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BRIEF COMMUNICATION: The effect of growth hormone on the intracellular amino acid profiles in the mammary gland of lactating cows

D. PACHECO, B.P. TRELOAR, and S.A. McCOARD*

AgResearch Grasslands, Private Bag 11-008, Palmerston North 4442, New Zealand
*Corresponding author: sue.mccoard@agresearch.co.nz

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INTRODUCTION

The dairy industry is a cornerstone of the New Zealand economy, and increasing milk production, especially milk protein, is essential to boost farmer profits and meet global demand for milk products. Increasing the milk protein output per unit of intake also has positive environmental effects, due to more nitrogen being partitioned towards product, rather than excreta (Pacheco & Waghorn, 2008).

Amino acids (AA), particularly the nutritionally essential ones, have received attention in dairy nutrition because of their obvious role as substrates for protein synthesis (Lapierre *et al.*, 2006). However, intracellular AA have also been described as important regulators of intracellular pathways controlling protein synthesis (Burgos *et al.* 2010). For example, the intracellular concentrations of arginine (Yao *et al.*, 2008) and leucine (Lynch, 2001) are key regulators of the mammalian target of rapamycin (mTOR) pathway, which is involved in the control of protein synthesis and other cellular processes in multiple tissues. Our previous research indicates that mTOR signalling may regulate milk protein synthesis in the bovine mammary gland in response to growth hormone (GH) (Hayashi *et al.*, 2009). It is not known if the GH-mediated increase in mammary protein synthesis is associated with changes in the concentration of specific intracellular AA.

We hypothesised that changes in the concentration and profile of intracellular AA would be observed, either as a cause or an effect of the increase in synthetic capacity in the mammary gland. Intracellular mammary concentrations of free (non protein bound) AA (FAA) were measured in mammary tissue collected from cows treated with GH.

MATERIALS AND METHODS

All procedures involving these animals were carried out in compliance with the guidelines of the Agresearch Grasslands Animal Ethics Committee.

Eight Jersey cows were injected with either a slow-release preparation of GH (GH Treatment group) or physiological saline (Control) (n = 4 per treatment). Details on the animals, diets, experimental procedures and results have been reported elsewhere (Hayashi *et al.*, 2009).

Parenchymal tissue samples were collected from the right hind-quarter of the mammary gland, avoiding large blood vessels and immediately frozen in liquid nitrogen. Samples were stored at -85°C until analysed for intracellular FAA concentrations.

A 200 mg tissue sample was homogenised in 1.75 mL of Seraprep (Pickering Laboratories, Alphatech Systems Ltd, Auckland, New Zealand) and 20 µL of aminoguanidinopropionic acid (25 µM/mL) added as an internal standard. Samples were left in ice for 20 minutes, and then 40 µL 5.88 M lithium (Li) hydroxide buffer added, followed by centrifugation at 13,680 *g* for five minutes. The resulting supernatant was analysed for FAA using a Shimadzu LC10Ai HPLC (Shimadzu Oceania Ltd., Auckland, New Zealand), fitted with a high-efficiency Li-ion exchange column (3 mm ID x 150 mm; Pickering Laboratories, Shimadzu Oceania Ltd., Auckland, New Zealand) and a Pickering PCX 3100 post-column reaction module (Pickering Laboratories, Shimadzu Oceania Ltd, Auckland, New Zealand). Injected volumes were 10 µL, a reagent flow rate of 0.3 mL/min and a run time of 162 minutes between injections, using Li buffers as eluants and ninhydrin post-column derivatisation.

Data were expressed both as absolute (nmol per g of tissue) and relative (g per 100 g of assayed FAA) concentrations. Percentage data were square-root transformed before the statistical analysis. Back-transformed results are presented. Differences between GH-treated and Control animals were assessed via two-sample *t*-tests for individual AA using the TTEST procedure in SAS 9.1. (SAS Institute Inc., Cary, North Carolina, USA). Initial data exploration indicated that for some AA the variances were not homogenous between treatments. Thus, the analysis was done using the Cochran option to adjust the probability values for those AA with unequal variance per treatment. Both the probabilities for the null hypotheses of equal mean and equal variance are reported.

