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REVIEW ARTICLE

Linking our understanding of mammary gland metabolism to amino acid nutrition

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Abstract Amino acids (AA) are not only building blocks of protein but are also key regulators of metabolic pathways in animals. Understanding the fate of AA is crucial to optimize utilization of AA for milk protein synthesis and, therefore, to reduce inefficiencies of nutrient utilization during lactation. By understanding the functional role of AA metabolism in mammary tissue, we can uncover pathways and molecular targets to improve AA utilization by mothers and neonates during the lactation period. The major objective of this article is to highlight recent advances in mammary AA transport, metabolism and utilization. Such knowledge will aid in refining dietary requirements of AA for lactating mammals, including women, sows and cows.

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Keywords Amino acid · Lactation · Metabolism · Mammary gland · Utilization

Abbreviations

AA	Amino acids
ASCT	Alanine, serine, cysteine amino acid transport protein
$ATB^{0,+}$	$B^{0,+}$-type amino acid transport protein
$b^{0,+}AT$	$b^{0,+}$-type amino acid transport protein
BCAA	Branched-chain amino acids
BCAT	Branched-chain aminotransferase
BCKA	Branched-chain α-keto acids
BCKAD	Branched-chain α-keto acid dehydrogenase
CAT-1	Cationic amino acid transport protein 1
CAT-2b	Cationic amino acid transport protein 2
CP	Crude protein
DETA-NO	Diethylenetriamine-NO
EAAT	Excitatory amino acid transporter
GOT	Glutamate oxaloacetate transaminase
GPT	Glutamate pyruvate transaminase
GS	Glutamine synthetase
LAT	L-type amino acid transporter
NOS	Nitric oxide synthase
NRC	National Research Council
OAT	Ornithine aminotransferase
ODC	Ornithine decarboxylase
P5C	Δ^1-L-Pyrroline-5-carboxylate
P5CD	Δ^1-L-Pyrroline-5-carboxylate dehydrogenase
P5CR	Δ^1-L-Pyrroline-5-carboxylate reductase
y^+LAT	y^+L-Type amino acid transporter
rBAT	Amino acid transporter rB
SNAT	Sodium-coupled neutral amino acid transporter
TAT	T-type amino acid transporter

Introduction

Growth of the mammalian neonate is dependent on milk quality and supply from the mother. Farm animals (e.g., sows and cows) have been extensively used to study protein nutrition during lactation (Wu et al. 2014). The traditional factorial approach of matching dietary amino acid (AA) profile to that of milk has allowed estimation of the dietary requirement for all indispensable AA for lactating swine to maximize growth of the nursing pig (NRC 2012). While the factorial concept is ideal in theory, its application remains very limited because the AA metabolic processes occurring between absorption and intracellular mammary utilization into milk protein are ignored. For instance, because coefficients of utilization of AA across the three major tissues, namely the small intestine, liver and mammary gland, are largely unknown, the current factorial estimates of dietary AA requirement reported by the NRC (2012) for the lactating sow remain to be validated. Such validation, however, will require tedious empirical testing of individual AA. Knowledge of the metabolic processes of AA utilization would allow for refinement of the existing factorially derived AA requirement estimates to maximize milk production by lactating mammals (Wu 2014). The goal of this review was to summarize the current body of knowledge on AA transport and metabolism in mammary tissue during lactation across the animal species studied to date and to discuss the relevance of these processes to AA nutrition.

Amino acid metabolism during lactation

It has long been recognized that the mammary gland is a site of extensive synthesis and degradation of AA. Tracer studies with the perfused goat, sheep and cow mammary glands (Verbeke et al. 1968, 1972; Roets et al. 1974), tissue explants and cell culture systems (Jorgensen and Larson 1968; O'Quinn et al. 2002) have been instrumental in identifying many of the metabolic transformations of AA. These pathways are tissue- and cell-specific, and may be more prominent in the mammary gland where net uptakes of so-called "nutritionally nonessential AA" by the gland are far less than that required for milk protein synthesis. This contrasts with skeletal muscle, where stoichiometric relationships mostly exist. In the last 20 years, the availability of a wider range of radio- and stable isotope labeled AA at an affordable price has led to a number of metabolic studies on the lactating cow, sow and goat mammary glands in vivo. Traditionally, AA and their metabolism have been categorized according to the balance between net arteriovenous (AV) uptake and milk casein-AA secretion. Excess uptake is assumed to represent catabolism, and, for this reason, those AA taken up in excess have traditionally not been considered to be limiting for milk protein synthesis. This point has also been argued on the basis that the K_m for activation of acyl-tRNA synthetases are 100-fold lower than those for catabolic enzymes (1×10^{-6} vs. 1×10^{-4}; Rogers 1976) and, therefore, catabolism should only proceed once the acyl-tRNA has become fully charged (DePeters and Cant 1992). This argument assumes that the end products of AA catabolism serve as rate-limiting substrates or regulators for protein synthesis, which we now know is incorrect (Appuhamy et al. 2011, 2012). It should also be borne in mind that K_m values obtained from in vitro enzyme assays may not be relevant to tissues or cells in vivo because of the complex intracellular compartmentation of substrates and cofactors, as well as complex interactions among proteins (Wu 2013a). Figure 1 illustrates, to the best of the authors knowledge, all of the AA metabolic pathways occurring in mammary tissue that have been studied to date across animal species, including those of bovids, ovids, suids and murids. Some of the enzymes may not have been identified per se, but their metabolic products are suggestive of their presence in the mammary tissue. On the other hand, some of the enzymes have been directly measured, hence suggesting the presence of their substrates and products.

Lysine

Lysine is often the first- or second-limiting AA for milk protein synthesis in lactating sows and cows, particularly when diets are based on corn and soybean meal as the primary protein sources (Tokach et al. 1993; Richert et al. 1997; Nichols et al. 1998). The fractional extraction of Lys by the mammary gland is among the highest of all essential AA, as reported in bovids, suids and ovids (Guinard and Rulquin 1994a, b; Trottier et al. 1997; Mabjeesh et al. 2000). While the metabolic fate of Lys in mammary tissue is primarily milk proteins, Hurley et al. (2000) reported that there is a significant, albeit small, proportion of Lys that is oxidized by the mammary tissue (Hurley et al. 2000), indicating that Lys is likely taken up by the mammary gland in greater quantities than that secreted in milk. However, this finding needs to be verified by directly determining CO_2 production from Lys. In fact, Kim et al. (1999) estimated a daily Lys retention of 0.11 g based on serial mammary gland isolation dissection during lactation. This value was also used by Guan et al. (2002) in their estimation of mammary protein synthesis during lactation. Despite these notions, Lys along with Phe, have been broadly used as internal markers for measurement of blood flow (Trottier et al. 1997; Nielsen et al. 2002; Guan et al. 2002, 2004) based on the Fick principle, with the assumption of no Lys oxidative losses or in situ utilization by mammary cells. The uptake of Lys by the mammary gland of dairy cows

