New Zealand Society of Animal Production online archive

This paper is from the New Zealand Society for Animal Production online archive. NZSAP holds a regular annual conference in June or July each year for the presentation of technical and applied topics in animal production. NZSAP plays an important role as a forum fostering research in all areas of animal production including production systems, nutrition, meat science, animal welfare, wool science, animal breeding and genetics.

An invitation is extended to all those involved in the field of animal production to apply for membership of the New Zealand Society of Animal Production at our website www.nzsap.org.nz

The New Zealand Society of Animal Production in publishing the conference proceedings is engaged in disseminating information, not rendering professional advice or services. The views expressed herein do not necessarily represent the views of the New Zealand Society of Animal Production and the New Zealand Society of Animal Production expressly disclaims any form of liability with respect to anything done or omitted to be done in reliance upon the contents of these proceedings.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

You are free to:

- **Share** — copy and redistribute the material in any medium or format

Under the following terms:

- **Attribution** — You must give appropriate credit, provide a link to the license, and indicate if changes were made. You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.
- **NonCommercial** — You may not use the material for commercial purposes.
- **NoDerivatives** — If you remix, transform, or build upon the material, you may not distribute the modified material.

http://creativecommons.org/licenses/by-nc-nd/4.0/
BRIEF COMMUNICATION: Effects of restricted fetal nutrition in utero on mTOR signalling in ovine skeletal muscle

Q. SCIASCIA1, D. PACHECO1, J. BRACEGIRDLE2, C. BERRY2, P.R. KENYON3, H.T. BLAIR3, M. SENNA SALERNO2, G. NICHOLAS2 and S.A. McCOARD1*

1AgResearch Grasslands, Private Bag 11-008, Palmerston North 4442, New Zealand
2AgResearch Ruakura, Private Bag 3123, Hamilton 3240, New Zealand
3Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11-222, Palmerston North 4442, New Zealand.

*Corresponding author: sue.mccoard@agresearch.co.nz

Keywords: lamb; skeletal muscle; hypertrophy; mTOR.

INTRODUCTION

High prolificacy (twins and triplets) is a desirable trait in sheep under intensive management systems. However, multiple-born lambs have lower birth weights resulting from placental insufficiency (McCoard, 1997), and carryover detrimental effects on lean deposition, feed efficiency, and lifelong performance compared to singletons (Greenwood et al., 1998; De Blasio et al., 2007), suggesting differential nutritional programming in utero.

We and others have shown that decreased birth weight in multiple-born lambs is associated with restricted skeletal muscle hypertrophy or myofibre size, but not number, reduced DNA accumulation, reduced MyoD-positive satellite cells (activated satellite cells) and protein synthesis (Greenwood et al., 1999; McCoard et al., 2001). However, the molecular mechanisms involved remain to be elucidated.

The mammalian target of rapamycin (mTOR) signalling pathway is critical both for sensing nutrient availability, fetal myogenesis, activation of myogenic satellite cells and for nutrient-stimulated muscle growth in both monogastrics and ruminants (Zhu et al., 2004; Du et al., 2005; Suryawan et al., 2008). The downstream targets of mTOR are proteins that control cell size, gene expression, mRNA translation and metabolism. Inhibition of mTOR leads to arrest of cells in the G1 phase of the cell cycle, suggesting mTOR may be involved in regulating muscle hypertrophy (Wang & Proud, 2006), a process dependent on protein synthesis and satellite cell activity.

The objective was to determine whether restricted skeletal muscle hypertrophy in twin fetuses in late gestation is associated with changes in translational capacity and/or efficiency and mTOR signalling.

MATERIALS AND METHODS

This study used M. semitendinosus samples collected from singleton and twin fetuses at 140 days gestation, collected as previously described (Kenyon et al., 2009). Briefly, a commercial flock of oestrus-synchronised Romney ewes with a live weight of 60.8 ± 0.2 kg and a body condition score of 3.02 ± 0.03 (1 = Emaciated; 5 = Obese) were mated to one of four Suffolk rams. Twin and singleton pregnancies were determined at 50 days post-insemination by transabdominal ultrasonographic examination. All ewes were fed on a maintenance pasture-only nutritional regimen from Day 1 post-insemination until 140 days of pregnancy, and grazed such that the total increase in maternal body weight during pregnancy approximately equalled the expected conceptus mass at term, that is the ewes maintained conceptus free weight.

At Day 140 of pregnancy, five twin-bearing ewes and ten singleton-bearing ewes were euthanised, fetal weight and crown-rump length recorded, and the M. semitendinosus excised, weighed and snap frozen in liquid nitrogen and stored at -80°C until analysis.

