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Amino acids and skeletal muscle growth in lambs

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ABSTRACT

The objectives of this thesis were three-fold. Firstly, to identify whether reduced muscle growth in twin compared to singleton lambs during late pregnancy and early post-natal life was associated with changes in the concentration of intracellular free amino acids (FAA) that may play a role in the regulation of pathways involved in muscle growth. Secondly, to evaluate if supplementation with a specific amino acid improved muscle growth in twin fetuses/lambs, and thirdly to examine the role of mTOR signalling. The first objective was investigated by examining the differences in muscle FAA concentrations between singleton and twin fetuses in late pregnancy from either heavy or light ewes, under a maintenance or *ad libitum* feeding regimen. Twin fetuses had lower intracellular FAA concentrations of arginine (Arg), leucine, valine, glutamine, while muscle mass was positively associated only with Arg concentration. A further study characterised the FAA concentrations of singleton and twin well-fed lambs at 140 days pregnancy and at weaning. High levels of Arg and glutamine were associated with muscle growth during pregnancy; however several FAA appeared to be associated with muscle growth to weaning. Objective 2 was tested by examining the effects of maternal Arg administration on fetal muscle growth and mTOR signalling. Well-fed twin-bearing ewes, received either an intravenous bolus of Arg or saline solution 3 times daily from 100 days of pregnancy to parturition. Female lambs from supplemented ewes had increased birth weight and muscle mass at market weight, associated with increased ribosome number and mTOR abundance at P140 and increased ribosome number at weaning, compared to control females. An additional experiment supplemented twin-born lambs with Arg via fortification of colostrum and milk replacer from birth to 28 days or from birth to 70 days of life. Supplementation increased body growth between 7 and 21 days of life. Only supplemented females expressed higher muscle weight at 70 days, compared with control females. Collectively, these results indicate that singleton and twin muscle differs in Arg concentration, and the use of Arg during pregnancy and early neonatal life improves muscle growth in females. This action potentially occurs through mTOR signalling, and ameliorates reduced females weight in at birth and growth from birth to weaning.

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TABLE OF CONTENTS

ABSTRACT	iii
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	ix
LIST OF TABLES	xiii
LIST OF FIGURES	xix
ABBREVIATIONS	xxiii
GENERAL INTRODUCTION	xxv
<i>Chapter 1: Literature review</i>	1
1.1 Introduction	3
1.2 Multiple births: Production and economic considerations for farmers.....	4
1.3 Skeletal muscle development and growth.....	6
1.4 Role of AA in muscle development and growth.....	10
1.5 The mTOR pathway: a nutrient sensing pathway signalled by amino acids	13
1.6 Evidence for AA activation of mTOR and skeletal muscle growth in livestock	17
1.7 Rationale for these studies	19
<i>Chapter 2: Muscle free amino acid profiles are related to differences in skeletal muscle growth between single and twin ovine fetuses near term</i>	21
2.1 Abstract	23
2.2 Introduction	24
2.3 Materials and methods	25
2.4 Statistical analysis	27
2.5 Results	27
2.6 Discussion	33

2.7	Conclusions.....	36
<i>Chapter 3: Identification of amino acids associated with skeletal muscle growth</i>		
	in late pregnancy and at weaning in lambs of well-nourished sheep.....	37
3.1	Abstract.....	39
3.2	Introduction.....	40
3.3	Materials and methods.....	41
3.4	Statistical analysis.....	43
3.5	Results.....	44
3.6	Discussion.....	57
3.7	Conclusions.....	61
<i>Chapter 4: Effect of maternal parenteral arginine supplementation during mid-late pregnancy on twin fetal muscle mass in late pregnancy and post-weaning.....</i>		
		63
4.1	Abstract.....	65
4.2	Introduction.....	66
4.3	Materials and Methods.....	67
4.4	Statistical analysis.....	72
4.5	Results.....	73
4.6	Discussion.....	92
4.7	Conclusions.....	96
<i>Chapter 5: Effect of oral supplementation of L-Arginine on skeletal muscle growth in artificially-reared twin born lambs from birth until weaning.....</i>		
		97
5.1	Abstract.....	99
5.2	Introduction.....	100
5.3	Materials and methods.....	101
5.4	Results.....	107

