR; 50% glucose solution). Predicted means ± s.e. at insulin infusion rates of 0.6 (grey line, open squares) and 6.0mU/kg.min (black line, closed symbols) during (a) pregnancy, (b) lactation, and (c) non-breeding. Closed and open symbols are adjusted raw data for 0.6 and 6.0mU/kg.min insulin infusion rates, each represents an experiment on a single sheep.
SSGIR did not differ between states ($P>0.05$) at the low level of IIR. However, at the high IIR the SSGIR was 17% lower ($P<0.05$) in the pregnant state than either lactating or non-breeding (Table 10.1). There was a positive correlation ($P<0.05$) between condition score and SSGIR across all states (Table 10.2), with SSGIR increasing by $3.33\pm1.28\text{mL/h}$ for each unit increase in condition score. The genetic effects on SSGIR described in the sections above were not affected by the inclusion of condition score, basal glucose nor liveweight in the models.

### 10.4 Discussion

The uptake of glucose in response to insulin infusion was found to be lower in sheep with higher muscling breeding values, and this effect did not diminish during pregnancy or lactation. This is in complete contrast with our initial hypotheses, the basis for which were partly associated with body composition. It was assumed that the high muscling groups would have a greater proportion of muscle (Hegarty et al 2006), resulting in increased uptake of glucose due to the relative importance of this tissue which accounts for over 80% of the insulin-dependent use of glucose (Kraegen et al. 1985). However, indicators of composition in this study, which included condition score recorded throughout the experiment, and DXA estimates of whole body fatness (taken during the non-breeding period only) suggested only minor differences in composition (positive association between leptin and HFAT; chapter 7). Furthermore,
the proposed shift towards a more glycolytic muscle type in response to selection for muscling (Greenwood et al. 2006), and the suggested increase in glucose use by these cells (Hocquette et al. 1995) was evidently not sufficient to affect whole body glucose uptake. Alternatively, this shift in muscle type may have had a direct impact on the insulin responsive glucose transporters in muscle – GLUT4. Dühlmeier et al. (2005) found that dairy cows with more oxidative muscle types had two to three times higher concentrations of GLUT4 and greater response to insulin. Furthermore, Duehlmeier et al. (2007) showed GLUT4 levels 1.5 to 6.3 times higher in oxidative rather than glycolytic muscles across a range of ruminant and monogastric species. The concentration of GLUT4 in muscle cells is positively correlated with the response to insulin in those muscles (Megeney et al. 1993; Dühlmeier et al. 2005), with this latter finding supported by results in rats, humans and pigs (Goodyear et al. 1991; Megeney et al. 1993; Hickey et al. 1995; Dühlmeier et al. 2005; Sirikul et al. 2006). Furthermore, calves with a higher proportion of glycolytic muscle fibres were less responsive to insulin infusion (Sternbauer and Essen-Gustavsson 2002). The only finding that is inconsistent with this explanation of our results is that by Hocquette et al. (1995) who reported a higher level of GLUT4 in glycolytic rather than oxidative muscles in calves and goats. The reason for this discrepancy is unknown; however these authors also reported a higher level of GLUT4 mRNA in oxidative rather than glycolytic muscle in cows and calves (Hocquette et al. 1996). A GLUT4-linked mechanism would be more likely to affect the responsiveness of muscle to insulin, as opposed to increasing the sensitivity of muscle by affecting its affinity for insulin at the receptor level. This aligns with the observation in this study that the differences between muscle groups were
only evident at the high insulin infusion rate, which is more indicative of an insulin responsiveness linked effect (Kahn 1978). We therefore suggest that high muscled ewes are less responsive to insulin as a result of a lower rate of glucose transport into muscle cells when stimulated by insulin which is associated with a more glycolytic muscle type and lower concentration of GLUT4 within their muscle.

Contrary to our initial hypotheses, ewes with higher HFAT breeding values had greater uptakes of glucose in response to insulin, particularly when pregnant and lactating. Our initial hypotheses were based largely on HFAT being positively correlated with whole body proportion of fat (Hall et al. 2002; Hopkins et al. 2007a), however these compositional effects were only subtle in this study (positive association between HFAT and leptin, chapter 7) and were not apparent in either condition score or DXA fat measurements. While a lack of a strong difference in body composition associated with HFAT explains why we did not see a negative association between HFAT and SSGIR when the ewes were non-breeding, it does not explain why the relationship was positive at the high IIR when the ewes were pregnant and lactating. However, a potential shift in muscle metabolism does provide a plausible explanation for these results. Genetically leaner animals have been shown to have a greater proportion of glycolytic muscle metabolism and hence less oxidative metabolism than their fatter counterparts (Greenwood et al. 2006). As detailed above, muscles with a more glycolytic metabolism have a lower glucose uptake in response to insulin due to a lower level of GLUT4 (Düllhmeier et al. 2005; Duehlmeier et al. 2007). Therefore, selection strategies that result in an increase in the proportion of muscle fibre types that
are more glycolytic, such as selection for more muscle or less fat, will result in a lower responsiveness to insulin. As glucose is required for both the fetus and lactation, this lower uptake of glucose by maternal tissues may improve the maternal ability of the ewes.

