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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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 phophatidylinositol-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.