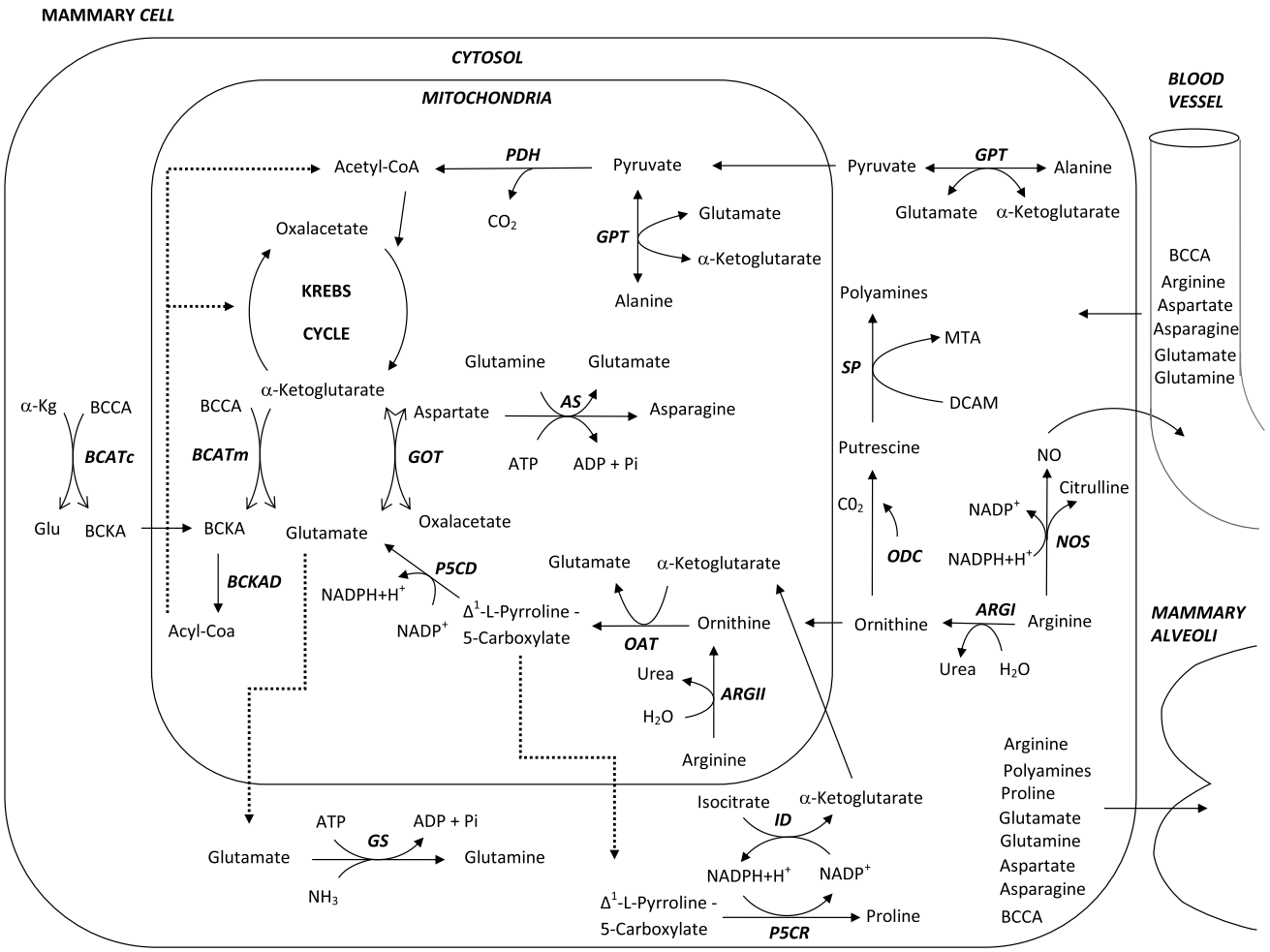

Fig. 1 Amino acid metabolism in the mammary epithelial cell during lactation. See text for description of pathways and symbols. Modified from L. I. Chiba (Ed) Sustainable Swine Nutrition. Wiley-Blackwell, Ames, Iowa

and goats has also been reported to be greater than milk Lys output (Guinard and Rulquin 1994b; Mabjeesh et al. 2000). Furthermore, administration of Lys in excess to lactating goats resulted in greater mammary uptake of Lys and increased oxidation, which accounted for the additional uptake of Lys. Why the mammary gland extracts Lys in excess only to be oxidized was addressed by Lapierre et al. (2009) in lactating dairy cows. Here, when Lys was limited and there was a corresponding decrease in milk protein output, the uptake of Lys by the mammary gland remained in excess relative to its secretion in milk protein, pointing to Lys serving other roles in addition to synthesis of milk proteins. Indeed, the authors found that even under limiting conditions the contribution of Lys nitrogen (^{15}N) to the synthesis of several nonessential AA remained relatively unchanged. Thus, there appears to be an obligate utilization of Lys in the milk protein synthesis process, at least under the experimental conditions used in the above studies. In the NRC (2012), the rate of Lys utilization post-absorption into milk protein Lys was estimated to be 67 %, indicating that 23 % of Lys is funneled into other processes, including oxidative losses. Clearly, oxidative losses accounting for as much as 23 % seem highly unlikely, supporting the observation by Lapierre et al. (2009) that Lys must serve other, presumably, essential roles. However, the validity of this conclusion depends on the accuracy of laboratory analysis of Lys and small peptides containing Lys. The Lys catabolic pathways in mammary tissue are yet to be characterized and assessed under different nutritional and physiological conditions. If there is an obligate utilization of Lys at least in part into nonessential AA synthesis as reported by Lapierre et al. (2009), a utilization rate of 67 % likely represents an underestimation of the global utilization of Lys for the milk protein synthetic process.

Methionine (and cysteine, taurine and glutathione)

The sulfur AA Met and Cys are essential for normal protein metabolism in all animals, and Met is often the first- or second-limiting AA in farm animal diets. Extraction of

Met by the mammary gland closely matches the amounts required for milk protein output (Bequette et al. 1998). In addition to incorporation into protein, Met is involved in multiple pathways leading to synthesis of phospholipids, carnitine, creatine and polyamines in a tissue-specific manner (Wu 2013a). At the same time, Met provides methyl groups for a number of transmethylation reactions involved in regulation of DNA activity and oncogene status, and it provides sulfur for Cys synthesis (Bequette et al. 1998). In lactating goats, 28 % of whole body Met methyl group flux contributes to plasma choline synthesis (Emmanuel and Kelly 1984). Cysteine, glutathione (GSH) and taurine are crucial intermediates in the maintenance of a variety of physiological functions, especially in monogastrics, including the human infant (Wu 2009). For instance, free taurine concentration in bovine milk is <10 % that of human milk and <100 % that of porcine milk (Erbersdobler et al. 1984; Wu and Knabe 1994; Pasantes-Morales et al. 1995). A number of studies have shown that supplementation of Met, either alone or together with Lys, significantly improved milk protein synthesis in dairy cows fed corn-based diets (Weekes et al. 2006). Such studies are not available in suids or other animal species. In fact, the available literature on dietary requirements of Met and Cys for the lactating sow is restricted to only two older studies (Ganguli et al. 1971; Schneider et al. 1992). Across the animal species studied to date, there is a clear deficit in our understanding of Met and Cys role specifically in mammary tissue other than their incorporation into milk proteins. None of the catabolic pathways of Met and Cys known to other tissues and organs has been addressed in mammary tissue. The swine NRC (2012) used a coefficient of utilization efficiency for Met and Cys incorporation into porcine milk proteins of 66 % in the factorial estimation of Met and Cys requirement. This value is likely grossly underestimated considering the notion that mammary extraction of Met closely matches the quantity required for milk protein output in ovids and suids (Bequette et al. 1998; Trottier and Guan 2000). In fact, in the study by Guan et al. (2004), the efficiency of Met incorporation into porcine milk protein was assumed to be 95 %.

