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Guoyao Wu *Editor*

Amino Acids in Nutrition and Health

Amino acids in systems function and health

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Contents

Amino Acids in Intestinal Physiology and Health

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Martin Beaumont and François Blachier

Abstract

Dietary protein digestion is an efficient process resulting in the absorption of amino acids by epithelial cells, mainly in the jejunum. Some amino acids are extensively metabolized in enterocytes supporting their high energy demand and/or production of bioactive metabolites such as glutathione or nitric oxide. In contrast, other amino acids are mainly used as building blocks for the intense protein synthesis associated with the rapid epithelium renewal and mucin production. Several amino acids have been shown to support the intestinal barrier function and the intestinal endocrine function. In addition, amino acids are metabolized by the gut microbiota that use them for their own protein synthesis and in catabolic pathways releasing in the intestinal lumen numerous metabolites such as ammonia, hydrogen sulfide, branched-chain amino acids, polyamines, phenolic and indolic compounds. Some of them (e.g. hydrogen sulfide) disrupts epithelial energy metabolism and may participate in mucosal inflammation when present in excess, while others (e.g. indole derivatives) prevent gut barrier dysfunction or regulate enteroendocrine functions. Lastly, some recent data suggest that dietary amino acids might regulate the composition of the gut microbiota, but the relevance for the intestinal health remains to be determined. In summary, amino acid utilization by epithelial cells or by intestinal bacteria appears to play a pivotal regulator role for intestinal homeostasis. Thus, adequate dietary supply of amino acids represents a key determinant of gut health and functions.

Keywords

Amino acids · Intestinal epithelial cells · Intracellular metabolism · Microbiota · Bacterial metabolites · Intestinal barrier

1.1 Introduction

The quantities of dietary proteins ingested every day by Humans, whatever their animal or plant origin, are vastly different according to food availability and cultural dietary habits. In Western Europe and United States for instance, protein consumption averages approximately 1.5-fold the recommended daily amount (Rand et al.

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2003; Dubuisson et al. 2010; Pasiakos et al. 2015). In sharp contrast, for instance in Southern Ethiopia, the prevalence of inadequate dietary protein intake represents as much as 94% in women (Asayehu et al. 2017).

Protein digestion in the mammalian digestive tract is globally a very efficient process, being generally higher than 90% (Bos et al. 2005; Tomé 2012); even if some dietary proteins, like for instance proteins in rapeseed, are digested with lower efficiency (Bos et al. 2007). The amino acids and oligopeptides that are released from dietary and endogenous proteins in the lumen of the small intestine are absorbed mainly in the proximal jejunum through the enterocytes by a variety of transporters present in the brush-border and baso-lateral membranes of enterocytes (Bröer 2008; Mailliard et al. 1995). The intestinal epithelium can be viewed as a selective barrier towards luminal compounds in a context of a renewal of the intestinal epithelium that is complete within a few days (Potten and Allen 1977; Potten 1997) through mitosis of pluripotent stem cells and differentiation in different phenotypes with specialized functions (Lin 2003; Barker et al. 2008; Moore and Lemischka 2006).

In this chapter, we will present how some amino acids are metabolized by the intestinal epithelial cells during their transcellular journey from the lumen to the bloodstream. The consequences of these processes for enterocyte functionality will be presented. Then, the regulatory roles of amino acids in intestinal homeostasis will be described with a focus on the gut barrier and endocrine functions. We will also give an overview on the ways by which the intestinal microbiota metabolizes amino acids; and how such metabolic capacity is linked to functional implications in both the small and large intestine. Then, we will examine how dietary amino acids have an impact on the intestinal microbiota composition. The aim of the authors is not to cover in an exhaustive way the different topics presented in this chapter, but rather to give some representative examples illustrating how amino acid and their derived compounds may have an impact on intestinal physiology.

