BRIEF COMMUNICATION: Relationship between profiles of free amino acids in fetal and maternal plasma with those in skeletal muscle, in twin and single fetuses from *ad-libitum* fed ewes in late gestation

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Introduction

Skeletal muscle growth depends on amino acid (AA) availability, not only as building components, but also as regulatory signals for protein accretion (Wu 2009). However, their signalling role for muscle growth in ruminants is poorly understood. We have previously reported that lower muscle weight in twin compared to single fetuses in late gestation, is associated with changes in the profile of specific intracellular muscle free AA (FAA), when ewes are fed a maintenance plane of nutrition during gestation (Pacheco et al. 2010a). This suggests that some AA may regulate muscle growth during fetal life.

Maternal nutrient intake is of particular relevance in sheep-production systems with high rates of prolificacy and high lamb growth rates. Even in well-nourished ewes, lower fetal weight and muscle growth are observed in twins (McCoard et al. 2010), suggesting factors other than maternal nutrition may affect fetal growth (Freetly et al. 2004). Changes in AA concentration in fetal plasma vary according to the level of ewe nutrition during gestation. However, the relationship between maternal or fetal plasma AA and FAA in muscle, and the potential effects on muscle growth are not well understood. Therefore, the objectives were to firstly study the relationship between maternal and fetal circulating levels of FAA and intracellular FAA profiles in skeletal muscle, and secondly to evaluate the relationship between muscle growth and muscle FAA profiles in twin compared to single fetuses of *ad-libitum* fed late-gestation ewes.

Materials and methods

The experimental procedures were approved by the AgResearch Ruakura Animal Ethics Committee.

Single- and twin-bearing ewes were sourced from a commercial population (Rissington Breedline Primera® terminal breeding program) and managed at AgResearch Ruakura as one group. They were fed an *ad libitum* (2,200-2,500 kg/dry matter per ha) pasture diet to maximize growth. At Day 140 of gestation, nine single- and ten twin-bearing ewes were euthanised. Samples of maternal and fetal plasma, and the *M. semitendinosus* were collected and stored at -80ºC for later analysis. For plasma AA determination, samples were subjected to reverse-phase high-performance liquid chromatography (HPLC) separation of phenylisothiocyanate derivatives using a Waters PicoTag® column and a Shimadzu LC-10A HPLC system. Intracellular FAA content in muscle was performed using a lithium-based HPLC as previously described (Pacheco et al. 2010b). Within and between assays variation was lower than 4% and 2% respectively for both plasma and muscle assays.

Maternal, fetal and *M. semitendinosus* weights and AA concentration were analysed using an analysis of variance MIXED procedure (SAS 2006), with a linear model, considering pregnancy rank of single and twin, sex, and their interaction. No transformation of the AA data was needed as it was normally distributed. Means separation was performed using the PDiff option of the LSMeans statement of SAS (2006). Pearson analysis was used to determine correlations between variables. Statistical significance was set at a probability value of 0.05 or less.

Results and discussion

No differences were observed in ewe live weight between single- and twin-bearing ewes at 90 days of gestation (60.4 ± 1.8 (standard error) kg vs 59.5 ± 1.6 kg, P = 0.73) or at 137 days of gestation (72.8 ± 1.7 kg vs 73.7 ± 2.2 kg, P = 0.75). Twin fetuses were 15% lighter (5.1 ± 0.2 kg vs 6.0 ± 0.3 kg, P = 0.02) and had a proportional 23% lower *M. semitendinosus* weight (8.6 ± 0.3 g vs 11.1 ± 0.9 g, P = 0.02) compared to singles, with no effect of sex or rank by sex interaction. In agreement with previous studies (Freetly et al. 2004) these observations highlight the effect of birth rank on fetal and muscle growth even under a maternal *ad libitum* nutrition regime. Many factors influence muscle growth (Brameld et al. 1998). This study suggests specific AA may also be involved in the regulation of muscle growth in sheep.

Maternal specific and total plasma FAA concentration did not differ between single- and twin-bearing ewes. Compared to maternal plasma, fetal plasma had a 1.4 times higher (P < 0.05) total AA concentration, both in single and twin fetuses

Table 1 Mean ± standard error of mean for free amino acid concentration in maternal plasma, fetal plasma and *M. semitendinosus* from single and twin fetal lamb at 140 days gestation. P value bolded indicates significance (P < 0.05). P value in italics indicates approaching significance with a P value between 0.05 and 0.10. ND = Not detected.

