J. Dairy Sci. 101:1–12
https://doi.org/10.3168/jds.2017-13844

Symposium review: Amino acid uptake by the mammary glands: Where does the control lie?[1]

John P. Cant,*[2] Julie J. M. Kim,* Scott R. L. Cieslar,* and John Doelman†
*Department of Animal Biosciences, University of Guelph, Ontario, N1G 2W1 Canada
†Nutreco Nederland BV, 5831, JN, Boxmeer, the Netherlands

ABSTRACT

Milk protein yield responses to changes in the profile of essential amino acids absorbed by the gastrointestinal tract or circulating in blood plasma do not follow the classic limiting amino acid response, in part because of an ability of the mammary glands to modify their blood flow rate and net clearance of amino acids out of plasma. The hypothesis that mammary blood flow is locally regulated to maintain ATP balance accounts for observed changes in flow due to postruminal glucose, insulin, and essential amino acid (EAA) infusions. An additional hypothesis that net mammary uptakes of metabolites from blood are affected by perturbations in their respective arterial concentrations and the rate of mammary blood flow also appears to hold for the energy metabolites glucose, acetate, β-hydroxybutyrate, and fatty acids. However, net EAA uptakes by the mammary glands are poorly predicted by models considering arterial concentrations and blood flow rates only. Evidence points to intramammary protein synthesis and secretion as the determinant of net EAA uptake. The intracellular signaling network anchored by the mechanistic target of rapamycin complex 1 stands as an excellent candidate to explain nutritional effects on milk protein synthesis because it integrates information on physiological and nutritional state to affect protein synthesis and cell metabolism, growth, proliferation, and differentiation in many cell types. In mammary cells in vitro and in vivo, the mechanistic target of rapamycin complex 1, integrated stress response, and glycogen synthase kinase-3 networks that contribute to regulation of initiation of mRNA translation are responsive to acute changes in nutrient supply and EAA profile. However, after several days of postruminal infusion of balanced and imbalanced EAA profiles, these signaling networks do not appear to continue to account for changes in milk protein yields. Gene expression evidence suggests that regulation of components of the unfolded protein response that control biogenesis of the endoplasmic reticulum and differentiation of a secretory phenotype may contribute to effects of nutrition on milk protein yield. Connections between early signaling events and their long-term consequences should be sought.
Key words: mammary gland, amino acid, milk synthesis

INTRODUCTION

The formulation and evaluation of diets fed to dairy cows is based on mathematical models that describe the flow of nutrients through the processes of digestion and absorption. Metabolizable protein and EAA supplies are typically predicted by estimating microbial, undegraded dietary and endogenous protein outflows from the reticulorumen (NRC, 2001; Sauvant and Nozière, 2016). A variable proportion of the absorbed EAA is captured in milk protein, depending on characteristics of the diet such as energy and MP content, level of intake, stage of lactation of the cow, health status, and so on. This variability in EAA capture can be accounted for in nutrient flow models if the underlying mechanisms of control are sufficiently understood. Progress in explaining variation in capture of metabolizable EAA in milk protein has been greatly facilitated by application of the arteriovenous difference technique by which net EAA uptakes by the mammary glands can be measured (Linzell, 1974). An estimate of mammary blood flow (**MBF**) rate is required to obtain uptake values from arteriovenous concentration differences, and through such measurements, nutritional effects on MBF have been recorded. Net uptake is a consequence of bidirectional transport across the plasma membranes of mammary epithelial cells. Net uptake values can be used to estimate rates of AA interconversion and catabolism and relations to other intra- and extra-mammary pathways of milk synthesis and nutrient metabolism. This review will attempt to disentangle relationships

Received September 15, 2017.
Accepted January 28, 2018.
[1]Presented as part of the ADSA Lactation Symposium: The New and Old of Mammary Gland Physiology and Milk Synthesis at the ADSA Annual Meeting, Pittsburgh, Pennsylvania, June 2017.
[2]Corresponding author: jcant@uoguelph.ca

between MBF, transmembrane transport, and net uptake, and focus on the main factor that regulates mammary EAA uptake, which is the control of milk protein synthesis. A framework for exploring variation in milk protein yield will be presented.