5.5	Discussion.....	124
5.6	Conclusions.....	127
<i>Chapter 6: General discussion.....</i>		<i>129</i>
6.1	Overview.....	131
6.2	Main findings and implications.....	133
6.3	Potential limitations.....	139
6.4	Future work.....	140
6.5	Concluding remarks.....	142
<i>Chapter 7: Appendices.....</i>		<i>143</i>
7.1	Appendix A.....	145
7.2	Appendix B.....	146
7.3	Appendix C.....	148
7.4	Appendix D: Statement of contribution to doctoral thesis containing publications.....	151
<i>Chapter 8: References.....</i>		<i>157</i>

LIST OF TABLES

<p>Table 2.1. Three-way interaction between maternal size, (S, Heavy vs. Light) plane of nutrition (N, <i>Ad libitum</i> vs. Maintenance) and pregnancy rank (R, Single vs. Twin) for the concentration (nmol/g wet tissue) of free amino acids in <i>M. semitendinosus</i> of fetuses at 140 days pregnancy. Values are expressed as least square mean (LSM). The average of the difference of the least square means (LSD, $\alpha = 0.05$) and probability of significance for the three way interaction (RxSxN) are presented.</p>	30
<p>Table 2.2. Rank effect (Single (S) vs. Twin (T)), two-way interaction between pregnancy rank and maternal size (Heavy (H) vs. Light (L)) and between pregnancy rank and plane of nutrition (<i>Ad libitum</i> (A) vs. Maintenance (M)) for the concentration (nmol/g wet tissue) of free amino acids (FAA) in <i>M. semitendinosus</i> of fetuses at 140 days pregnancy. Values are expressed as least square mean (LSM). The average of the difference of the least square means (LSD, $\alpha = 0.05$) is presented.</p>	31
<p>Table 3.1. Effect of birth rank of the fetus/lamb on body (kg) and <i>M. semitendinosus</i> weight (ST; g) at 140 days of pregnancy (P140) for singletons (n = 9) and twin (n = 20) fetuses and at weaning, considering singleton (n = 20) and twin lambs (Twin(age), n = 17) at same age and twin lambs at same weight (Twin(wt), n = 17) compared to singletons.</p>	46
<p>Table 3.2. Free amino acid (AA) concentration ($\mu\text{Mol/L}$) in maternal plasma, fetal plasma and intracellular free amino acid concentration (nMol/g) in <i>M. semitendinosus</i> from singleton and twin fetal lamb at 140 days of pregnancy. Values are expressed as mean \pm standard error of mean (SEM).....</p>	50
<p>Table 3.3. Ratio of fetal to maternal plasma amino acid (AA), and <i>M. semitendinosus</i> to fetal plasma AA concentration for singleton and twin fetuses at 140 days pregnancy.</p>	51

Table 3.4. Partial correlation between fetal plasma and <i>M. semitendinosus</i> free amino acid concentration for singleton and twin fetuses at 140 days pregnancy.....	52
Table 3.5. Intracellular free amino acid concentration (nMol/g) in <i>M. semitendinosus</i> at weaning for singleton lambs and one twin lamb of a twin pair (Twin(age)) at the same age (85 days of life) and the other twin lamb at same weight (Twin(wt), 98 days of life). Values are expressed as mean \pm standard error of mean (SEM). The effects of treatment and sex of lamb are shown.	54
Table 3.6. Biochemical indices (DNA, RNA, protein and ratios) of <i>M. semitendinosus</i> in singleton and twin fetuses at 140 days pregnancy (P140) and at weaning considering singleton and one of twin lamb of a twin-pair (Twin(age)) at same the age and the other twin lamb at the same weight (Twin(wt)) compared to singletons. Data are presented as least square means \pm standard error of the mean (SEM).	56
Table 3.7. Blood β -hydroxybutyrate (BHBA), glucose, non-esterified fatty acids (NEFA) and urea concentration for twin lambs at the same age (Twin(age), 85 days of life) or weight (Twin(wt), 98 days of life) compared to singletons. Least square means \pm standard error of mean (SEM) are presented. Back transformed values and CI (95%) are shown for NEFA.....	57
Table 4.1. Body weight of fetuses at day 140 of pregnancy and birth weight of fetuses/lambs born to ewes either supplemented with arginine (Arg) or saline (controls). Data shows least square means \pm standard error of mean (SEM). ^{ab} Different superscripts within rows represent $P \leq 0.05$. Values for treatment (T), sex of the lamb/fetus (S) and the interaction between treatment and sex of the lamb/fetus (T x S) are presented. In brackets number (n) of animal.	75
Table 4.2. Individual muscle weights (g) at 140 days of pregnancy (P140) of fetuses born to ewes either supplemented with arginine (Arg) or saline (controls) from P100-P140. Table shows least square means \pm	

standard error of the mean (SEM) of muscle weight (unadjusted) or using fetal weight as a covariate in the model (adjusted). Values for treatment (T), sex of the fetus (S) and the interaction of treatment by sex of fetus (T x S) are presented. Significance was established at $P \leq 0.05$ and a trend for $P \leq 0.10$ 77