Branched-chain AA (leucine, isoleucine and valine)

The BCAA, in particular Leu, are extracted by the mammary gland of the cow, goat and sow in quantities that exceed their secretion in milk protein (Viña and Williamson 1981; Bequette et al. 1996a, b; Bequette and Douglass 2010; Trottier et al. 1997; Trottier and Guan 2000; Nielsen et al. 2002). The first step in catabolism of the BCAA occurs extensively in extrahepatic tissues, including skeletal muscle and the small intestine, where branched-chain aminotransferase (BCAT) transaminates the BCAA to their corresponding keto acids (Shimomura et al. 2001). The second and irreversible step in BCAA catabolism occurs largely in the liver, where the branched-chain α-keto acid dehydrogenase enzyme complex (BCKAD) catalyzes the oxidative decarboxylation of the BCAA keto acids (Shimomura et al. 2001). Studies in lactating sows, cows, and rats indicate that activities of both BCAT and BCKAD are also substantial in mammary tissue. In this regard, Viña and Williamson (1981) and Bequette et al. (1996a, b), (Bequette and Douglass 2010) reported Leu oxidation by the rat, and the cow and goat mammary tissue in vitro and in vivo, respectively. Significant gene and protein expression of BCAT and BCKD has also been observed in mammary tissues of lactating rats and sows (DeSantiago et al. 1998; Li et al. 2009). Two different BCAT isoenzymes have been reported in porcine mammary epithelial tissue: BCAT located in the cytosol, and $BCAT_m$ located in the mitochondria (Li et al. 2009). Similar results have been reported for bovine mammary epithelial cells (Lei et al. 2012a, 2013). By contrast, only $BCAT_m$ was present in rat mammary gland (DeSantiago et al. 1998). During BCAA transamination, the α-amino group of Leu, Ile and Val is transferred to the Krebs intermediate α-ketoglutarate to form Glu. Subsequently, the corresponding α-keto acids (BCKA; α-ketoisocaproate, α-keto-ß-methylvalerate and α-ketoisovalerate, respectively) are catabolized to their acyl-CoA derivative by BCKAD (Shimomura et al. 2001). The BCKAD is a multi-enzyme complex located on the inner surface of the mitochondrial membrane (Harper et al. 1984); thus, if BCAA transamination occurs in the cytoplasm of mammary epithelial cells, BCKA are either channeled into the mitochondria or released from cells. Studies with bovine mammary epithelial cells indicate that most of the BCKA produced from BCAA do not undergo oxidation to CO_2 and instead are released into the extracellular medium (Lei et al. 2012b).

The activity of BCKAD is regulated by both insulin and BCAA via phosphorylation. When plasma insulin is elevated or BCAA concentrations are low, the enzyme remains inactive (Razook-Hasan et al. 1982). Indeed, oxidation of Leu by the mammary gland is reduced in goats subjected to a hyperinsulinemic–euglycemic clamp (Bequette et al. 2002). Furthermore, mammary expression of BCAT is highly upregulated in lactating sows and rats (DeSantiago et al. 1998; Tovar et al. 2001; Li et al. 2009) compared to the non-lactating state. Porcine and rat mammary tissue obtained in the lactating state also had very high activity of BCKAD (DeSantiago et al. 1998; Li et al. 2009); it was estimated that approximately 60 % of BCKA formed in porcine mammary gland was catabolized (Li et al. 2009). Oxidation of BCKA results in formation of acetyl-CoA and succinyl-CoA (Harper et al. 1984) which ultimately leads to energy generation and carbon skeletons

for the de novo synthesis of nonessential AA and fatty acids (Shimomura et al. 2001). Alternatively, the BCKA can be reaminated to their corresponding BCAA (Tovar et al. 2001; Bequette et al. 2002). It has been suggested that changes in oxidation of Leu and perhaps of Ile and Val occur in relationship to milk production. Under conditions of increased milk production, however, such as increased milking frequency (Bequette and Douglass 2010) and hyperinsulinemic–euglycemic clamp (Bequette et al. 2002), changes in Leu oxidation by the goat mammary gland were not associated with milk production. As such, while mammary gland oxidation of the BCAA may serve a role in milk biosynthesis, their role is not obligatory for milk synthesis. However, it is unknown whether the amount of Leu oxidized is sufficient to affect lactation. Based on these studies, an estimated rate of Leu utilization of 72.3 % into porcine milk protein (NRC, 2012) appears high given that Lys was estimated to be only 67 % (NRC, 2012). Guan et al. (2002) reported that the proportion of mammary intracellular valine utilized for metabolism other than protein synthesis was 0.34 and this proportion appeared to remain unaffected by dietary AA regime, indicating that valine may participate considerably in metabolism, including oxidation, other than protein synthesis in the mammary. As discussed above, the catabolism of the BCAA in the rat mammary gland increases significantly during lactation due to increased expression of mitochondrial BCAT and increased activity of BCKAD. Based on the study by Guan et al. (2002), mammary Val utilization for milk protein synthesis may be estimated at 66 %, which is in contrast to the estimated coefficient of 58.3 % reported by NRC (2012).

Arginine (and proline)

Net quantities of Arg for maintenance of protein synthesis are synthesized de novo by the kidneys from citrulline produced from Gln, Glu and Pro by the small intestine (Wu and Morris 1998), but additional Arg demand for growth, reproduction and lactation (Visek 1985) makes Arg a dietary essential AA for these functions (Wu 2013b; Wu et al. 2013a, b). For instance, feeding an Arg-free diet to gestating rats decreased birth weight and increased perinatal mortality, and decreased both mammary gland development and mammary protein synthesis during lactation (Pau and Milner 1982). In practice, Arg has traditionally not been considered to be a limiting AA for milk synthesis because of the relatively high content of Arg in feeds (Wu 2010). However, the content of Arg in animals is also relatively high and Arg is extensively catabolized in the body (Wu et al. 2010). Results of a study indicate that Arg supplementation to a conventional lactating sow diet for instance did not increase litter performance (Pérez-Laspiur and Trottier 2001). However, the effect of Arg supplementation depends on the stage of lactation (Mateo et al. 2008). Nonetheless, Wu et al. (2004) reported that piglet growth was limited by Arg availability in sow's milk. In support of this view, Arg supplementation in the diet increased the growth of artificially reared piglets (Kim and Wu 2004).