RESULTS

GH tended to increase the absolute intracellular concentration of total FAA (P = 0.08), associated with increases in the absolute intracellular concentrations of glycine (P = 0.02), serine (P = 0.03) and glutamate (P = 0.01). The absolute

concentrations of glutamine and lysine in the GH-treated group had smaller variances ($P <0.05$) than those observed in the control group (Table 1).

The relative concentrations of FAA did not change in response to GH treatment except for a reduction in arginine ($P = 0.04$) and an upward trend for serine ($P = 0.07$). However, the variance of the relative concentrations of the essential AA histidine, isoleucine, leucine, lysine, phenylalanine, tyrosine and valine, and the non-essential AA alanine, glutamine, glutamate, were smaller ($P = 0.05$) for the GH-treated group (Table 2).

DISCUSSION

Protein synthesis has been described as one of the processes defining the overall level of metabolic activity in the mammary gland (Cant *et al.*, 1999; Volpe *et al.*, 2010). The mammary gland is able to modulate blood flow and the extraction of FAA for milk production in response to changes in circulating FAA in plasma, thus matching inputs to synthetic activity (Volpe *et al.*, 2010). Although the importance of AA for milk protein synthesis has been recognised for a long time, most of the published literature has focused on the relationships between AA inputs from blood or plasma and outputs in milk, with limited published data on the relationships between intracellular FAA and metabolic capacity of the mammary gland. However, it is accepted that increased intracellular concentrations of FAA will increase protein synthesis in *in vitro* models of the bovine mammary gland (Clark *et al.*, 1980) and presumably *in vivo* as well, according to mathematical models of mammary metabolism (Hanigan *et al.*, 2000; Volpe *et al.*, 2010). Thus, the upward trend in intracellular FAA concentrations is compatible with the increased milk protein reported from the same animals, which in turn might be mediated via downstream signalling from the mTOR pathway (Hayashi *et al.*, 2009).

In this study, GH treatment caused significant reductions in the variance of the FAA concentrations, rather than their means. This observation needs to be confirmed in future research with greater number of samples and experimental conditions. We speculate that the reduction in variance in relative concentrations of FAA could be an orchestrated event to optimise their supply relative to the protein synthetic set point and the energy status of the cell. However, the paucity of data regarding mean intracellular AA concentrations, let alone variances, frustrates further elaboration of this mechanism.

Histidine, isoleucine, leucine, phenylalanine, tyrosine and valine are among the group of AA in which GH treatment changed the variance of the relative concentrations (Table 2). These AA are substrates of the L-system of AA transporters present in the mammary gland. The L-system has been described as a target of mTOR in other tissues (Roos *et al.*, 2009). The effect on the variance of both lysine and glutamine suggests that other AA transporter systems may be affected by intracellular signals. Further research is needed to establish a cause-effect link between mTOR and FAA transporters activity in the mammary cell, which would provide further evidence that mTOR-mediated intracellular signalling is an important control point for protein synthesis in the mammary gland. Here, we

TABLE 1: Mean ± standard error of the mean of the effect of bovine growth hormone treatment on the absolute intracellular concentrations (nmol per g of tissue) of free amino acids (FAA) in the mammary gland of lactating Jersey cows. P value of difference between treatment means has been adjusted for those amino acids with unequal variances ($P <0.05$). SEM = Standard error of the mean.

Amino acid	Treatment		P value	
	Control	Growth hormone	Null hypothesis of equal means	Null hypothesis of equal variances
Essential				
Arginine[1]	57 ± 23	9 ± 9	0.10	0.14
Histidine	54 ± 11	43 ± 3	0.34	0.09
Isoleucine	60 ± 25	85 ± 8	0.37	0.09
Leucine	124 ± 34	114 ± 12	0.80	0.11
Lysine	125 ± 43	91 ± 4	0.49	<0.01
Methionine	22 ± 14	17 ± 6	0.74	0.18
Phenylalanine	52 ± 30	61 ± 9	0.79	0.08
Tyrosine[1]	40 ± 25	47 ± 7	0.78	0.08
Valine	163 ± 55	190 ± 14	0.65	0.05
Non-essential				
Alanine	1,898 ± 438	1,908 ± 157	0.984	0.13
Asparagine	92 ± 8	107 ± 7	0.196	0.78
Aspartate	575 ± 101	642 ± 29	0.545	0.07
Glutamine	337 ± 251	179 ± 35	0.593	0.01
Glutamate	4,688 ± 640	7,168 ± 353	0.015	0.46
Glycine	1,779 ± 101	2,279 ± 109	0.015	0.91
Proline	213 ± 30	214 ± 31	0.992	0.99
Serine	411 ± 40	560 ± 26	0.021	0.51