1.2 Amino Acid Metabolism by the Intestinal Cells and Functional Implications

From experimental works performed in animal models, mostly rodents and pigs, and from more limited clinical studies with human volunteers, it appears clearly that a significant part of several dispensable and indispensable amino acids present in the small intestine content are metabolized during their journey from the luminal side of the intestinal epithelium to the portal bloodstream (Baracos 2004). The *in vitro* studies of amino acid metabolism in the small and large intestine epithelial cells generally used isolated living absorptive enterocytes (Blachier et al. 1993) and colonocytes (Cherbuy et al. 1995) for determining their metabolic capacities towards the different amino acids and their metabolites produced within the luminal content. This *in vitro* design allows to document the metabolic capacity of intestinal cells towards amino acids, but not to fully extrapolate to the *in vivo* situation when numerous substrates are present at the same time in the luminal content. A major *in vivo* experimental design used to estimate the apparent amino acid intestinal absorption and metabolism consists of measuring the amino acid concentrations at different time after a meal in both the arterial and portal blood, as well as measuring continuously the blood flow in the portal vein (Rérat et al. 1988). These experiments help to determine if a given amino acid is globally degraded (e.g. glutamine and glutamate) or produced (e.g. aspartate and alanine) in the intestinal mucosa (Blachier et al. 1999). The limitation of such experiments resides in the fact that the portal vein does not exclusively drain amino acids from the intestine, but also from several other visceral tissues. The utilization of alimentary proteins labelled with stable isotope allows for following more precisely the metabolic fate of amino acids during their intestinal absorption (Morens et al. 2003). Utilization of amino acids in intestinal epithelial cells supports not only protein and nucleotide synthesis, but also the synthesis of various compounds with important biological functions like for instance the tripep-

tide glutathione (Reeds et al. 1997). It is worth noting that in the enterocytes from the small intestine, the amino acids can be supplied from both the luminal route (notably in the post-prandial period), but also from the baso-lateral (blood) side (Windmuller and Spaeth 1975); while for the colonocytes, the amino acid supply is believed to be from the blood side exclusively (Darragh et al. 1994), even if this latter point remains somewhat controversial as some amino acid transporters have been identified on the luminal side of colonocytes (van der Wielen et al. 2017).

1.2.1 Glutamate, Glutamine, Arginine and Related Amino Acid Metabolism in Intestinal Absorptive Cells

Glutamate and glutamine are extensively metabolized in enterocytes (Darcy-Vrillon et al. 1994) and colonocytes (Darcy-Vrillon et al. 1993). Glutamate can be used in enterocytes for protein synthesis or can be extensively metabolized in other pathways including those involved in enterocyte ATP production (Blachier et al. 2009) (Fig. 1.1). Indeed, glutamine and glutamate are among the most important contributors for energy metabolism in mammalian enterocytes (Ashy et al. 1988) and colonocytes (Ardawi and Newsholme 1985). ATP production and utilization are intense in enterocytes. This corresponds to the fact that although the gastrointestinal tract represents approximately 5% of the body weight, it is responsible for around 20% of whole body oxygen consumption (Vaugelade et al. 1994; Yen et al. 1989). The intestinal epithelium presents a high energy demand (Watford et al. 1979) due to the rapid renewal of the epithelium, thus requiring intense anabolic metabolism. In addition, sodium extrusion through the Na/K ATPase activity following nutrient and electrolyte absorption is likely to represent a major ATP-consuming