The finding that Arg availability in milk limits piglet growth (Kim and Wu 2004) is intriguing because as compared to all other AA, the mammary gland of lactating sows, dairy cows and goats extracts Arg in the greatest quantities relative to milk protein secretion (Trottier et al. 1997; Bequette et al. 1998). The large uptake of Arg coupled with its relatively lower milk abundance can only point to substantial Arg catabolism by mammary tissue. Arginine is metabolized by the sow mammary gland to Pro, ornithine and urea via the arginase pathway, and to polyamines and NO via the arginase and NOS pathways, respectively (O'Quinn et al. 2002). Trottier et al. (1997) reported a significant net production of Pro (10 g/day) and of urea by the mammary gland during lactation, indicative of mammary Pro synthesis. Trottier et al. (1997) also reported significant negative arteriovenous differences of NH_3 across the porcine mammary gland during the peak phase of lactation, suggesting net production of NH_3 and active AA catabolism. Urea N also has been shown to be formed by the mammary gland of lactating goats (Mepham and Linzell 1967). Studies in lactating bovine (Clark et al. 1975; Basch et al. 1997), porcine (O'Quinn et al. 2002) and murine mammary gland (Glass and Knox 1973) observed two different arginases: (1) arginase I, located in the cytosol, and (2) arginase II, located in the mitochondria. Both enzymes catalyze the cleavage of Arg into urea and ornithine in mammary tissue (Basch et al. 1997; O'Quinn et al. 2002). Ornithine can be used for polyamine synthesis by the cytoplasmatic enzymes, ornithine decarboxylase (ODC) and spermidine synthase (Wu and Morris 1998), leading to relatively high polyamine concentrations in rat, human, and sow's milk (Pollack et al. 1992; Motyl et al. 1995; Dai et al. 2014). The importance of milk polyamines has been demonstrated in mammals, where polyamines have been shown to regulate lactogenesis (Oka and Perry 1974) and enterocyte proliferation (Johnson 1988).

Alternatively, ornithine can be transported into the mitochondria and converted to Δ^1-L-pyrroline-5-carboxylate (P5C) by the enzyme ornithine aminotransferase (OAT). The presence of OAT activity has been reported in murine, cow and porcine mammary tissue (Yip and Knox 1972; Basch et al. 1995; O'Quinn et al. 2002). The α-ketoglutarate used by OAT can be formed from glucose via pyruvate and oxaloacetate and from Glu by Glu dehydrogenase (Farrell et al. 1990; Wu 2013a). Mammary tissue lacks P5C synthase and Pro oxidase and, therefore, cannot synthesize P5C from Gln, Glu or Pro (O'Quinn

et al. 2002). In murine, bovine, and porcine mammary tissue, ornithine-derived P5C is either converted to Glu by Δ^1-L-pyrroline-5-carboxylate dehydrogenase (P5CD), or exported to the cytosol and converted to Pro by Δ^1-L-pyrroline-5-carboxylate reductase (P5CR; Mezl and Knox 1977; Basch et al. 1996, 1997; O'Quinn et al. 2002). The activity of P5CR in lactating porcine mammary tissue is 56-fold greater than P5CD, thus favoring the conversion of Arg to Pro rather than to Glu (O'Quinn et al. 2002). The high concentration of Pro in porcine milk protein parallels the requirement for Pro by the piglet neonate, since Pro biosynthetic activity during the neonatal period is low (Wu and Knabe 1994; Bertolo et al. 2003; Wu et al. 2011a).

Nitric oxide (NO) synthesis is quantitatively a minor pathway for Arg degradation in the lactating mammary gland (O'Quinn et al. 2002). Nitric oxide is produced from Arg and molecular oxygen in a reaction catalyzed by the enzyme NO synthase. Once synthesized, NO rapidly diffuses into the tissue where it acts as a vasodilator (Lacasse et al. 1996; Meininger and Wu 2002) and angiogenic factor (Wu and Meininger 2000). By enhancing the synthesis of NO in the mammary endothelial cells, Arg may increase blood flow to the mammary gland, thus increasing supply of nutrients for milk production (Kim and Wu 2009).

While the quantitative importance of the Arg metabolic products in the mammary tissue remains to be assessed, the information available to date has allowed for a better understanding of the significant production of Pro in milk. Milk-borne Pro is utilized by neonatal enterocytes to produce citrulline, which is converted to Arg both locally and in the kidneys (Wu 1997; Wu et al. 2009). This helps ameliorate, in part, Arg deficiency in the milk of most mammals, including humans and sows.

Glutamate and glutamine (and aspartate and asparagine)

Glutamate and Gln are major metabolic fuels for the mammalian small intestine (Rezaei et al. 2013a; Wu 1998) and represent the most abundant free and protein-bound AA in milk of all mammals (Davis et al. 1994a, b; Wu and Knabe 1994; DeSantiago et al. 1999; Ramirez et al. 2001), and these AA are considered to be essential during the first days of life of the suckling newborn (Rezaei et al. 2013b; Wu et al. 2011b). In sows and rats, mammary uptake of Glu and Gln is quantitatively the highest of all AA (Viña and Williamson 1981; Trottier et al. 1997). In sows, while Glu uptake is stoichiometrically balanced with Glu secretion in milk, the uptake of Glu accounts for less than half of Gln secretion in milk (Trottier et al. 1997). By contrast, dairy cow and goat mammary uptakes of Glu and Gln, as well as Asp and Asn, are low and significantly less than (i.e., <50 %) their secretions in milk protein (Guinard and Rulquin 1994a; Mepham and Linzell 1966), indicating that these AA must be synthesized in the gland. In the sow mammary, despite the large uptake of Glu, Li et al. (2009) observed significant mammary synthesis of Glu, a portion of which is derived from Ile and Val carbon chains. In porcine mammary epithelial cells, there is a lack of glutaminase to convert Gln to Glu, and a lack of Pro oxidase that catabolizes Pro to Gln (O'Quinn et al. 2002). Glutamine is subsequently amidated, however, to form Gln by the cytosolic Gln synthetase (GS), and then used for protein synthesis or secreted into the mammary alveolar lumen (Li et al. 2009). Alternatively, Glu can be transaminated either by the enzyme Glu oxaloacetate transaminase (GOT) to form Asp, or by the enzyme Glu pyruvate transaminase (GPT) to form Ala (Li et al. 2009). Nevertheless, Gln synthesis predominates over Ala and Asp, suggesting that Glu transamination represents a minor pathway in porcine mammary tissue during lactation (Li et al. 2009). Similarly, rat mammary gland lacks the glutaminase enzyme (Watford et al. 1986), and therefore BCAA transamination is also a major contributor to Glu in rat milk (Matsumoto et al. 2013).

Phenylalanine and tyrosine

In balance studies across the lactating mammary gland, the uptake of Phe is generally balanced with its secretion in milk proteins (Guinard and Rulquin 1994a; Trottier et al. 1997; Bequette et al. 1999). An observation that cultured bovine mammary tissues do not require Tyr to synthesize casein suggests that sufficient Tyr can be synthesized from Phe via the Phe hydroxylase pathway (Jorgensen and Larson 1968). However, the serum used in the culture medium might have provided a sufficient amount of Tyr for protein synthesis. Studies with the perfused sheep udder estimated that 10 % of casein-Tyr can be derived via Phe hydroxylation (Verbeke et al. 1972). Similarly, Bequette et al. (1999) estimated from stable isotope kinetics that the lactating goat mammary gland converts 5 to 9 % of extracted Phe to Tyr. Future studies are required to identify expression of Phe hydroxylase in mammary epithelial cells.