[1]Deemed as "semi-essential" or conditionally essential. For GH treatment, three out of four animals had non-detectable concentrations of this amino acid.

report that non-essential AA make a major contribution to total intracellular FAA, in agreement with the previous reports for bovine mammary tissue (Shennan *et al.*, 1997). The non-essential FAA have not received much attention by researchers, as it is normally assumed that they will be synthesised from excess uptake of essential AA to support a protein synthesis setpoint (Mepham, 1982). Further investigation is required to determine the mechanisms behind the changes in non-essential FAA, especially as their synthesis involves metabolites such as acetyl CoA and pyruvate, which are central to the energetic status of the mammary epithelial cell.

The findings of the current study, demonstrate that the galactopoietic effect of GH results in changes in the mean and variance of intracellular FAA profiles. Coupled with our previous work (Hayashi *et al.*, 2009), it is suggested that this may be mediated via activation of mTOR signalling. Future work is needed to explore the mechanisms by which mTOR may exert its effect via modulation of AA transporters or directly through intracellular FAA interconversion. A better understanding of the interactions between FAA supply and the intracellular pathways involved in the control of protein synthesis, such as mTOR, will provide new strategies to enhance the lactation performance of dairy cows and other livestock.

TABLE 2: Effect of GH treatment on the relative intracellular concentrations of amino acids AA: (g AA per 100 g of total AA) in the mammary gland of lactating Jersey cows. P value of difference between treatment means has been adjusted for those amino acids with unequal variances P <0.05). CI = 95% confidence interval of the mean.

Amino acid	Treatment				P value	
	Control		Growth hormone		Null hypothesis of equal means	Null hypothesis of equal variances
	Mean	CI	Mean	CI		
Total AA	100	-	100	-		
Essential						
Arginine[1]	0.6	0.1 - 0.7	0.1	0.1 - 0.4	0.04	0.930
Histidine	0.6	0.2 - 1.2	0.4	0.3 - 0.5	0.20	0.022
Isoleucine	0.4	0.1 - 2.0	0.7	0.5 - 0.8	0.57	0.004
Leucine	1.1	0.3 - 2.6	0.9	0.7 - 1.1	0.48	0.024
Lysine	1.3	0.2 - 3.3	0.8	0.7 - 0.8	0.35	<0.001
Methionine	0.1	0.1 - 1.0	0.1	0.1 - 0.5	0.97	0.323
Phenylalanine	0.3	0.2 - 2.5	0.6	0.4 - 0.8	0.57	0.009
Tyrosine[1]	0.3	0.2 - 2.2	0.5	0.3 - 0.7	0.56	0.012
Valine	1.3	0.2 - 3.5	1.3	1.1 - 1.5	0.96	0.005
Non-essential						
Alanine	11.7	5.7 - 19.9	9.9	8.4 - 11.6	0.47	0.048
Asparagine	0.9	0.6 - 1.2	0.8	0.7 - 0.9	0.66	0.071
Aspartate	5.4	3.5 - 7.6	5.0	4.0 - 6.1	0.66	0.311
Glutamine	2.8	3.6 - 27.3	1.5	0.6 - 2.9	0.66	0.029
Glutamate	53.1	31.9 - 79.5	61.9	59.8 - 64.0	0.26	0.008
Glycine	9.7	8.2 - 11.3	10.0	9.2 - 10.9	0.54	0.351
Proline	1.8	0.9 - 3.0	1.4	0.9 - 2.0	0.36	0.349
Serine	3.1	2.8 - 3.4	3.5	3.0 - 4.0	0.10	0.492

[1]Deemed as "semi-essential" or conditionally essential.

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