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BRIEF COMMUNICATION: mTOR signalling in the lactating bovine mammary gland
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Introduction

The mammalian target of rapamycin (mTOR) is a protein kinase that has a well established role in the regulation of a range of cellular functions in response to hormones, growth factors and amino acids (Wang & Proud 2011). The role of mTOR signalling in the regulation of milk protein synthesis in the mammary gland of ruminants is not well understood. Understanding this mechanism is an important step towards developing intervention strategies on farm, such as the supply of nutrients, to modulate milk protein output.

Rates of protein synthesis largely depend on translation initiation and elongation rates, with the rates being regulated by several eukaryotic initiation and elongation factors. In bovine mammary tissue, we and others have reported that modulation of mTOR signalling in response to hormone/growth factors and/or nutrients such as amino acids and starch, may be an important control point in the regulation of both the initiation and elongation steps of the protein synthetic pathway (Appuhamy et al. 2012; Hayashi et al. 2009; Rius et al. 2010). Increasing the non-milking interval in lactating dairy cows decreases milk and milk protein output (McCoard et al. 2012; Singh et al. 2012).

The objective of this study was to test the hypothesis that reduced milk protein output in response to increasing non-milking interval is associated with altered mTOR signalling.

Material and methods

Samples used for this work were sourced from a trial described elsewhere (Singh et al. 2012). Briefly, milk protein output by the gland was determined by comparing milk protein yield for each animal on the day of slaughter following adjustment for pre-treatment yield values. Milk protein yield data unadjusted for pre-treatment yields has been presented previously (McCoard et al. 2012). Adjusted data is presented here for ease of interpretation of the mTOR data. Mammary parenchymal tissue was collected post-mortem from five cows per group cows that were not milked for either 7, 14 or 28 days, and then re-milked for a period of 7 days prior to euthanasia.

Total protein was extracted from tissue samples and the abundance of total (native and phosphorylated forms) and phosphorylated forms of mTOR, RPS6, eEF2 and eIF4E determined as previously described (Hayashi et al. 2009; Sciascia et al. 2013). All antibodies were procured from Cell Signaling Technology (Boston, Massachusetts, USA), and included anti-total mTOR, anti-phospho-mTOR (Ser2448), anti-total eEF2, anti-phospho-eEF2 (Thr56), anti-total RPS6, anti-phospho-RPS6 (Ser235/236), anti-total-eIF4E, anti-phospho-eIF4E (Ser209). All samples were run on the same gel to enable direct comparison between groups, and triplicate Western blots prepared. Measurement and calculation of signal intensities is described by Sciascia et al. (2013). The treatment effect was determined using one-way analysis of variance and confirmed with both the Kruskal-Wallis rank sum test and permutation test. A significance level threshold of P ≤0.05 with corresponding least significant differences were used to compare specific treatment means. All analyses were undertaken in R (R Development Core Team 2012).

Results and discussion

Milk protein yield on the day of slaughter was 39.7 ± 2.9, 29.7 ± 3.2 and 13.4 ± 3.2 g for 7-day, 14-day and 28-day non-milking interval groups respectively (P <0.001), indicating a large effect of non-milking interval on milk protein output by the gland. Phosphorylation of mTOR at Ser2448 is widely used as a biomarker of activation of the mTOR pathway (Appuhamy et al. 2012; Hayashi et al. 2009; Rius et al. 2010). Increasing the non-milking interval in lactating dairy cows decreases milk and milk protein output (McCoard et al. 2012; Singh et al. 2012).

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Figure 1 Abundance of total and phosphorylated ribosomal protein (RPS6), eukaryotic elongation factor 2 (eEF2) and mammalian target of rapamycin (mTOR) in mammary tissue collected from five lactating Friesian cows in response to a 7-, 14- or 28-day non-milking period followed by a 7-day milking period. Data are presented as mean ± standard error of the mean. Significance indicated as ** P ≤ 0.001, * P ≤ 0.05, † P ≤ 0.10.

Figure 2. Relative protein activation (ratio of phosphorylated to total abundance) of ribosomal protein (RPS6), eukaryotic elongation factor 2 (eEF2), eukaryotic initiation factor 4E (eIF4E) and mammalian target of rapamycin (mTOR) in mammary tissue collected from in lactating Friesian cows in response to a 7-, 14- or 28-day non-milking period followed by a 7-day milking period (n = 5/group). Data are presented as mean ± standard error of the mean. ** P ≤ 0.01, * P ≤ 0.05.

Phosphorylation of RPS6 appears to be a key link between mTOR and cell size, however the physiological role is yet to be fully elucidated (Wang & Proud 2011). Increasing non-milking interval was associated with a decrease in the amount of total-RPS6 in the glands but did not change the abundance of phosphorylated RPS6 (Figure 1). The relative activation of RPS6 was lower in the 7-day non-milked glands compared to the 14-day and 28-day non-milked glands (Figure 2). It is known that phosphorylation of RPS6 in mammary cells is increased in response to nutrients (Appuhamy et al. 2012; Rius et al. 2010), hormones and growth factors (Hayashi et al. 2009; Sciascia et al. 2013). However, the mechanism involved in the control of RPS6 phosphorylation in relation to non-milking interval remains to be determined.

The elongation step of protein translation is regulated by eEF2 with phosphorylation at Thr56 by eEF2 kinase inhibiting its function (Ryazanov et al. 1988). Abundance of total eEF2, phosphorylated eEF2 and the ratio of phosphorylated to total eEF2 were unaffected by the duration of the non-milking interval (Figures 1 and 2). These observations indicate that the effect of non-milking interval on milk protein output is not mediated by altered rates of translation elongation. Previous studies report inconsistent effects of environmental signals on the elongation step of protein synthesis (Hayashi et al. 2009; Rius et al. 2010), indicating further research is
required to elucidate the role of translation elongation in the regulation of protein synthesis in the lactating bovine mammary gland.

The eukaryotic translation initiation factor 4E is implicated in the development of the mammary gland and onset of lactation (Long et al. 2001) and increased milk protein output in response to GH is associated with increased abundance of total and phosphorylated eIF4E while the relative ratio is unaffected (Sciascia et al. 2013). Changes in the duration of non-milking interval had little effect on neither the abundance of total and phosphorylated eIF4E nor the relative activation, suggesting that eIF4E is unlikely to be rate limiting in this experimental model.

In summary, these results indicate that mTOR signalling may be involved in mediating the effect of environmental stress such as non-milking interval, on milk protein synthesis in the mammary gland. These results contribute to the growing body of evidence that mTOR signalling may be a key regulator of milk protein synthesis in the bovine mammary gland. Such knowledge may support the development of new approaches to increase production performance of lactating ruminants.

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References