Other amino acids

Despite their importance in nutrition of the lactating sow there are no studies reporting on the metabolism of Thr and Trp in mammary tissue. Trottier et al. (1997) reported a negative net retention of His by the sow mammary gland during lactation, indicating that His may be produced by the mammary tissue. However, small peptides containing His should be analyzed to calculate their contribution to His in the lactating gland. The presence and activity of the zinc metalloenzyme carnosinase, responsible for hydrolysis of the dipeptide carnosine, or the presence of carnosine itself, have not been reported so far in mammary tissue.

Although there is growing interest in glycine nutrition in young mammals (Wang et al. 2013, 2014a, b), little is known about the metabolism of this AA in mammary tissue.

Amino acid transport across the mammary epithelial cell

The ability of AA to enter into mammary cells depends on both the affinity of a specific AA to a transporter domain (i.e., as defined by the K_m), and the number and activity of functional transporters on the cell membrane (i.e., as defined by the V_{max}; Souba and Pacitti 1992). It has been long known that the capacity of the mammary gland for AA transport is upregulated during lactation, as shown by an increase in V_{max} in the mammary tissue of lactating compared to that of pregnant mice (Verma and Kansal 1993). It was also reported much earlier that milk accumulation decreased A-V differences of AA across the mammary gland in lactating rodents (Viña and Williamson 1981), while the uptake of amino-isobutyric acid, an AA analog, decreased in mammary explants obtained from glands not suckled for 24 h compared to that of suckled glands (Shennan et al. 1994). Shennan et al. (1994) and Sharma and Kansal (1999, 2000) observed that specific AA transporter systems were upregulated by prolactin and insulin at the onset of lactation. The intracellular availability of dietary AA is controlled by a coordinated activity of AA transport systems and their respective carrier proteins. Such AA transporters are located in the cellular membrane and are responsible for channeling AA from the arterial blood across the cell membrane and within membranes of organelles (Devés and Boyd 1998; Palacín et al. 1998; Shennan and Peaker 2000; Bröer 2008). Regulation of AA transport is complex because many transporters not only handle multiple AA, but also co-transport them in and out of the cells (Shennan and Peaker 2000). Amino acid transporters have been classified into systems based on their transport mechanism (energy and non-energy dependent) and substrate specificity (Hyde et al. 2003; Bröer et al. 2004; Hundal and Taylor 2009). Energy-dependent AA carriers function as secondary active transporters, promoting the concentrative uptake and intracellular accumulation of specific AA by coupling AA transport to the inward movement of Na^+ (Fig. 2). Non-energy-dependent AA transporters are Na^+ independent and function as tertiary exchangers, moving AA in opposite directions; such transporters facilitate the uptake of extracellular AA in exchange for cytoplasmic AA accumulated via secondary active transporters (Hundal and Taylor 2009). Similar to the active transport processes, the facilitated diffusion for AA transport is stereospecific, and unlike the active transport processes, requires no metabolic energy; thus AA transport is passively mediated by uniport transporters down concentration or electrochemical gradient, and can operate bidirectionally (Hundal and Taylor 2009). The amino acid transporter systems and proteins described below are those that have been reported and characterized in mammary tissue of suids, bovids, ovids and murids.

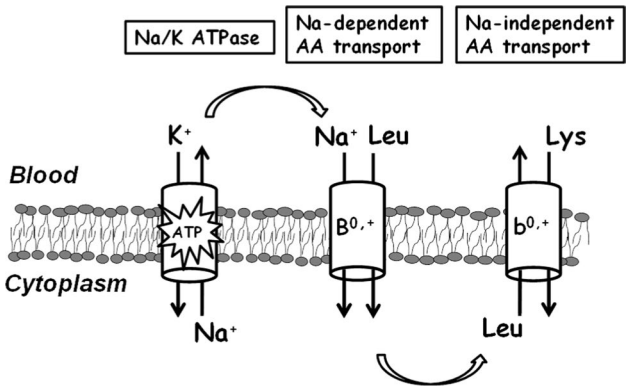

Fig. 2 Primary (Na/K ATPase), secondary (Na^+-dependent AA transporter), and tertiary (Na^+-independent AA transporter) active transport mechanisms in the cellular membrane. Secondary active transporters (e.g., System $B^{0,+}$) generate net movement of amino acids from the extracellular to the intracellular pool, whereas tertiary active transport (System $b^{0,+}$) allows for redistribution of individual amino acids without affecting total pool sizes (Modified from Hundal and Taylor 2009)

Cationic AA transporters

Proteins known to transport Lys into mammalian epithelial cells include the y^+ system (Table 1; Closs 1996, 2000; Palacín et al. 1998), two of which have been identified in the mammary gland of lactating sows, cows and rats, namely CAT-1 (*SLC7A1*) and CAT-2b (*SLC7A2*; Pérez-Laspiur et al. 2004; Aleman et al. 2009; Bionaz and Loor 2011; Manjarín et al. 2011). Transporters CAT-1 and CAT-2b are structural homologous proteins, and consist of a single polypeptide with several trans-membrane domains and glycosylation sites (Closs 1996). Studies in *Xenopus* oocytes indicate that CAT-1 and CAT-2b are Na^+-independent transporters selective for cationic AA, such as Lys, Arg and ornithine (Kim et al. 1991; Wang et al. 1991; Closs et al. 1993; Kakuda et al. 1993), although they have a weak interaction with neutral AA when Na^+ is present (Wang et al. 1991; Kakuda et al. 1993). As opposed to systems $b^{0,+}$ and y^+L, which work as AA exchangers (Chillarón et al. 1996), CAT-1 and CAT-2b promote unidirectional transport of AA into the cells (Devés and Boyd 1998), a mechanism that in human fibroblasts and oocytes is pH independent and sensitive to both membrane potential and trans-acceleration by intracellular AA concentrations (White et al.

Table 1 Transporters for basic and acidic AA identified in mammary tissue

System	Protein	Gene	Substrates[a]	Species[b]
Na^+-dependent				
$B^{0,+}$	$ATB^{0,+}$	*SLC6A14*	Lys, Arg, His, Leu, Ile, Met, Ala, Ser, Thr, Val	Pig, human, rat
y^+L	y^+LAT1/4F2hc	*SLC7A7/SLC3A2*	Lys, Arg, His, Gln, Leu, Met, Ala, Cys	Pig
	y^+LAT2/4F2hc	*SCL7A6/SLC3A2*		
Na^+-independent				
y^+	CAT-1	*SLC7A1*	Lys, Arg, His, Ornithine	Pig, human, rat, cow
	CAT-2b	*SLC7A2*		
$b^{0,+}$	$b^{0,+}$AT1/rBAT	*SLC7A9/SLC3A1*	Lys, Arg, Ornithine, Cystine, Leu	Pig
X^-_{AG}	EAAT1	*SLC1A3*	Asp, Glu	Mouse, rat
	EAAT2	*SLC1A2*		