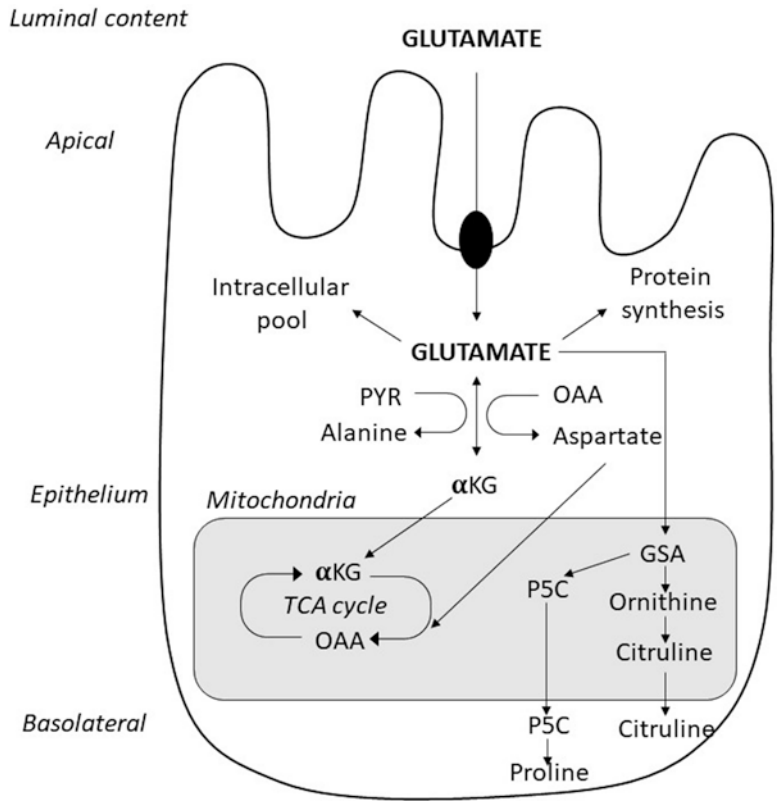

Fig. 1.1 Glutamate metabolism in intestinal absorptive cells Glutamate is metabolized to alpha ketoglutarate (alpha KG) by transamination with pyruvate (PYR) and oxaltoacetate (OAA). Alpha KG then enter the TCA cycle. Glutamate is also a precursor for the stepwise production of citrulline and proline

process in enterocytes and colonocytes (Buttgereit and Brand 1995). The metabolic steps involved in glutamate utilization in enterocytes involve transamination with oxaloacetate to produce alpha-ketoglutarate and aspartate (Fig. 1.1). Incidentally aspartate, in addition to glutamine and glutamate, represent a major fuel for the absorptive enterocytes (Windmueller and Spaeth 1976). Glutamate can also be transaminated in the presence of pyruvate to produce alanine and alpha-ketoglutarate, these latter compounds entering the tricarboxylic cycle in the mitochondria. In contrast, for glutamine oxidation, an initial conversion of glutamine into glutamate and ammonia by the phosphate-dependent glutaminase activity has to proceed in the mitochondria of enterocytes (Pinkus and Windmueller 1977, Duée et al. 1995) (Fig. 1.2).

Glutamate, together with cysteine and glycine, are the amino acid precursors for the synthesis of glutathione in mammalian cells including intestinal epithelial cells (Coloso and Stipanuk 1989); and inhibition of mucosal glutathione synthesis is associated with alteration of intestinal functions that can be prevented by giving glutathione monoester orally (Martensson et al. 1990). In addition to their capacity to synthesize glutathione, human enterocytes take up extracellular glutathione (Iantomasi et al. 1997). Glutathione in intestinal mucosa appears to derive largely from the metabolism of enteral glutamate (Reeds et al. 1997). The ratio of reduced to oxidized glutathione is an important parameter for fixing the intracellular redox status and controlling the intracellular concentrations of both oxygen-reactive and nitrogen-reactive species (Chakravarthi et al. 2006; Kemp et al. 2008).

Glutamate and glutamine allow the net production of of proline (Wu et al. 1994a), ornithine (Henslee and Jones 1982), and citrulline (Wu et al. 1994b) (Fig. 1.1). Although neither ornithine nor citrulline are present in proteins, they represent important compounds for inter-organ metabolism. Ornithine that is mainly produced

