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**BRIEF COMMUNICATION:** The effect of growth hormone on translation initiation and elongation in liver and skeletal muscle tissue in the lactating dairy cow

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**Keywords:** growth hormone; mTOR; liver; skeletal muscle.

**INTRODUCTION**

Administration of bovine growth hormone (GH) has been shown to increase milk production in dairy cows by coordinating the metabolism of various organs and tissues, so that nutrients are repartitioned to the mammary gland to support increased requirements for milk synthesis (Keys *et al.*, 1991). Some of the targets for GH are adipose tissue (Gallo *et al.*, 1990), skeletal muscle, liver (Bush *et al.*, 2003) and the mammary gland (Hayashi *et al.*, 2009). In muscle GH decreases glucose uptake and increases amino acid catabolism efficiency (Vallimont *et al.*, 2001), whilst the liver responds by increasing the rate of basal gluconeogenesis through lypolysis of adipose reserves (Gallo *et al.*, 1990).

We have previously shown that the increase in total milk protein yield following a six day treatment of dairy cows in mid-lactation with GH is associated with a change in protein translation initiation and elongation (Hayashi *et al.*, 2009). However, whether these effects are tissue-specific has not been explored.

The objective of this study was to determine if GH administration influences protein translation initiation and elongation in liver and skeletal muscle of lactating dairy cows.

**MATERIALS AND METHODS**

**Western blots**

Frozen liver and skeletal muscle tissues from mid-late lactation dairy cows treated for six days with bovine GH or saline controls, were sourced from a previously described study (Hayashi *et al.*, 2009) and homogenised in extraction buffer (in mM: 20 Tris-HCl (pH 7.4), 1 EDTA (pH 8.0), 1 EGTA (pH 8.0), 0.5 Vanadate, 1 Benzamidine Hydrochloride, 1 DTT, 0.1 PMSF; in μg: 1 Pepstatin, 1 Antipain, 1 Leupeptin; 1% Triton X-100 - all purchased from Sigma, Australia). Lysates were centrifuged at 7,000 rpm for 10 minutes at 4°C to remove debris, and supernatant protein concentrations determined by Bradford assay (Bradford, 1976). Equal amounts of protein lysate were used for SDS-PAGE and Western blotting, as described by Kimball *et al.* (1996), using the TransBlot and immunoblot PVDF membranes (BioRad, Hercules, California, USA), and chemiluminescent visualisation (ThermoScientific, Rockford, Illinois, USA). Primary antibodies were: total and phospho-4E-BP1 (Thr70 and Ser65); total and phospho-eIF4E (Ser209); total and phospho-RPS6 (Ser235/236); and total and phospho-eEF2 (Thr56), and the secondary antibody horseradish peroxidise-linked goat anti-rabbit (Cell Signaling Technology, Beverly, Massachusetts, USA). Primary antibodies were raised against synthetic peptides that correspond to regions that are highly conserved across a number of species, including bovine (Cell Signalling Technology Inc., Personal communication).

**Analysis**

Triplicate western blots with GH and control samples were generated for each primary antibody target. Quantity One Image Software (BioRad, Hercules, California, USA) was used to measure the density of each band, whose values were then used to generate an average and standard error of the mean across cows within treatments. Tests were performed using the two-sided *t*-test procedure in SAS v9.1 (SAS Institute Inc., Cary, North Carolina, USA) with a probability value of *P* <0.05 being considered statistically significant.

**RESULTS AND DISCUSSION**

**Effect of GH treatment on 4E-BP1 and eIF4E abundance and phosphorylation**

Protein translation can be initiated when 4E-BP1 and eIF4E are uncoupled from each other via hierarchical phosphorylation of 4E-BP1 at four distinct sites, Thr37, Thr46, Thr70 and Ser65 (Gingras *et al.*, 2001). eIF4E can then form the eIF4F pre-initiation complex with two other factors in preparation for ribosome recruitment and the initiation of protein synthesis. The role of eIF4E in the eIF4F pre-initiation complex is to bind mRNA at a unique 7-methyl site (Yanagiya *et al.*, 2009). The affinity of eIF4E binding is enhanced when phosphorylation at Ser209 occurs, leading to a fourfold increase in the efficiency of protein synthesis.