Table 4.3. Individual muscle weights (g) of offspring at 153 days of age born to ewes either supplemented with arginine (Arg) or saline (controls) from P100 to birth. Table shows unadjusted and adjusted values for carcass weight. Data is expressed as least square means \pm standard error of the mean (SEM). Significance was stated at $P \leq 0.05$ and a trend for $P \leq 0.10$. Values for treatment (T), sex of the lamb (S) and the interaction between treatment and sex of the lamb (T x S) are presented. 79

Table 4.4. Treatment effect for free amino acid (AA) concentration in maternal plasma ($\mu\text{mol/L}$), fetal plasma ($\mu\text{mol/L}$) and intracellular free amino acid concentrations in *M. longissimus dorsi* ($\mu\text{mol/g}$) at 140 days of pregnancy (P140), of fetuses born to ewes either supplemented with arginine (Arg) or saline (control) from P100-P140. Data are presented as least square means and averaged standard error of the mean (SEM). Significance was established at $P \leq 0.05$ 81

Table 4.5. Treatment and sex of lamb effect for plasma FAA concentration ($\mu\text{mol/L}$) at 153 days of life (PN153) of twin lambs born to ewes supplemented with arginine (Arg) or saline (control) from 100 days of pregnancy (P100) to birth. Data corresponds to least square means \pm standard error of the mean (SEM). Values for treatment (T) and sex of the lamb (S) are presented. Significance was established at $P \leq 0.05$ 83

Table 4.6. Intracellular free amino acids (FAA) concentrations ($\mu\text{mol/g}$) in *M. longissimus dorsi* post-weaning (PN153), of twin lambs born to twin-bearing ewes either supplemented with arginine (Arg) or saline (control) from day 100 of pregnancy (P100) to birth. Treatment and sex of lamb effects are presented. Data corresponds to least square

means \pm standard error of the mean (SEM). Significance was established at $P \leq 0.05$ 84

Table 4.7. Biochemical indices (DNA, RNA, protein and ratios) of *M. longissimus dorsi* (LD) in control and Arg-treated groups at 140 days pregnancy (P140), of fetuses born to ewes either supplemented with arginine (Arg) or saline (control) from P100-P140. Data are represented as least square mean \pm standard error of the mean (SEM). Significance was established at $P \leq 0.05$ and values are presented for effect of treatment (T), sex of fetus (S) and the interaction between treatment and sex of the fetus (T x S). ^{ab}Different superscripts represents statistical difference ($P \leq 0.05$). 86

Table 4.8. Biochemical indices (DNA, RNA, protein and ratios) of *M. longissimus dorsi* (LD) in control and Arg-treated groups post-weaning (PN153). Data are represented as least square mean \pm standard error of the mean (SEM). Significance was established at $P \leq 0.05$ and values are presented for effect of treatment (T), sex of lamb (S) and the interaction between treatment and sex of the lamb (T x S). ^{ab}Different superscripts represents statistical difference ($P \leq 0.05$). 87

Table 4.9. Maternal and fetal plasma concentrations of hormones and metabolites 1 hour after final supplementation at 140 days of pregnancy (P140), either supplemented with arginine (Arg) or saline (control) from P100-P140. Table shows least square means and averaged standard error of the mean (SEM). Values for treatment (T), sex of the fetus (S) and the interaction of treatment by sex of fetus (T x S) are presented. 90

Table 4.10. Plasma concentrations of hormones and glucose at PN153 of lambs born to ewes either supplemented with arginine (Arg) or saline (controls) from day 100 of pregnancy (P100) to birth. Table shows least square means \pm averaged standard error of the mean (SEM). Values for treatment (T), sex of the lamb (S) and the interaction of treatment by sex of lamb (T x S) are presented. 91