We propose that changes in muscle metabolism associated with high HFAT resulted in muscle tissue being more responsive to insulin, resulting in higher SSGIR in high fat ewes. However, differences in fat tissue response to insulin may also have contributed. A potential mechanism for the higher glucose uptake in response to insulin in high fat ewes is an increased glucose transport into fat cells. Fat cells from mice that were genetically fatter had higher insulin-stimulated transport of glucose compared to fat cells from normal mice. The cells from the genetically fatter mice had both higher sensitivity and responsiveness to insulin (Eberhart et al. 1994). This suggests that differences in adipose tissue responsiveness to insulin may account for some of the observed higher uptake of glucose. However this mechanism would be only a minor contributor to measured differences in SSGIR because on a weight basis fat tissue accounts for only around 10% of the total uptake of glucose under insulin stimulation with uptake into muscle tissue being by far the dominant process (Kraegen et al. 1985). A higher uptake of glucose into fat cells when circulating levels of insulin are elevated may be a characteristic of high HFAT ewes but associated changes in muscle fibre type are likely to dominate the observed relationship between HFAT and SSGIR.
The uptake of glucose in response to insulin as measured by SSGIR was lower during pregnancy than the other two physiological states studied. The lower SSGIR was evident at both levels of insulin. This finding agrees with previous work which has shown a reduction in peripheral tissue sensitivity (Pettersen et al. 1993) and fat tissue responsiveness in pregnant sheep (Pettersen et al. 1994). This adaptation is the result of a systematic shift toward glucose sparing allowing preferential uptake by the uterus. This coordinated shift in whole body metabolism to support a physiological state is termed homeorhesis and is common to all species (Bauman and Currie 1980). The SSGIR was not different between the lactating and non-breeding states. However, both the uptake of glucose by the mammary gland and the higher rate of gluconeogenesis in lactation need to be considered when comparing those states. The mammary gland accounts for up to 40% of the glucose used by lactating sheep, although it can vary with the amount of milk produced (Pethick and Lindsay 1982; Oddy et al. 1985). The uptake of glucose by the mammary gland is not responsive to insulin (Vernon et al. 1990) and under normal conditions this increased glucose requirement is met by a 125% increase in gluconeogenesis in lactating compared with non-breeding ewes (Wilson et al. 1983). However, under insulin stimulated conditions there is both an increase in peripheral tissue uptake of glucose and a reduction in gluconeogenesis (Bergman et al 1989; Faulkner and Pollock 1990). In non-breeding ewes, gluconeogenesis would have been almost non-existent at the high rate of insulin infusion (Bergman et al 1989), and therefore SSGIR would represent the rate of insulin-stimulated uptake of glucose by peripheral tissues. In lactating ruminants, insulin infusion also results in much lower rates of gluconeogenesis (Debras et al. 1989).
However unlike in non-breeding ewes, the mammary gland is reliant on a high rate of hepatic gluconeogenesis to maintain constant milk production. The short fall created by insulin infusion would have needed to be met by infused glucose. Therefore, SSGIR in lactating ewes represents both the requirements of the mammary gland that are not being met by gluconeogenesis and the maximum uptake of glucose in peripheral tissues. Considering SSGIR did not differ between lactating and non-breeding states and that the SSGIR in lactating ewes was partly required to meet mammary gland requirements we conclude that peripheral tissues are less responsive to insulin in lactating ewes. This result is supported by previous work showing that fat tissue is less sensitive and muscle tissue less responsive in lactating compared with non-breeding ewes (Vernon and Taylor 1988; Vernon et al. 1990). We therefore conclude that the uptake of glucose by peripheral tissues in both pregnant and lactating sheep is less responsive to insulin than that in non-breeding sheep.