[a] Based on data from Arriza et al. (1993), Chillarón et al. (1996), Millar et al. (1997), Devés and Boyd (1998), Closs (2002), and Bröer (2008)

[b] Species where AA transporter has been identified in mammary tissue. Based on data from Millar et al. (1997), Aleman et al. (2009), Sloan and Mager (1999), Pérez-Laspiur et al. (2004), Karunakaran et al. (2011), Manjarín et al. (2011), Bionaz and Loor (2011)

1982; Kavanaugh 1993; Nawrath et al. 2000). Studies with murine, bovine and porcine mammary glands have shown CAT-1 gene expression to increase with the onset of lactation (Aleman et al. 2009; Bionaz and Loor 2011; Manjarín et al. 2011) and to be related to changes in β-casein and α-lactalbumin mRNA abundance (Manjarín et al. 2011), and unaffected by dietary protein levels. In contrast, CAT-2b mRNA levels decreased in vivo in response to higher levels of protein intake (Pérez-Laspiur et al. 2009). Consequently, CAT-1 and CAT-2b may play a critical role in adapting to fluctuating plasma Lys levels to ensure that high intracellular Lys concentrations are maintained to keep tRNA fully charged. In addition, CAT-1 transports Arg in mammary epithelial and endothelial cells, as previously shown in endothelial cells of human umbilical vein (Bussolati et al. 1993; Sobrevia et al. 1995), bovine aorta (Ogonowski et al. 2000), and porcine pulmonary artery (Woodard et al. 1994; Mcdonald et al. 1997). As noted previously, Arg is the substrate for synthesis of the vasodilator NO (Meininger and Wu 2002; Kim and Wu 2009), and NO has been shown to increase the rate of blood flow to mammary glands in lactating goats (Lacasse and Prosser 2003), thus increasing the amount of nutrients provided to the mammary cells (Kim and Wu 2009). Accordingly, arterial Arg extraction rate by the porcine mammary gland increased during lactation (Trottier et al. 1997). Moreover, supplementing Arg or the NO donor diethylenetriamine-NO (DETA) to diets of primiparous sows enhanced litter weight gain and milk production during a 21-days lactation period (Mateo et al. 2007, 2008).

Lysine may also be transported into the mammary cells using shared systems with BCAA, such as system $B^{0,+}$, y^+L, and $b^{0,+}$, of which AA transporters $ATB^{0,+}$, y^+LAT1, y^+LAT2, and $b^{0,+}$AT (*SLC6A14*, *SLC7A7*, *SLC7A6*, and *SLC7A9*, respectively; Table 1) have been identified in porcine and human mammary tissue (Sloan and Mager 1999; Pérez-Laspiur et al. 2004, 2009; Manjarín et al. 2011). Transporter $ATB^{0,+}$ works as a secondary AA exchanger, coupling the transfer of AA to the inward movement of Na^+ and Cl^- (Van Winkle et al. 1990; Sloan and Mager 1999). Transporter $ATB^{0,+}$ has broad substrate specificity (Palacín et al. 1998), and higher affinity for neutral than for cationic AA (Sloan and Mager 1999). Studies with *Xenopus* oocytes suggest that $ATB^{0,+}$ is adaptively regulated by dietary AA, being upregulated in vitro in response to AA starvation and down-regulated by AA supplementation (Taylor et al. 1996). Expression of $ATB^{0,+}$ is also upregulated in ER^+ breast tumors in women, likely to meet the increased AA demand of tumor cells (Karunakaran et al. 2011).

System y^+L is characterized by the heteromeric Na^+-dependent transporters y^+LAT1/4F2hc and y^+LAT2/4F2hc, both composed of a catalytic light chain (y^+LAT) and a heavy subunit (4F2hc) linked by a disulfide bond (Devés et al. 1992; Torrents et al. 1998). In polarized epithelia such as kidney and intestinal cells, y^+LAT1/4F2hc and y^+LAT2/4F2hc are located in the basolateral cell membrane, and function as obligatory asymmetric AA exchangers, favoring the release of cationic AA in exchange for extracellular neutral AA and Na^+ (Chillarón et al. 1996; Kanai et al. 2000). In the absence of Na^+, only cationic AA are channeled in and out of the epithelial cells (Devés et al. 1992). Transporter y^+LAT1/4F2hc is mainly expressed in renal and intestine epithelial cells, whereas y^+LAT2/4F2hc has a wider tissue distribution, including brain, heart, testis, kidney, small intestine and parotids (Bröer et al. 2000a). Transporter rBAT/$b^{0,+}$AT is a heteromeric AA transporter composed of a catalytic light subunit named $b^{0,+}$AT (light chain) and the covalently associated type II glycoprotein

Table 2 Neutral amino acid transporters identified in mammary tissue

System	Protein	Gene	Substrates[a]	Species[b]
Na^+-dependent				
A	SNAT2	*SLC38A2*	Ala, Ser, Gly, Pro, Cys, Gln	Rat, mouse[c], cow[c]
ASC	ASCT1	*SLC1A4*	Ala, Ser, Gly, Val, Thr, Cys, Gln	Pig, human, rat, cow
	ASCT2	*SCL1A5*		
Na^+-independent				
L	LAT1/4F2hc	*SLC7A5/SLC3A2*	Ala, Ser, Val, Thr, Leu, Ile, Met, Phe, Tyr, Trp, His	Mouse, rat, cow
	LAT2/4F2hc	*SLC7A8/SLC3A2*		
T	TAT1	*SLC16A10*	Phe, Tyr, Trp	Mouse

[a] Based on data from Sharma and Kansal (2001), Bröer (2008)

[b] Species where AA transporter has been identified in mammary tissue. Based on data from Aleman et al. (2009), Shennan and Peaker (2000), Shennan et al. 2002, Pérez-Laspiur et al. (2004), López et al. (2006), Bionaz and Loor (2011)

[c] Undefined isoform, only identified as System A. Based on data from Baumrucker (1985), and Sharma and Kansal (1999)

heavy subunit (heavy chain) rBAT, linked by a disulfide bridge (Chillaron et al. 2001; Dave et al. 2004). Similar to y^+L system, $b^{0,+}$AT is distributed ubiquitously in the organism, such as kidneys, heart, liver, placenta, and lungs (Wagner et al. 2001). In addition, $b^{0,+}$AT is Na^+-independent and localized only to the apical pole of the intestinal and kidney cells (brush border membrane), where $b^{0,+}$AT mediates the exchange of cationic AA (influx) for neutral AA (efflux) with a stoichiometry of 1:1 (Chillarón et al. 1996; Bauch et al. 2003). As a result, rBAT/$b^{0,+}$AT and y^+LAT1/4F2hc may act as a functional unit in kidneys and intestinal cells, to reabsorb cationic AA and Cys from the lumen in exchange for neutral AA (Chillarón et al. 1996; Bauch et al. 2003; Sperandeo et al. 2008). Whether y^+L and $b^{0,+}$ systems work as an unit in mammary tissue remains to be elucidated.