MAMMARY AA UPTAKE IS REGULATED BY CONTROL OF MILK PROTEIN SYNTHESIS

Net mammary AA uptake represents loss into milk, mostly as protein, tissue protein accretion, and catabolism. Milk protein yield accounts for approximately 90% of net mammary AA uptake (Cant et al., 1993). Milk protein yield of cows is stimulated by an increased MP supply (Daniel et al., 2016), apparently because of higher concentrations of EAA in blood and faster delivery of these EAA to the mammary glands. Attempts to identify which EAA is most limiting for milk protein yield have shown that milk protein yield is not limited by supply of a single EAA. Evidence against the limiting EAA phenomenon includes stimulation of milk protein yield by mutually exclusive sets of EAA in cows (Schwab et al., 1976) and mice (Liu et al., 2017), equal losses in milk protein yield when Met, Lys, His, Phe, or Leu are subtracted from the duodenal EAA supply (Weekes et al., 2006; Doelman et al., 2015a,b), and stimulation of milk protein yield by insulin or glucose when EAA concentrations in blood decrease (Mackle et al., 2000; Toerien et al., 2010).

Mammary cells that synthesize milk protein are exposed to EAA concentrations that are a function of their concentrations in arterial blood and the rate of MBF. Reasons why milk protein yield does not respond according to a limiting AA phenomenon may include the effect of extra-mammary organs on arterial EAA concentrations and local regulation of MBF. For example, when graded levels of Met from 0 to 32 g/d were infused into the duodenum of lactating cows, MBF decreased in a quadratic fashion and net mammary uptake of Met was not affected (Guinard and Rulquin, 1995). When His was added to an abomasal infusate of EAA lacking His, MBF in goats decreased and net mammary uptake of His was not affected (Bequette et al., 2000). Increasing Lys concentration in an otherwise complete mix of AA + glucose infused into the jugular veins of fasted goats also caused MBF to decrease (Guo et al., 2017). Guo et al. (2017) reported a linear decrease in concentrations of $NO_3^- + NO_2^-$ in samples of the mammary venous outflow as Lys was added to infusates, indicating lower output of the short-lived vasodilator NO. Synthesis of NO and other vasodilators in the mammary glands may be regulated by AA supply, but a potential mechanism has received very little study. Vascular NO is produced by endothelial NO synthase from oxidation of a guanidino N of Arg. The synthase is regulated by phosphorylation, acylation, and calmodulin binding in response to numerous chemical and mechanical stimuli acting upon the endothelium (Dudzinski et al., 2006). Whether and how physiological concentrations of the Arg substrate influence rates of endothelial NO synthesis is a matter of some debate. The K_m of endothelial NO synthase for Arg is 50 times lower than normal intracellular Arg concentration (Cardounel et al., 2007), so reaction velocity would not be expected to respond to increased Arg supply. However, adding 2% Arg to the drinking water of rabbits partially restored aortic endothelial NO synthesis and the vasodilatory response to acetylcholine that had been impaired by cholesterol feeding (Böger et al., 1997). Supplementary Arg for lactating sows did not affect MBF (Krogh et al., 2017) but subtraction of Arg from a mixture of EAA infused abomasally into cows tended to decrease MBF (Doepel and Lapierre, 2011). The subtraction of Arg did not affect milk protein yield (Doepel and Lapierre, 2011). An Arg-induced elevation in blood flow, when it occurs, may be due to competition between Arg and endogenous methylarginine inhibitors that can accumulate (Böger et al., 1997; Cardounel et al., 2007).

It is tempting to speculate that the mammary vasculature is endowed with a mechanism to respond to individual EAA supplies. However, the involvement of a canonical energy-sensing vasoactive pathway has not yet been ruled out. Much as has been described for cardiac and skeletal muscle (Deussen et al., 2012; Hellsten et al., 2012), the mammary glands release end products of oxidative metabolism and ATP synthesis into the interstitium, which activate endothelial synthesis of NO and another vasodilator, prostacyclin (Cieslar et al., 2014). This set of mechanisms (Figure 1) allows MBF to increase or decrease according to the rate of milk synthesis. Mammary uptake and catabolism of the energy metabolites glucose, acetate, and BHB is dependent to some degree on their respective arterial concentrations (Cant et al., 2016), so the vasodilatory mechanisms also cause MBF to decrease when concentrations of energy metabolites rise (Cant et al., 1993, 2002; Cieslar et al., 2014). Computer simulation of intracellular ATP turnover and its effect on capillary geometry, and ensuing changes in rates of blood flow and net metabolite uptake (Cant et al., 2003), indicates that elevated concentrations of acetate and BHB in circulation during single EAA infusions (Guinard and Rulquin, 1995; Weekes et al., 2006) may be responsible for the decreased MBF rates that have been observed. Whether the drop in mammary NO release and MBF observed by Guo et al. (2017) as i.v. Lys supply increased was caused by an imbalance in mammary supply of energy metabolites or AA, possibly Arg, remains to be determined.