The breeding value for growth (HWT) was negatively correlated with response to insulin at the low level of infusion when ewes were lactating. However, during the pregnant and non-breeding states there was an increase in the uptake of glucose in response to insulin with increasing HWT at the highest level of IIR. It is possible that this effect is simply due to the total size of the insulin responsive tissue. However, insulin was infused on a per kilogram liveweight basis and we would therefore expect these differences to be accounted for. The result that ewes with higher HWT are less responsive to insulin when lactating is of interest. It is possible that this effect is
associated with a difference in growth hormone secretion associated with the HWT breeding value. Growth hormone concentration increased during lactation in the ewes in this study (chapter 7) as is expected (Hatfield et al. 1999). Furthermore, in lactation, high HWT ewes had higher growth hormone concentrations (Chapter 7). Previous work has shown that growth hormone treatment of cattle and sheep reduced the response of glucose use to insulin (Dunshea et al. 1995; Rose and Obara 1996). These effects of growth hormone result in mobilisation of maternal tissues, particularly fat (Brockman and Laarveld 1986). Although the reduction in glucose use in response to insulin in higher HWT ewes was only seen at the low IIR, this is consistent with a reduction in insulin sensitivity (Kahn 1978) one of the known impacts of high circulating levels of growth hormone in ruminants (Dunshea et al. 1995). The finding of differential effects of the HWT ASBV on glucose use in response to insulin depending on physiological state is difficult to explain. Differences in circulating growth hormone concentrations may partially explain this result, although further work is required to confirm these results.

Condition score was positively correlated with basal glucose and the slope of the relationship increased as demand for glucose amplified from non-breeding to pregnancy and then to lactation. In support of these findings, the increase in basal glycaemia in fatter sheep was also amplified in pregnancy in the study of Petterson et al. (1993). Furthermore, Caldeira et al. (2007a; 2007b) also found higher basal glucose concentration in non-breeding ewes in higher condition score when all were fed at maintenance. Similarly, a two-fold increase in whole body fatness resulted in a 25%
increase in basal glucose concentration in sheep studied by Bergman et al. (1989). However, McCann et al. (1986) found no effect of condition score on basal glucose in fed or fasted sheep. Condition score was also positively correlated with SSGIR in all states and IIR. Considering the consistency of this result across states and IIR it is likely to be a direct result of the higher amount of insulin sensitive tissue in ewes of higher condition score. The proportion of fat increased by 17% for each one unit increase in condition score in the ewes studied here and Yates and Gleeson (1975) report that the amounts of both fat and muscle increase with condition score. Considering muscle and fat tissue account for most of the insulin-stimulated uptake of glucose (Weekes 1991), it is not surprising that increasing the proportion of these tissues resulted in higher SSGIR. However, increasing condition score may have been expected to have the opposite effect because obesity in sheep is known to reduce the level of glucose uptake in response to insulin (McCann et al. 1986). A finding thought to be the result of reduced tissue sensitivity to insulin in obese sheep (Bergman et al. 1989). However, this lower sensitivity to insulin associated with fatness is normally induced by large differences in body composition produced through differential feeding so is not likely to be a factor with the more modest range of condition scores in this study. It is probably that the higher proportion of insulin-sensitive fat and muscle tissue in ewes of higher condition score resulted in an increase in glucose uptake in response to insulin.

Selection for muscling and fatness based on ASBVs in Merino ewes results in changes to the whole body glucose use in response to insulin. Sheep selected for increased
muscling have reduced glucose use in response to insulin across all physiological states. In addition, in both pregnant and lactating ewes, those with lower HFAT breeding values have lower uptakes of glucose in response to insulin. We suggest that both high muscled ewes and low fat ewes are less responsive to insulin as a result of a lower rate of glucose transport into muscle cells associated with a more glycolytic muscle type and lower concentration of GLUT4 within their muscle. In addition we propose that part of the higher uptake of glucose in high HFAT ewes is a result of them having a higher uptake of glucose into fat cells when circulating levels of insulin are elevated.

Sheep genotypes that express either higher muscling or lower fatness are less responsive to insulin. It is suggested that this is a result of an increase in the proportion of muscle fibre types that are more glycolytic in these genotypes. Since glucose is the key limiting nutrient for both the growing fetus and the lactating mammary gland, this lower uptake of glucose by maternal tissues may improve the maternal ability of ewes with higher muscling or lower fatness.
Chapter 11 General Discussion

This series of experiments has described a range of associations between measures of body composition, maternal performance and the underlying physiology with breeding values for weight, muscling and fatness in Merino ewes. The findings highlight the need to understand the potential impacts of selection for performance or carcass traits on maternal traits and on body composition and metabolic processes. The results also highlight the need for a balanced approach to designing breeding strategies. Some of the findings here demonstrate that the most appropriate breeding strategies are likely to differ across environments or production systems. Care must be taken when defining a selection strategy because while rapid genetic progress is readily achieved with modern genetic technologies, it is important that this genetic change does not have undesirable side effects on metabolism, reproduction or fitness.