Anionic AA transporters

System X^-_{AG} is a Na^+-dependent transporter with high affinity for Asp and Glu (Table 1). There are 5 different isoforms: EAAT1 or GLAST (*SLC1A3*), EAAT2 or GLT-1 (*SLC1A2*), EAAT3 or EAAC1 (*SLC1A1*), EAAT4 (*SLC1A4*) and EAAT5 (*SLC1A5*). Studies with mice (Kansal et al. 2000) and rats (Millar et al. 1996) indicate the presence, on the mammary tissue, of a Glu and Asp transporter with similar characteristic to system X^-_{AG}. Such transporter is likely localized to the basolateral membrane of mammary epithelial cells (Millar et al. 1996), and is able to function as an exchanger and a co-transporter (Millar et al. 1997). Likewise, two different studies have shown both EAAT1 and EAAT2 to be expressed in the mammary tissue of lactating rats (Martínez-Lopez et al. 1998; Aleman et al. 2009), and EAAT2 seems to be responsible for the basal transport of Glu into the mammary cells (Martínez-Lopez et al. 1998). None of these transporters, however, is regulated at the mRNA level in rat mammary tissue during pregnancy, lactation and post-weaning (Aleman et al. 2009).

Neutral AA transporters

Proteins known to facilitate neutral AA transport into mammary epithelial cells are classified into 4 different systems (Table 2): Na^+-dependent systems A (SNAT2 and SNAT4) and ASC (ASCT1 and ASCT2), and Na^+-independent systems L (LAT1 and LAT2) and T (TAT1). Transporters ASCT1 (*SLC1A4*) and ASCT2 (*SLC1A5*) are Na^+-dependent, and have affinity for small neutral AA, such as Ala, Ser and Cys (Utsunomiya-Tate et al. 1996). These transporters have been identified in porcine (Pérez-Laspiur et al. 2004), murine (Aleman et al. 2009) and bovine mammary glands (Bionaz and Loor 2011). Although these transporters do not contribute to the net transport of neutral AA across the apical membrane due to an obligatory exchange of AA (Bröer et al. 2000b), gene expression of ASCT2 in the mammary gland of sows (Pérez-Laspiur et al. 2009) and of ASCT1 in the mammary gland of rats (Aleman et al. 2009) increased in vivo during lactation, suggesting a role in regulation of mammary gland AA uptake, such as the equilibration of the neutral AA pool in the cytoplasm (Bionaz and Loor 2011).

System A is a Na^+-dependent transporter with high affinity for short-chain neutral AA (i.e., Ala, Gly, Ser) and low affinity for Thr and BCAA (Barker and Ellory 1990; Sharma and Kansal 1999). It has been identified in the mammary tissue of mice (Neville et al. 1980; Sharma and Kansal 1999), rats (López et al. 2006) and cows (Baumrucker 1985) during pregnancy and lactation. System A has several isoforms designated as SNAT1 (*SLC38A1*), SNAT2 (*SLC38A2*) and SNAT4 (*SLC38A4*), although only SNAT2 has been described in mammary tissue (López et al. 2006). Studies in rats indicate that SNAT2 activity and expression are upregulated by a decrease of AA substrates in the medium (Tovar et al. 2001). In addition, SNAT2 appears to be regulated by hormones, with an increase in mRNA abundance from pregnancy to lactation in mouse mammary

tissue in vivo, and in the presence of estradiol and prolactin in vitro (López et al. 2006).

System L is characterized by the heteromeric Na^+-independent transporters LAT1/4F2hc and LAT2/4F2hc, composed of a catalytic light chain (LAT) and a heavy subunit (4F2hc) linked by a disulfide bond (Chillaron et al. 2001; Palacín and Kanai 2004). Both transporters have been identified in the mammary glands of lactating rats, cows and mice (Baumrucker 1985; Shennan et al. 2002; Rudolph et al. 2007; Bionaz and Loor 2011). Transporter LAT1 has affinity for BCAA and aromatic AA (Kanai et al. 2000), while LAT2 has a broad substrate specificity including all neutral AA except Pro (Pineda et al. 1999; Segawa et al. 1999). Such versatility makes system L as one of the most important mechanisms for the uptake of neutral AA in lactating mammary tissue (Shennan et al. 2002). Studies with lactating rats indicate that both LAT1/4F2hc and LAT2/4F2hc transporters are localized in the basolateral membrane of the mammary epithelium (Shennan et al. 2002), where they function as obligatory AA exchangers (Pineda et al. 1999; Meier et al. 2002). The finding that AA uptake, but not efflux, is *trans*-stimulated suggests that system L in mammary tissue operates with asymmetrical kinetics, favoring AA retention by the mammary gland (Shennan and Peaker 2000). The LAT-1 mRNA abundance was found to be upregulated during lactation in mammary tissue of mice and rats (Rudolph et al. 2007; Aleman et al. 2009) and also in mammary tumor cells (Liang et al. 2011), likely representing a potential target for breast cancer therapy (Shennan and Thomson 2008).

Transporter TAT1 (*SLC16A10*) is a low affinity Na^+-independent aromatic AA carrier (Kansal and Kansal 1996). In contrast to LAT1 and LAT2, studies in murine kidney and intestinal tissue suggest that TAT1 mediates a unidirectional transport of AA across the basolateral membrane of the epithelial cells, hence contributing to the net influx/efflux of aromatic AA (Kim et al. 2001; Ramadan et al. 2006). In mammary tissue, TAT1 has only been described for mice (Kansal and Kansal 1996).

Evidence for interactions between AA in the mammary gland

Several ex vivo and in vivo studies support the notion that an interaction exists between cationic and BCAA for transport across the basolateral membrane of the mammary epithelial cell. In this regard, high concentrations of Leu were shown to inhibit Lys uptake and increased Lys efflux from rat mammary explants (Shennan et al. 1994; Calvert and Shennan 1996). Subsequently, a similar response was reported with mammary tissue explants collected from lactating sows (Jackson et al. 2000). Lysine also inhibited up to 67 % valine uptake by lactating sow mammary tissue explants (Hurley et al. 2000). Although the nature of these interactions between cationic and neutral AA in the mammary gland determined on mammary tissue (i.e., ex vivo) explants remains to be understood, a sequence of in vivo study clearly corroborates the ex vivo findings. Dietary over-supplementation with purified L-Lys·HCl in sow diets appeared to lead to a decrease in Val utilization (Richert et al. 1996, 1997). Conversely, Guan et al. (2002) found that dietary over-supplementation of crystalline L-Val decreases Lys transport in the mammary gland of lactating sows by stimulating Lys outward movement. A Na^+-dependent AA transporter ($hATB^{0,+}$) cloned from human mammary gland has expressed the same kinetic properties of system $B^{0,+}$ (Sloan and Mager 1999). This AA transport system has high affinity for both Lys and Val (K_m ~ 110–140 μM), is trans-stimulated, and is adaptively regulated. In the study by Guan et al. (2002), arterial plasma concentrations of Lys and Val were, respectively, 250 and 650 μM, therefore, uptake of Lys by the mammary gland might be inhibited by physiological (or high) concentrations of plasma valine through stimulation of outward trans-membrane transport of lysine.