Table 5.1 Amino acid content (mg/g DM) in colostrum, milk replacer and pasture.	104
Table 5.2. Lamb weight (kg) at birth for Cohort 1 and Cohort 2, from lambs supplemented with Arg or unsupplemented (control). Table shows least square means and average standard error of the means (SEM). The effect of treatment (T, Arginine or control), Sex of lamb (S) and treatment by sex of lamb interaction (T x S) are shown. In brackets, number of animal.	108
Table 5.3. Cohort 1; muscle weight (g) from lambs supplemented with Arg or unsupplemented (control) at 28 days of age. Table shows least square means and average standard error of the means (SEM). The effect of treatment (T, Arginine and control), Sex of lamb (S) and treatment by sex of lamb interaction (T x S) are shown.	112
Table 5.4. Cohort 2; muscle weight (g) from lambs supplemented with Arg or unsupplemented (control) at 70 days of age. Table shows least square means and average standard error of the means (SEM). The effect of treatment (T, Arginine and control), Sex of lamb (S) and treatment by sex of lamb interaction (T x S) are shown.	114
Table 5.5. Free amino acid concentration in Cohort 1 plasma ($\mu\text{Mol/L}$) and in <i>M.</i> <i>longissimus dorsi</i> muscle (nMol/g) from arginine and control lambs from birth to 28 days. The effect of treatment (T), sex of lamb (S) and treatment by sex of lamb interaction (T x S) are presented. Data corresponds to least square means and average standard error of the means (SEM).	116
Table 5.6. Free amino acid concentration in Cohort 2 plasma ($\mu\text{Mol/L}$) and in <i>M.</i> <i>longissimus dorsi</i> (nMol/g) from arginine supplemented and control lambs from birth to 70 days. The effect of treatment (T), sex of lamb (S) and treatment by sex of lamb interaction (T x S) are presented. Data corresponds to least square means and average standard error of the means (SEM).	118
Table 5.7. Effect of arginine supplementation on biochemical indices of arginine treated and control lambs from Cohort 1 and Cohort 2. The effect of	

treatment (T), sex of lamb (S) and treatment by sex interaction (T x S) are presented. Data corresponds to least square means \pm averaged standard error of the mean (SEM). 123

Table 5.8. Insulin concentration (mMol/L) for Cohort 1 and Cohort 2. The effect of treatment (T), sex of lamb (S) and treatment by sex of lamb interaction (T x S) are presented. Data corresponds to least square means \pm averaged standard error of the mean (SEM). 124

LIST OF FIGURES

- Figure 2.1. The bars graphic represent the *M. semitendinosus* weight (g) of the eight groups of fetuses (singletons and twins from Heavy and Light ewes offered an *ad libitum* or maintenance feeding regime) at 140 days pregnancy, not adjusted by fetal weight. Data are presented as least square means and standard error of the mean (SEM). Bars with different letters are significantly different at $P \leq 0.05$28
- Figure 2.2. Partial correlation plot for *M. semitendinosus* (ST) weight with arginine concentration. The plot graphic shows the partial correlation analysis for ST muscle weight (g) with arginine concentration (nmol/g wet tissue). The analysis considered pooled data of all fetuses and was performed after accounting for the effects of pregnancy rank, maternal size and nutrition.....32
- Figure 2.3. Partial correlation plot for *M. semitendinosus* (ST) weight with taurine concentration. The plot graphic shows the partial correlation analysis for ST muscle weight (g) with taurine concentration (nmol/g wet tissue). The analysis considered pooled data of all fetuses and was performed after accounting for the effects of pregnancy rank, maternal size and nutrition.....32
- Figure 3.1. Pre-weaning live weight of singleton (n = 20) and twin lambs (n = 34) from 13 days after birth to slaughter, adjusted by birth weight as covariate. Data for twins at 81 days corresponds to all lambs, while at 98 days it represents the live weight of Twin(wt) (n = 17) lambs. Data are expressed as least squares means \pm standard error of the mean (SEM). * $P \leq 0.05$47
- Figure 3.2. Average daily gain (kg) of singleton and twin lambs, adjusted by birth weight and age as covariates, from birth to 28 days of life, which accounted for the pre-ruminant stage ⁽¹⁾, 29 days to 80, which corresponded to the time all lambs were raised together and 81 to 98 days, corresponded to the time only Twin(wt) lambs were maintained with their dam before slaughter. Data for twins at 81 days corresponds to all lambs, while at 98 days it represents the average

