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Decline in mammary translational capacity during intravenous glucose infusion into lactating dairy cows

R. V. Curtis,*[1] J. J. M. Kim,* D. L. Bajramaj,* J. Doelman,† V. R. Osborne,* and J. P. Cant*
*Department of Animal and Poultry Science, University of Guelph, Ontario, N1G 2W1, Canada
†Nutreco Canada AgResearch, Guelph, Ontario, N1G 4T2, Canada

ABSTRACT

The objective of this study was to determine effects of glucose on milk protein yield and mammary mammalian target of rapamycin (mTOR) activity in dairy cattle in early lactation. Eight multiparous cows at 73 ± 8 d in milk were randomly assigned to 2 treatments in a crossover design for two 6-d periods. Treatments were jugular infusion of either saline (Sal) or 896 g/d glucose (Glc). All cows were fed a total mixed ration with 42% neutral detergent fiber, had free access to water, and were milked twice a day. Within each period, blood samples were taken (d 5) and mammary tissue was collected by biopsy (d 6) from each hindquarter for Western blot analysis. In addition to Sal and Glc treatments, on d 6, rapamycin dissolved in 50% dimethyl sulfoxide was administered via the teat canals into the left quarters, with a control solution administered into the right quarters. Rapamycin had no effect on milk protein yields or phosphorylation state of mTOR signaling proteins. Infusions of Glc significantly increased milk yield but only tended to increase milk protein yields. Milk fat tended to be decreased in cows infused with Glc, whereas lactose yields were significantly increased. Glucose infusion did not increase plasma glucose levels, but insulin and nonessential AA concentrations increased by 21 and 16%, respectively, branched-chain AA concentrations decreased 24%, and essential AA concentrations tended to decrease by 14%. Infusion of Glc significantly decreased abundances of both phosphorylated and total ribosomal S6 kinase 1 (S6K1) in mammary tissue by 27 and 11%, respectively. Abundance of phosphorylated eukaryotic initiation factor 4E-binding protein 1 (4EBP1) decreased significantly by 25%, whereas total 4EBP1 exhibited a tendency to decrease by 16%. We conclude that the mTOR signaling pathway is not the only regulator of milk protein synthesis. Decreases in essential AA concentrations in plasma suggest that protein synthesis was stimulated in nonmammary tissues of the body, presumably skeletal muscle.

Key words: glucose infusion, dairy cow, mammary protein synthesis, mammalian target of rapamycin (mTOR)

INTRODUCTION

Milk protein synthesis in the lactating dairy cow is greatly influenced by 2 major nutritional sources, protein and energy, with recent research attention being heavily focused on energy. Many studies have found that glucose infusion in dairy cows increases milk protein yield (Rulquin et al., 2004; Al-Trad et al., 2009; Toerien et al., 2010), whereas others have shown glucose to have no effect on milk protein yield (Hurtaud et al., 1998; Cant et al., 2002; Lemosquet et al., 2009). As a result of these inconsistencies, the mechanism behind how glucose carries out its effects on milk protein synthesis remains unknown.

Glucose infusion does not increase essential AA (**EAA**) concentrations in circulation but Raggio et al. (2006) found that mammary uptake of nonessential AA (**NEAA**) was increased during propionate infusion. Rulquin et al. (2004) suggested that glucose directs AA to the mammary glands by stimulating mammary blood flow. This hyperemia could be mediated by insulin, which stimulates mammary blood flow and milk protein yield in cows (Mackle et al., 2000). However, elevating mammary blood flow with vasodilators does not increase milk protein yield (Lacasse and Prosser, 2003); therefore, blood flow itself cannot explain the effect of glucose or insulin on milk protein yield. If, instead, glucose or insulin stimulated milk protein synthesis, the associated ATP expenditure would be expected to increase mammary blood flow according to a metabolic control hypothesis (Cant et al., 2003). Toerien et al. (2010) found that glucose infusion into fasted cows caused activation of mammary eukaryotic initiation factor 2 (**eIF2**) by dephosphorylation of its α subunit; eIF2 is an important controller of the rate of initiation of mRNA translation (Proud, 2005). Rius et al. (2010) found that the mammalian target of ra-

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[1]Corresponding author: rcurtis@uoguelph.ca

pamycin (**mTOR**) pathway of translational regulation was activated in mammary glands of cows infused abomasally with starch. Both insulin and intracellular energy charge are known activators of the mTOR pathway (Wullschleger et al., 2006) and have been shown to activate mTOR in mammary epithelial cells in culture (Appuhamy et al., 2011; Burgos et al., 2013).