Reductions in maternal performance may reduce enterprise profitability since the profitability of Merino production systems is increasingly reliant on the sale of lambs and adult sheep. Selection for growth, muscling or fatness will ultimately change a range of associated traits and processes.

11.1 Growth

Higher weight at yearling or hogget age resulted in higher mature liveweight and was associated with a range of other changes. One of the obvious changes was an increase in the total amount of muscle and fat tissue. While ewes with a higher growth
genotype had greater total amounts of muscle and fat there was no change in the relative proportions of these tissues. Generally high growth ewes produced more lambs through an increase in the proportion that gave birth to twins, and also had lambs that were heavier at birth and at weaning. The increase in weaning weight in lambs from high growth ewes can, at least partly, be explained by higher milk production in high growth ewes. The increased milk production is likely to be associated with higher circulating levels of growth hormone in high growth ewes during lactation.

Based on the results here, selection for higher early growth is expected to increase ewe mature weight and importantly generally improve the reproductive rate and milk production of ewes. There were only two potential negative associations between growth selection and maternal performance. Firstly, the increase in lamb birthweight from high growth ewes has the potential to cause dystocia problems so concomitant downward pressure on lamb birthweight is advised. Secondly, a higher circulating level of growth hormone suggests that the high growth ewes may mobilise more tissue during lactation and may be in lower condition at the subsequent mating particularly in low nutritional environments. Finally, while not considered in this thesis, the increase in mature liveweight associated with selection for early growth will increase the maintenance requirements of these ewes which may result in higher feeding costs.
11.2 Muscling

Selection for higher muscling is advocated to improve the value of lamb carcasses. This series of experiments has described a range of other changes associated with this selection strategy. The finding that ewes with higher muscling produce a higher proportion of twin lambs than their low muscled equivalents is the most significant of these findings for industry. This result may be partly explained by a higher level of circulating glucose in higher muscled ewes in the non-breeding state given that blood glucose plays an important role in reproduction.

We were expecting the high muscled ewes studied to have a markedly leaner body composition than low muscled ewes which would flow on to have a negative effect on reproduction in high muscled ewes. However there was very little evidence of a negative association between muscling and body fatness in the experiments completed and the association between muscling and reproduction was a positive one as already discussed.

Ewes with higher muscling produced lambs that were lighter at birth in two separate experimental flocks. Importantly the lighter weights at birth did not result in lower survival of lambs. Ewes with higher muscling maintained higher condition score across the breeding cycle in one of the experiments. This may be at least partly
attributed to a lower response to adrenaline at the level of the muscle in higher muscled ewes. These findings suggest that high muscled ewes may retain energy and muscle weight by mobilising less muscle glycogen and protein in response to stress and therefore be more robust in production systems. Similarly higher muscled ewes had lower growth hormone concentration in lactation which would result in lower mobilisation of tissues.

The association between muscling and milk production was variable with no consistent effect. A reduction in milk is supported by the finding of lower circulating levels of growth hormone in higher muscled ewes during lactation. However peripheral tissues were less responsive to insulin in high muscled ewes which would enable higher levels of blood glucose to be maintained ultimately increasing milk output.

11.3 Fatness

Increases in fatness breeding values were associated with both higher total amount of fat in the body as well as an increase in the proportion of fat in the body. Therefore altering body composition by selecting for a breeding value of subcutaneous fat depth is effective. These associations were consistent across DXA measurements, chemical composition of ground whole carcasses and plasma leptin levels. The change in body composition may be partly a result of differences in the growth hormone axis. The higher fatness breeding values were associated with lower growth hormone
concentrations during lactation and non-breeding states. Furthermore, the response to insulin was higher in ewes with higher HFAT breeding values providing a further potential mechanism for the observed higher fatness. Lastly the response to adrenaline within the liver was greater in ewes with higher HFAT breeding values, suggesting improved ability to release glucose into the blood stream under times of stress. It is hypothesised that this is in part due to a reduced gluconeogenic capacity in lean ewes and if proven correct, selection for reduced fatness is likely to have adverse effects on maternal performance.