daily gain of Twin(wt) lambs. Data are expressed as least squares means \pm standard error of the mean (SEM). * $P \leq 0.05$. ⁽¹⁾ (Wardrop and Coombe, 1961).....	48
Figure 4.1. Experimental design presenting the time of mating, number of ewes and rams.....	68
Figure 4.2. Post-natal live weight change of lambs ($n = 30$) from ewes either supplemented with arginine (Arg, $n = 16$, 6 male and 10 female) or saline (controls, Con, $n = 14$, 10 males and 4 females) from P100 to birth. Data shows least square means \pm standard error of the mean (SEM) for treatment (Arg and control) by sex (Male (M) and Female (F)) by time interaction. * $P \leq 0.05$, † $P < 0.10$ for female lambs from ewes supplemented with Arg (Arg-F) vs. female lambs from control ewes (Con-F) comparison.....	75
Figure 4.3. Arginine concentration in ewe plasma ($\mu\text{mol/L}$, $n = 6/\text{group}$), fetal plasma ($\mu\text{mol/L}$, $n = 10/\text{group}$), and fetal muscle ($\mu\text{mol/g}$, $n = 10/\text{group}$), at 140 days of gestation (P140). Data shows least square means \pm standard error of the mean (SEM).....	82
Figure 4.4. Treatment by sex of fetus interaction for total abundance of mechanistic target of rapamycin (mTOR) in <i>M. longissimus dorsi</i> from fetuses born to ewes either supplemented with arginine (Arg, $n = 10$) or saline (controls, $n = 10$) from day 100 to 140 of pregnancy (P100 to P140). The figure shows the least square mean \pm standard error of the mean (SEM). ^{ab} Different superscript for each target represents statistical difference ($P \leq 0.05$).....	88
Figure 4.5. Treatment by sex of fetus interaction for total abundance of phosphorylated mTOR (Ser ²⁴⁴⁸) in <i>M. longissimus dorsi</i> from fetuses born to ewes either supplemented with arginine (Arg, $n = 10$) or saline (controls, $n = 10$) from day 100 to 140 of pregnancy. The figure shows the least square mean \pm standard error of the mean (SEM). ^{ab} Different superscript represents statistical difference ($P \leq 0.05$; † $P < 0.10$).	89

Figure 4.6. Proposed model for increased protein synthesis as an effect of Arginine (Arg) supplementation in <i>M. longissimus dorsi</i> from fetuses born to ewes supplemented from day 100 to 140 of pregnancy.....	95
Figure 5.1. Lamb live weight for Cohort 1, either supplemented with arginine (Arg, n = 15) or unsupplemented (controls, Con, n = 14) from birth to day 28. Data shows least square means \pm standard error of the means (SEM) for treatment (Arg vs control) by time interaction. $\dagger P < 0.10$; $*P \leq 0.05$	108
Figure 5.2. Average daily gain (kg/day) of Cohort 1 from lambs supplemented with Arginine (Arg, n = 15) or unsupplemented (control, Con, n = 14) during the first 28 days of life. Data shows least square means and standard error of the mean (SEM). $*P \leq 0.05$	109
Figure 5.3. Live weight change for Cohort 2, either supplemented with arginine (Arg, n = 16) or unsupplemented (controls, Con, n = 11) from birth to day 28. Data shows least square means \pm standard error of the mean (SEM) for group by time interaction. $*Arg$ vs. Control $P < 0.01$	110
Figure 5.4. Live weight change for Cohort 2, either supplemented with arginine (Arg, n = 16) or unsupplemented (controls, Con, n = 11) from birth to day 28. Data shows least square means \pm standard error of the mean (SEM) for group by time interaction.....	110
Figure 5.5. Treatment (Arginine vs. Control) by sex of lamb (male vs. female) interaction for concentration and total abundance (concentration * weight of muscle) of (A) mechanistic target of rapamycin (mTOR) and (B) phosphorylated mTOR (Ser2448) in <i>M. longissimus dorsi</i> from Cohort 1 lambs supplemented with arginine or unsupplemented (control), from birth to 28 days of life. The figure shows the least square mean \pm standard error of the mean (SEM).	120
Figure 5.6. Treatment (Arginine vs. Control) by sex of lamb (male vs. female) interaction for concentration and total abundance of (A) mechanistic target of rapamycin (mTOR), (B) phosphorylated mTOR (Ser2448) and ratio of Ser2448 :mTOR (C) in <i>M. longissimus dorsi</i> from	

Cohort 2 lambs supplemented with arginine or unsupplemented (control), from birth to 70 days of life. The figure shows the least square mean \pm standard error of the mean (SEM). Different superscript for each variable (^{a,b}total abundance; ^{c,d}concentration) represents $P \leq 0.05$, $\dagger P < 0.10$ 121