It has been also observed that lactating sows fed diets with increasing dietary CP levels responded by decreasing mammary transport of cationic Lys and Arg and by increasing transport of BCAA (Guan et al. 2004). Finally, a reduction in dietary CP % coupled with crystalline AA inclusion lowered arterial BCAA:Lys and increased efficiency of Lys and Arg transport by the mammary gland (Manjarín et al. 2012). Similarly, in lactating dairy cows fed dietary CP above requirement, Leu oxidation by the mammary gland increased with no increase in milk protein yield (Bequette et al. 1996a, b). As discussed early, others have also demonstrated in vivo, a high capacity for BCAA oxidation by the mammary gland in rats (DeSantiago et al. 1998) and sows (Richert et al. 1997). The physiological relevance of large mammary uptake capacity and oxidation of BCAA demonstrated in vivo and ex vivo is unknown. As reported in the dairy cow, Guan et al. (2004) demonstrated that feeding excessive quantities of CP (24 %) resulted in a negligible increase (i.e., 1.6 %) in true milk protein concentration, but a significant increase in daily Leu and Ile (22 and 28 %, respectively) uptake per suckled gland compared to sows fed an 18 %-CP diet. Bequette et al. (1996a, b) suggested earlier that reducing metabolic activities, such as oxidation, not seemingly crucial for milk synthesis, might improve the efficiency of AA conversion into milk proteins.

Dietary AA supply to the dam below the requirement level depresses milk protein yield and subsequent neonatal pig growth. Likewise, excessive dietary AA supply to the dam depresses piglet growth and does so to a greater extent than that of dietary AA deficiency (King et al. 1993; Yang et al. 2000). These changes are accompanied by what

seems to be some adaptive mechanisms whereby the mammary gland modulates the extraction rates of circulating AA in response to dietary AA availability, on one hand in an attempt to meet the need of milk protein demand and on the other hand to limit the unnecessary uptake of certain AA (Guan et al. 2004). Limiting uptake of superfluous AA is, however, costly and inefficient; not only because of the metabolic processes associated with AA catabolism and N excretion, but because the mammary gland's ability to down-regulate nutrient flow and uptake. In a recent study (Pérez-Laspiur et al. 2009) using a nutritional stress model, lactating sows were fed a deficient-protein diet containing 12 % CP, an adequate diet (18 % CP) and an excess-protein diet (24 %). As expected, feeding a 12 % CP diet led to lower sow plasma IAA concentrations which directly limited milk casein yield and piglet growth; in contrast, feeding an excessive dietary CP (24 %), despite higher IAA plasma concentrations, clearly depressed milk casein yield and piglet growth. There was a clear curvilinear response of piglet growth and casein yield such that under both conditions of nutritional stress, either protein deficiency or protein excess, low milk casein yield appeared to limit piglet growth. In addition, high amounts of ammonia produced from excessive AA oxidation can impair blood flow and thus nutrient uptake into cells by increasing glutamine formation and reducing NO synthesis in endothelial cells (Wu et al. 2001). It was also shown in an earlier study that lactating sows fed diets with increasing dietary protein (7.8–23.5 % CP) respond by decreasing mammary transport of cationic (Lys and Arg) and certain neutral AA (Thr) and by increasing transport of Leu and Ile (Guan et al. 2004).

Manjarín et al. (2012) examined if a similar response was relevant in sows fed diets formulated to match an ideal AA profile using that of NRC (1998). The extraction rate of AA by the mammary gland was measured using the A-V difference approach, in response to different levels of dietary AA and CP concentrations fed to lactating sows. A diet containing 13.5 % CP and crystalline AA was formulated to both meet requirements and match AA profile reported in NRC (1998; labeled as "ideal") and a diet containing 17.5 % CP also formulated to match NRC (1998) profile (labeled as "standard"). A diet deficient in CP (9.5 %) was used to estimate additional efficiency gain of dietary protein reduction under a scenario of AA deficiency. Sows fed the ideal diet exhibited higher AA extraction rates in particular for those typically dietary limiting compared to sows fed the standard diet, indicating that the ideal-fed sows were more efficient at utilizing circulating AA presumably via decreased competitive AA inhibition uptake at the membrane interface. Thus, balanced and adequate provision of all protein-AA in diets is necessary for maximal milk production by lactating mammals. The recommended optimal values are recently provided for lactating sows (Wu 2014).

Regulation of lactation by amino acids

Transcriptional regulation of milk protein synthesis in response to lactogenic hormones has been extensively studied over the past years (Rosen et al. 1999; Plaut et al. 1989; Casey and Plaut 2007; Johnson et al. 2010; Prosser et al. 1987; Burgos et al. 2010). Only recently the effect of individual dietary AA in regulating milk protein synthesis at the translational level has become the focus of attention (Rhoads and Nogalska 2007). Numerous studies in pigs, cows, and mice have shown a dual effect of dietary AA, and especially Leu, in increasing protein synthesis, by acting both as building blocks and as anabolic factors through the mammalian target of rapamycin (mTOR) pathway (Frank et al. 2005; 2006; Moshel et al. 2006; Kimball and Jefferson 2006; Stipanuk 2007). The mTOR pathway regulates both cell growth and cell cycle progression through its ability to integrate signals from nutrients and growth factors to increase protein translation (Wang and Proud 2006, 2009; Ma and Blenis 2009). All components of the mTOR pathway have been previously characterized in the lactating mammary gland (Bionaz et al. 2007 Hayashi and Proud 2007; Hayashi et al. 2009). However, the molecular mechanisms by which dietary AA regulate the activation of mTOR and increase protein synthesis in mammary tissue are just beginning to be elucidated. In this regard, an in vitro study with murine mammary cell lines suggested a positive effect of dietary AA, and specifically Leu, on the initiation of β-lactoglobulin mRNA translation, through the activation of mTOR (Moshel et al. 2006). Similarly, Prizant and Barash (2008) indicated that Leu, Ile and Val were able to induce protein synthesis by increasing S6K1 phosphorylation in vitro in bovine and murine mammary epithelial cells, whereas addition of Lys, His, and Thr to the medium inhibited the activation of S6K1. More recently, Wang et al. (2014b) have shown that LeuRS, an aminoacyl-tRNA synthetase, may act as an intracellular Leu sensor for the mTOR pathway in bovine mammary epithelial cell lines, promoting cellular proliferation and lactation in response to changes in intracellular Leu concentrations. However, despite the growing evidence of a regulatory effect of AA in mammary gland protein translation, in vivo studies are lacking, and therefore further research is warranted to elucidate whether dietary AA can be used to improve milk protein synthesis in farm animals.

Conclusion and future directions

Over the last 20 years, discoveries of the metabolic pathways of AA in mammary tissue highlight the critical need of re-examining the AA requirement for milk production in sows in the future. In this review, we have highlighted the major pathways of AA metabolism in the mammary gland

during lactation along with the transport mechanism of AA into the mammary cells. Progress in accurate factorial estimation of AA requirements in swine using milk AA profile will depend on direct quantitative assessment of arterial AA incorporation into milk protein and (or) conversion to other end products. Furthermore, continued characterization of novel AA transporter systems across different animal species has shed light on the possible AA interactions at the cell membrane interface. It is proposed that these interactions play a role in the mechanisms governing the efficiency of mammary AA utilization for milk synthesis.

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