In liver, GH reduced the total abundance of 4E-BP1 (26%, *P* = 0.0009) and eIF4E (40%, *P* = 0.02), whilst Ser65 phosphorylation was increased 95% relative to controls (*P* = 0.03) (Figure 1). GH had no
FIGURE 1: The effect of growth hormone on the total abundance and ratio of phosphorylated eIF4E and 4E-BP1 to total expressed as a percentage relative to controls. The graph shows the mean ± standard error (n = four per treatment; Solid bars = Controls; Open bars = Growth hormone treated.

significant effect on 4E-BP1 Thr70 (P = 0.60) or eIF4E Ser209 phosphorylation (P = 0.18) and no effect on 4E-BP1 or eIF4E abundance (P = 0.46; P = 0.36) or phosphorylation (4E-BP1 Ser65 P = 0.61; 4E-BP1 Thr70 P = 0.34; eIF4E Ser209 P = 0.45) in skeletal muscle.

These results indicate that despite reduced abundance of eIF4E, decreased abundance and increased Ser65 phosphorylation of 4E-BP1 that there may be more eIF4E available for the initiation of protein translation in the liver of GH treated cows. We have previously shown GH administration increases eIF4E abundance in the mammary gland, consistent with increased translation initiation (Hayashi et al., 2009). Therefore, these results indicate that GH has tissue-specific effects, and suggest GH may be involved in the activation of translation initiation in liver and mammary tissues via differential regulation of the eIF4F complex (Shen et al., 2002), a potential mechanism that warrants further investigation.

Effect of GH treatment on eEF2 abundance and phosphorylation

Once protein synthesis has been initiated, elongation of the new protein strand is regulated by eEF2 (Taylor et al., 2007). Phosphorylation of eEF2 at Thr56 inhibits its activity, shutting down protein synthesis prior to elongation. GH had no effect on total abundance or phosphorylation of eEF2 in either liver (total: P = 0.98, Thr56: P = 0.38) or skeletal muscle (total: P = 0.87, Thr56: P = 0.35), indicating the rate of protein synthesis is unchanged in these tissues. In contrast, GH treatment significantly increases the abundance of eEF2 and reduces phosphorylation in the mammary gland (Hayashi et al., 2009), further suggesting the effects of GH are tissue-specific.

Effect of GH treatment on RPS6 abundance and phosphorylation

The role of ribosomal protein S6 is not yet fully elucidated; however it has been implicated in the regulation of cell growth and increased translation of 5' TOP mRNAs, which exclusively encode for components of the translation machinery (Jafféries et al., 1994). GH administration had no effect on the total abundance or phosphorylation of S6 in liver (total: P = 0.95; phospho: P = 0.74) or skeletal muscle (total: P = 0.19; phospho: P = 0.48). These observations contrast the stimulatory effect of GH on S6 phosphorylation in the mammary gland (Hayashi et al., 2009), and indicate that the rate of ribosome biogenesis in liver and skeletal muscle remains unaffected.

In summary, the results of this study indicate that the effects of GH on translation initiation and elongation in the liver, skeletal muscle and mammary gland during established lactation are tissue-specific.

ACKNOWLEDGEMENTS

The authors thank N. Roy, W. McNabb, B. Sinclair, K. Broadley, J. Peters, B. Treloar, S. Davis, P. Schreurs, A. Death, M.C. Reynolds, A-C. Pupin and J. Lane for their help with the animal experiment. Tissues used in this experiment were sourced from an experiment conducted as part of a Joint Venture between AgResearch Limited and Primary Industries Research, Victoria, Australia. This work was funded by a Foundation of Science, Research and Technology Postdoctoral Fellowship (Dr McCoard); the Capability Fund, a project in partnership with Livestock Improvement Corporation and the Foundation of Science, Research and Technology, (C10X0702).

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