ABBREVIATIONS

4E-BP1 eukaryotic initiation factor 4E binding protein

AA Amino acid

AMP-activated protein kinase AMPK

Arg Arginine

BCAA Branched-chain amino acid

EAA Essential amino acid

FAA Free amino acid

i.v. intravenous

IGF-I/PI(3)K insulin growth factor-1/ Phosphatidylinositide 3-kinases

mTOR mechanistic target of rapamycin

NEAA Non-essential amino acid

NO nitric oxide

P70S6K1 ribosomal protein S6 kinase S6Ks

PI(3)K-Akt phosphatidylinositol-3 kinase/Akt kinase

ST Semitendinosus

GENERAL INTRODUCTION

The improvement of lambing percentage has been one of the key factors leading to increased productivity and profitability for New Zealand sheep farmers. The increase of the national lambing percentage of over 26% in the last 40 years, reaching an average of 124% (Anon, 2013a) and the increase of market weight of lambs have both compensated for the 32% decrease in the national sheep stock number during the last 15 years, and maintained the productivity as measured by kg of meat produced (Anon, 2013b). However, while the increase in litter size and thus the number of multiple-born lambs can impact positively on farm productivity, there are important constraints compared to singletons. Reduced fetal growth, higher mortality rates in the first 3 weeks of life (Scales et al., 1986; Greenwood et al., 2000b; Morris and Kenyon, 2004; Everett-Hincks et al., 2005; Gootwine, 2005; Gootwine et al., 2007), reduced neonatal growth rate and lower muscle mass (Bennett et al., 1991; McCoard et al., 1997; McCoard et al., 2010), are key factors in multiple-born lambs for which farmers demand feasible solutions. When considering that fetal skeletal muscle accounts for 25–30% of body mass at term of pregnancy (Forhead et al., 2002), and up to 45% of the animal body weight post-birth (Teleni, 1993), reduced muscle growth in twins compared to singletons becomes a key component behind the lower productivity in twins. However, the mechanisms resulting in differences between singles and twins are not clear.

Nutrition during pregnancy and post-natally plays a crucial role in skeletal muscle growth. The relevance of nutrition has been enhanced by the discovery that amino acids (AA) have roles other than as building blocks of proteins and other nitrogenous substances, glucose and fatty acids (Galli, 2007; Grillo and Colombatto, 2007; Li et al., 2007; Sugita et al., 2007). Changes in intracellular concentration of specific AA can also regulate cellular signalling pathways, such as the mechanistic target of rapamycin (mTOR), which controls protein accretion and therefore, muscle growth (Jobgen et al., 2006; Kim et al., 2007). Whether reduced muscle growth in twin lambs during pregnancy and post-natally is associated with lower intracellular AA concentrations in muscle and down regulation of mTOR signalling is not known. The objectives of this thesis were therefore to, firstly, identify possible intracellular AA which could act as limiting factors for muscle growth during late pregnancy and post-natally in twin sheep, secondly, to evaluate the *in vivo* the effect of supplementing a potentially regulatory AA on muscle

growth, and thirdly to examine the role of mTOR signalling in mediating any observed effects on muscle growth. The objectives of each chapter in this thesis were:

Chapter 1: Review the existing literature on the role of AA in the regulation of the development and growth of skeletal muscle.

Chapter 2: Identify intracellular FAA in muscle of singleton and twin fetuses at term, in order to determine which FAA could be acting as limiting signalling AA for skeletal muscle growth in twin fetuses.

Chapter 3: Identify intracellular FAA in muscle of singleton and twin fetuses at term and at weaning, in order to determine which ones could be acting as limiting signalling AA for skeletal muscle growth in twin lambs.

Chapter 4: Arginine (Arg) was shown in Chapter 2 and Chapter 3 to be associated with muscle mass during pregnancy. This chapter evaluated the effect of maternal Arg supplementation during mid-late pregnancy on fetal muscle growth and the effect on mTOR signalling. Further, this chapter also evaluated post-natal skeletal muscle growth to determine possible carry-over effects of maternal Arg supplementation on post-natal muscle growth stage in the offspring.

Chapter 5: To determine if oral Arg supplementation of twin-born lambs, had an effect on skeletal muscle growth while the lamb is a monogastric (from birth until 28 days of life) and from birth until 70 days of life, and determine if mTOR plays a role on muscle growth.

Chapter 6: A summary of the main findings, potential limitations and future work which could complement the results of the research presented in this thesis.