An important finding in this series of experiments is that genetic fatness of ewes is positively associated with the birthweight of their lambs but only when nutrition during pregnancy is restricted. This result has important ramifications for selection strategies regarding genetic fatness and may also explain why the effect of nutrition during pregnancy has variable associations with lamb birthweight in the literature.

11.4 Associated changes in muscle metabolism

In this series of experiments neither muscle fibre type nor muscle biochemistry were directly measured but the results from infusions of both adrenaline and insulin support previous evidence of changes to muscle metabolism as a result of selection for either changes in muscling or changes in fatness. Both high muscled and low fat ewes were less responsive to insulin which may be explained by a more glycolytic muscle metabolism in these genotypes. This would result in a lower rate of glucose transport
into muscle cells when stimulated by insulin because of a lower level of GLUT4 glucose transporters within these more glycolytic muscle types. Furthermore, higher muscled ewes had a lower response to adrenaline within their muscle tissue when non-breeding, potentially as a result of a lower predominance of oxidative muscle metabolism and lower density of β-adrenergic receptors associated with higher muscling.

11.5 Future breeding direction in the Australian Merino

This thesis has described a range of inter-relations between breeding values for growth, muscling and fatness and their association with maternal performance and metabolic processes. The results show that there are many potential improvements to flock performance by positive selection for growth and muscling as long as selection results in simultaneous gain in these two traits. Furthermore, there are apparent benefits from selection for higher fatness in the Australian Merino at least to the top of the range studied here. From this body of work it seems that this is particularly true in harsher or more variable environments where genetic fatness has positive influence on productivity. There is a considerable opportunity to select for all three traits of growth, muscle and fat at the same time since the genetic correlations between all traits is positive. However care must be taken to not negatively influence some of the wool production traits in this process. Given that the profitability of the Australian sheep industry is increasingly reliant on sale of lambs and adult sheep, positive selection pressure for growth, muscling and fatness are strongly encouraged.
11.6 Reflection on methodologies used

This thesis relied heavily on EBVs (or ASBV termed in the Australian sheep industry) as a proxy for genetic differences between animals. The potential issue with interpreting EBVs as the genotype of the animal is two fold: Firstly, part of the calculation of the EBV incorporates data measured on the individual animal. Thus the associations we derive may be biased by phenotypic correlations and some of the expected associations may not persist in the next generation. Secondly, EBVs on young animals that do not have any measured progeny have relatively low accuracies on the EBVs generated. With unlimited resources and time it would be preferable do the studies conducted here on animals that have resulted from divergent selection lines or that have been generated by using widely different sires. Ultimately it would be preferably to measure thousands of animals for the traits studied here and calculate true genetic and phenotypic correlations between traits. Unfortunately neither the time nor resources were available for this to occur in these studies. Because of the way the experiments and analyses were conducted we do not believe a strong bias from phenotypic correlations would have impacted on the outcomes of this research. However we do acknowledge that the relationships generated in this way are at risk of potential bias. Models were meticulously fitted to ensure current phenotype was accounted for wherever necessary. We are certain that if the time and resources were available more thorough testing of the hypotheses tested here would not alter the ultimate findings.
The results from the largest study in this thesis which measured the hormonal response of ewes across three physiological states were somewhat biased by nutritional differences across the three states. While care was taken to ensure ewes were provided with a maintenance ration at each time point. The result reveals that this was not adequately achieved at all times. Within this same experiment it would have also been favourable to complete insulin infusions across more rates of infusion to help with interpretation of sensitivity versus responsiveness to insulin.
12. Bibliography


Hall DG, Gilmour AR, Fogarty NM and Holst PJ (2002) Growth and carcass composition of second-cross lambs. 2. Relationship between estimated breeding values


Karamichou E, Richardson RL, Nute GR, McLean KA and Bishop SC (2006) Genetic analyses of carcass composition, as assessed by X-ray computer tomography, and meat quality traits in Scottish Blackface sheep. Animal Science 82, 151-162.


Lewis RM and Beatson PR (1999) Choosing maternal-effect models to estimate (co)variances for live and fleece weight in New Zealand Coopworth sheep. Livestock Production Science 58, 137-150.

Lewis RM and Emmans GC (2007) Genetic selection, sex and feeding treatment affect the whole-body chemical composition of sheep. Animal 1, 1427-1434.


McGilchrist P, Pethick DW, Bonny SPF, Greenwood PL and Gardner GE (2011) Beef cattle selected for increased muscularity have a reduced muscle response and increased adipose tissue response to adrenaline. Animal 5, 875-884.