We hypothesized that signaling to the translation apparatus in mammary tissue is responsible for the milk protein response to glucose. Therefore, the objective of the present study was to determine effects of glucose on milk protein yield and mammary mTOR activity in dairy cattle in early lactation. Intramammary infusions of the mTOR inhibitor rapamycin were given to test whether mTOR is involved in the effects of glucose on milk protein synthesis.

MATERIALS AND METHODS

Animals and Housing

The Animal Care Committee at the University of Guelph approved all experimental procedures in this study. Eight multiparous Holstein cows began the experiment at 73 ± 8 DIM and 674 ± 84.7 kg of BW. Cows were housed in a tiestall barn at the Ponsonby Livestock Research Station (Ponsonby, ON, Canada) and had free access to feed and water throughout the study. A high-forage diet (Table 1) containing 42% NDF was formulated for 32 kg/d of ME-allowable and MP-allowable milk according to the Cornell Net Carbohydrate and Protein System (CNCPS v6.1; AMTS LLC, Cortland, NY). Feed offered and refused was recorded throughout the study for determination of daily ad libitum feed intakes of individual cows. Feed samples were collected daily and pooled weekly over the 5-wk study and submitted for nutrient composition analysis by wet chemistry at a commercial laboratory (Agri-Food Labs, Guelph, ON, Canada). Orts from individual cows were sampled daily and pooled weekly. Dry matter contents of feed and orts samples were determined using a forced-air oven at 60°C.

Treatments

Cows were randomly assigned to a 6-d continuous infusion into the jugular vein of either a physiological saline (**Sal**) treatment or 896.2 ± 12.9 g/d of glucose (**Glc**) treatment via peristaltic pump. After a 7-d rest period between infusion periods, cows were switched to the opposite treatment. One day before each infusion period, cows were weighed and fitted with long-term catheters (14-gauge, 20 cm; MILA International Inc., Erlinger, KY) in the left jugular vein. Ceftiofur (Excede; Zoetis Canada, Kirkland, QC, Canada) was administered after catheter insertion as a precautionary measure to prevent infection. Glucose was completely dissolved in 3 L of 0.9% NaCl saline and both infusates were sterilized via autoclave.

To test the role of mTOR signaling in effects of glucose on milk protein synthesis, 10 mg of rapamycin dissolved in 150 mL of 50% dimethyl sulfoxide (**DMSO**) solution was rapidly infused, following the 1530 and 0500 h milkings on d 5 and 6, respectively, of each period, into the 2 left mammary glands of each cow via the teat canals. As a control, 150 mL of 50% DMSO was infused into each of the right mammary glands.

Milking and Milk Sampling

During infusion periods, cows were milked twice daily at 0500 and 1530 h using a bucket milker modified to collect milk from the 2 udder halves separately. Milk yields from each udder half were recorded daily and samples were collected and analyzed for fat, lactose and protein by spectroscopy at the Laboratory Services Division, University of Guelph (Guelph, ON, Canada).

Blood Sampling and Metabolite and Hormone Concentrations

Blood samples were collected from the tail vein on d 5 of each period after the morning milking and on d

Table 1. Ingredient and chemical composition (% of DM unless otherwise noted) of the experimental TMR (DM basis) fed to lactating dairy cattle (n = 8) infused i.v. with physiological saline or glucose for 6 d

Item	Value
Ingredient composition	
Alfalfa silage	29.4
Corn silage	24.6
Soybean hulls, ground	11.1
Mixed grains, chopped	7.8
Tripro soy plus[1]	7.3
Corn grain, ground fine	6.9
Straw	5.0
Corn distillers	2.1
Wheat shorts	1.0
Soybean meal	0.9
Canola meal	0.9
Vitamin and minerals	2.9
Chemical composition	
CP	15.1
Soluble CP (% of CP)	35.6
NDICP[2] (% of CP)	32.4
NDF	42.1
ADF	29.1
Lignin	5.7
NFC	37.8
Starch (% of NFC)	40.2
Ether extract	3.1
NE_L (Mcal/kg)	1.4

[1]West Central (Ralston, IA).
[2]Neutral detergent-insoluble CP.