Proceedings of the Nutrition Society (2008), **67 (OCE)**, E155 doi:10.1017/S0029665108007878

Joint meeting of the Société Française de Nutrition and The Nutrition Society, 6–7 December 2007

Maternal protein supplementation differentially promotes uncoupling protein 1 expression in the fetus that is dependent on the time of gestation

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Maternal nutrient restriction during critical stages of gestation (e.g. mid-gestation, coincident with maximal placental growth) compromises fetal growth and development[1]. However, late gestational protein supplementation in twin-bearing pregnancies improves lamb vigour, immune competence and colostrum production[2]. The extent to which nutritional supplementation of the mother with protein in the form of fishmeal can promote expression of the thermogenic brown adipose tissue-specific uncoupling protein (UCP) 1 is unknown. Thus, the present study aimed to determine whether protein supplementation of the maternal diet at defined stages of gestation promotes the fetal growth or the regulation of mitochondrial protein abundance in brown adipose tissue.

Twenty-eight twin-bearing sheep of similar body weight and parity were randomly allocated into four feeding groups from 10 d of gestation. Each animal was fed a standard control diet, which was supplemented with fishmeal (66% (w/w) crude protein plus an equal amount of molasses to aid palatability) and fed to each of the three treatment groups during early (10–40 d; *n* 7), mid- (40–70 d; *n* 7) or late (110 d; *n* 7) gestation. Each mother was then humanely killed with an overdose of barbiturate (100 mg pentobarbital sodium/kg; Euthanal) at 140 d of gestation to enable fetal tissue sampling. mRNA and protein abundances were determined using fully-validated real-time RT–PCR techniques and antibodies respectively. Results, in arbitrary units (au), are expressed as a percentage of a reference sample present on all quantitative PCR plates and SDS–PAGE gels. Significant differences in relation to nutritional supplementation were determined by GLM analysis.

Protein supplementation had no effect on fetal body, placental or organ weights. UCP1 expression of the protein (but not mRNA) was increased in offspring supplemented during mid-gestation (control 79 (SE 10) au *v.* 126 (SE 140 au; $P<0.05$) despite a reduction in total mitochondrial protein. UCP2, PPARγ, PPARγ co-activator-1a (PGC-1a), glucocorticoid receptor (GR), 11β-hydroxysteroid dehydrogenase (11β HSD)-1 and -2, β-adrenergic receptor 3 (β-AR 3) and AMP-activated protein kinase α2 (AMPK α2) mRNA levels were all unaffected by fishmeal supplementation.

	Control		Early gestation		Mid-gestation		Late gestation		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*P* value
Body wt (kg)	5.0	0.6	5.2	0.3	5.7	0.3	5.4	0.3	NS
Placental wt (g)	703.6	58.8	631.7	83.8	753.1	39.4	712.4	56.2	NS
Fat mass (g)	18.7	1.3	21.3	1.4	22.8	1.5	23.6	1.8	NS
UCP2	1.0	0.4	1.7	0.6	0.9	0.4	1.2	0.5	NS
PPAR γ	1.0	0.3	1.5	0.4	1.2	0.3	2.4	0.9	NS
PGC-1a	1.0	0.6	0.3	0.02	0.1	0.03	0.3	0.1	NS
GR	1.0	0.5	0.5	0.1	0.3	0.5	0.7	0.3	NS
11β HSD-1	1.0	0.3	1.8	0.7	0.9	0.3	1.6	0.7	NS
11β HSD-2	1.0	0.5	1.0	0.3	0.3	0.1	0.5	0.2	NS
β-AR 3	1.0	0.3	1.3	0.3	0.7	0.2	1.5	0.5	NS
AMPK α2	1.0	0.3	1.9	0.5	1.0	0.2	3.1	1.4	NS

Increased UCP1 expression with protein supplementation specifically during mid-gestation is likely to improve thermogenesis in the newborn. However, this adaptation appears unrelated to sympathetic activation or glucocorticoid stimulation and is, therefore, likely to be mediated by translational modification and/or stabilisation of UCP1.

1. Heasman L, Clarke L, Stephenson TJ & Symonds ME (1999) *Proc Nutr Soc* **58**, 283–288.
2. Robinson JJ, Sinclair KD & McEvoy TG (1999) *Anim Sci* **68**, 315–331.

https://doi.org/10.1017/S0029665100590028 Published online by Cambridge University Press