Amino acid	Ewe plasma (μMol/L)			Fetal plasma (μMol/L)			Muscle (nMol/g)		
	Single	Twin	P value	Single	Twin	P value	Single	Twin	P value
Essential									
Arginine	203 ± 16	199 ± 21	0.85	150 ± 21	140 ± 13	0.67	312 ± 26	281 ± 27	0.42
Glutamine[1]	266 ± 12	291 ± 21	0.28	402 ± 26	489 ± 30	**0.05**	3,143 ± 123	3,484 ± 190	0.16
Histidine	89 ± 6	86 ± 7	0.73	89 ± 9	140 ± 24	*0.07*	290 ± 29	276 ± 18	0.7
Isoleucine	114 ± 6	122 ± 11	0.54	83 ± 6	84 ± 7	0.97	294 ± 74	238 ± 39	0.51
Leucine	154 ± 14	157 ± 17	0.89	166 ± 16	150 ± 11	0.42	37 ± 5	32 ± 4	0.38
Lysine	221 ± 17	234 ± 22	0.64	164 ± 11	151 ± 9	0.35	101 ± 12	116 ± 11	0.33
Methionine	49 ± 2	55 ± 3	0.14	106 ± 10	153 ± 12	**<0.01**	68 ± 6	112 ± 12	**<0.01**
Phenylalanine	80 ± 9	83 ± 10	0.84	116 ± 7	119 ± 9	0.82	44 ± 3	44 ± 6	0.98
Threonine	150 ± 22	167 ± 27	0.64	385 ± 29	362 ± 46	0.68	981 ± 49	800 ± 73	**0.05**
Tryptophan	50 ± 4	52 ± 9	0.85	ND	ND		ND	ND	
Valine	287 ± 32	292 ± 39	0.93	353 ± 19	316 ± 26	0.27	143 ± 9	119 ± 11	0.11
Non-essential									
Alanine	226 ± 8	227 ± 16	0.98	463 ± 32	457 ± 34	0.89	1,893 ± 180	1,767 ± 149	0.59
Asparagine	52 ± 14	56 ± 10	0.79	43 ± 4	45 ± 6	0.86	70 ± 15	49 ± 11	0.3
Aspartate	22 ± 3	24 ± 4	0.75	69 ± 4	59 ± 2	**0.03**	505 ± 47	359 ± 28	**0.01**
Carnosine	13 ± 1	11 ± 1	*0.06*	13 ± 2	11 ± 1	0.43	48 ± 8	41 ± 7	0.51
Cystine	10 ± 3	6 ± 2	0.38	6 ± 1	4 ± 1	0.5	5 ± 1	6 ± 1	0.43
Glutamate	100 ± 16	130 ± 14	0.19	217 ± 31	127 ± 15	**0.02**	1,166 ± 148	1,134 ± 113	0.87
Glycine	544 ± 44	575 ± 34	0.58	715 ± 100	732 ± 60	0.88	1,739 ± 252	1,769 ± 156	0.92
Proline	330 ± 33	343 ± 23	0.75	393 ± 54	412 ± 58	0.81	250 ± 34	277 ± 27	0.54
Serine	114 ± 28	120 ± 15	0.85	495 ± 67	530 ± 55	0.68	1,015 ± 163	1,071 ± 153	0.8
Tyrosine	101 ± 7	102 ± 9	0.94	153 ± 14	162 ± 15	0.66	73 ± 7	67 ± 10	0.65
Non-protein									
Citrulline	244 ± 24	210 ± 15	0.24	152 ± 16	112 ± 10	**0.05**	80 ± 9	127 ± 21	**0.05**
Ornithine	116 ± 9	95 ± 10	0.14	169 ± 18	122 ± 10	**0.03**	64 ± 7	84 ± 9	0.12
Taurine	30 ± 3	25 ± 2	0.15	34 ± 4	25 ± 2	*0.08*	5,594 ± 487	5,192 ± 338	0.5
Total	3,514 ± 189	3,608 ± 187	0.73	4,907 ± 213	4,902 ± 182	0.98	17,855 ± 623	16,913 ± 750	0.35

[1]Conditionally essential in neonates.

(Table 1) in agreement with other studies (Ashworth et al. 2011). However, twin fetuses had lower plasma concentrations (P < 0.05) of aspartate, glutamate, citrulline, and ornithine, and higher concentrations of methionine and glutamine compared to single fetuses, although total plasma AA concentration did not differ (Table 1). These results indicate amino acid transport across, and utilisation by the placenta and/or fetal AA turnover may differ between singles and twins. This warrants further investigation.

Differences in specific but not total FAA concentration in the muscle were found between single and twin fetuses. Intracellular concentrations of aspartate and threonine were lower, while citrulline and methionine were higher in twins compared to singles (Table 1). When compared to fetal plasma, these results suggest differential muscle AA uptake or utilisation in twins compared to singles. Intracellular concentrations of FAA also influence signalling pathways involved in protein accretion (Wu 2009) indicating changes in specific FAA between singles and twins may influence cell signalling pathways, which warrants further investigation. The effect of birth rank on muscle FAA concentration in the present study, contrasts with that described by Pacheco et al. (2010a), with only methionine exhibiting a higher concentration in twins compared to singles in both studies. Breed and nutrition are known modifiers of FAA concentration, at least in fetal plasma (Ashworth et al. 2011; Kwon et al. 2004) which may account for the differences observed between the studies. Differences in methionine warrant further investigation, as methionine plays a major role in fetal growth and DNA methylation (Rees et al. 2006).

In singles, muscle weight was positively correlated with intracellular glutamine (r = 0.67, P = 0.05) and arginine (r = 0.62, P = 0.007) but negatively correlated with histidine (r = -0.86, P = 0.003) concentrations. In twins, muscle weight was positively correlated only with arginine (r = 0.67, P = 0.03) concentration. Arginine is a known regulator of muscle growth in non-ruminants, acting as a signal to activate pathways involved in protein accretion such as mTOR (Wu 2009). Arginine supplementation of nutrient-restricted ewes also enhances fetal growth

(Lassala et al. 2010). Therefore, this study suggests arginine may also be a key regulator of muscle growth in singles and twins in well-fed ewes.

The observed differences in specific but not total FAA in the muscle, between single and twins, notably methionine, and the strong correlation between intracellular arginine concentration and muscle weight, indicate that methionine and arginine may be involved in the regulation of muscle growth during late gestation in sheep.

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