Total RNA and DNA was extracted from frozen muscle tissue from singleton and twin fetuses using Tri-reagent (Invitrogen, Auckland, New Zealand) according to the manufacturer’s instructions. RNA and DNA were quantified using a NanoDrop Spectrophotometer ND-1000 (Nanodrop Technologies, Wilmington, Delaware, USA). Protein was isolated using lysis buffer (50 mM Tris, pH 7.6; 250 mM NaCl; 5 mM EDTA; 0.1% Nonidet P-40; Complete (Roche Molecular Biochemicals, Mannheim, Germany) protease inhibitor). The muscle extracts were centrifuged to pellet the cell debris, and the supernatants were frozen at -80°C. Bradford reagent (Bio-Rad, Hercules, California, USA) was used to estimate total protein content (Bradford, 1976).

Equal amounts of protein were separated electrophoretically using SDS-PAGE and transferred to nitrocellulose membranes using the iBlot system (Invitrogen). Membranes were probed with primary antibodies against total ribosomal protein S6 (RPS6), phospho-S6 (Ser235/236), total 4EBP1, phospho-4EBP1 (Thr70 and Ser65), total eIF4E and phospho-eIF4E (Ser209), and...
RESULTS AND DISCUSSION

Twin fetuses had 25% reduced body weights and 43% reduced *M. semitendinosus* weights compared to singletons (Table 1). Reduced muscle mass in twins compared to singletons was associated with a 30% lower protein to DNA ratio suggesting restricted myofibre hypertrophy (Table 1). Twins also had 32% less DNA and ~40% less total RNA compared to singles, indicative of fewer myonuclei and reduced ribosome number respectively and thus capacity for muscle growth (Table 1). Because 85% of total RNA is ribosomal RNA (Nader et al., 2005), and increased rRNA is an indicator of increased ribosome biogenesis (Camacho et al., 1990), an increase in total RNA is indicative of increased ribosome number. Furthermore, twin fetuses had a 16% lower protein to RNA ratio compared to singles, indicating that twins had reduced protein synthetic efficiency compared to single fetuses (Table 1). The findings of the present study are consistent with previous observations (Greenwood et al., 1999; McCoard et al., 2001), highlighting the importance of fetal nutrition on skeletal muscle growth and development in utero.

The mTOR pathway senses nutrient availability and is critical for nutrient-stimulated muscle growth, and proteins downstream of mTOR including 4EBP1, eIF4E, RPS6 and eEF2 control the initiation and elongation phases of mRNA translation (Wang & Proud, 2006). The relative content (µg protein⁻¹) of both the native and phosphorylated forms of all the proteins evaluated (eIF4E, 4EBP1, RPS6 and eEF2) was increased 144 to 222% in muscle from twin compared to single lambs, with the exception of RPS6-Ser235/236 which did not differ. However, the absolute abundance of these translational components per muscle was reduced 46 to 55% in twins compared to singles, suggesting decreased capacity for protein synthesis consistent with decreased muscle mass in twins. The ratio of phosphorylated to native protein for each of the targets did not differ, suggesting that the short-term activation of mRNA translation did not differ between singles and twins. However, mTOR also increases the translational capacity of the cell in the longer-term by increasing the number of ribosomes and other translation components (Wang & Proud, 2006). Thus, using total RNA as an estimate of ribosome number, we observed in twins a decrease in the abundance of total eEF2, 4EBP1, and eIF4E per ribosome (Figure 1), consistent with decreased translational capacity in muscle from twin compared to single fetuses. We suggest that long-term restriction of nutrient availability to twin compared to singleton fetuses in late gestation down-regulates mTOR signalling in late gestation, leading to reduced ribosome number and abundance of the translational machinery and ultimately leads to retarded myofibre hypertrophy and muscle mass.

These potential mechanisms warrant further investigation under differing feeding regimes to determine whether nutrient availability is a key driver or whether other environmental signals are involved. The ability of mTOR to link environmental cues to ribosome biogenesis provides

### TABLE 1: Characteristics of *M. semitendinosus* in singleton and twin foetuses (n = 9 per group) from maintenance-fed ewes at 140 days gestation. Data are represented as the mean ± standard error of the mean.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Singletons</th>
<th>Twins</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>6.4 ± 0.2</td>
<td>4.8 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>M. Semitendinosus</em> weight (g)</td>
<td>11.4 ± 0.4</td>
<td>6.6 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total DNA per muscle (mg)</td>
<td>34.9 ± 2.9</td>
<td>23.9 ± 2.1</td>
<td>0.008</td>
</tr>
<tr>
<td>Total RNA per muscle (mg)</td>
<td>15.7 ± 0.6</td>
<td>9.6 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total protein per muscle (mg)</td>
<td>358 ± 24</td>
<td>182 ± 12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein : RNA</td>
<td>23.0 ± 1.2</td>
<td>19.1 ± 0.4</td>
<td>0.008</td>
</tr>
<tr>
<td>Protein : DNA</td>
<td>11.0 ± 1.2</td>
<td>7.9 ± 0.5</td>
<td>0.039</td>
</tr>
</tbody>
</table>
an efficient mechanism for skeletal muscle cells to alter their overall protein biosynthetic capacity.

ACKNOWLEDGEMENTS

This work was funded by the AgResearch Capability Fund, and the National Research Centre for Growth and Development (NRCGD).

REFERENCES