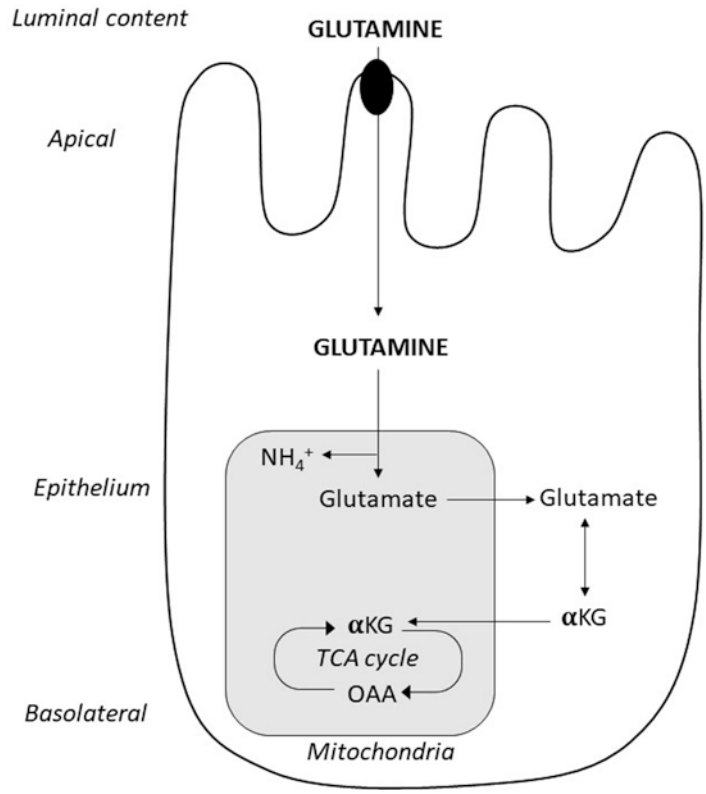

Fig. 1.2 Glutamine metabolism in intestinal absorptive cells Glutamine is converted to glutamate and ammonia. Then glutamate is converted to alpha ketoglutarate (alpha KG) that enter the TCA cycle

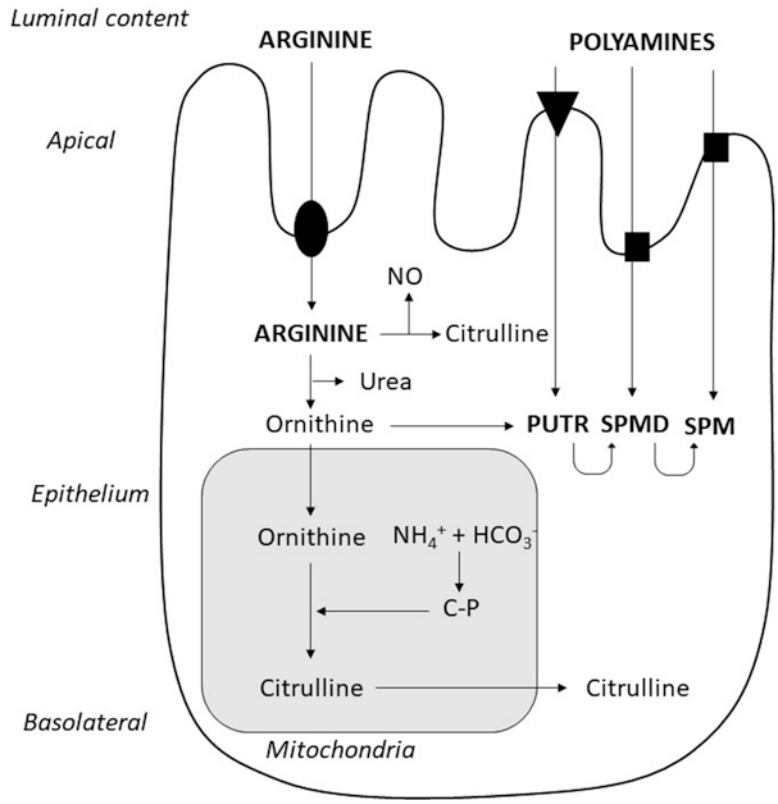

Fig. 1.3 Arginine metabolism in intestinal absorptive cells
Arginine is converted to ornithine and urea, and to a much lower extent to nitric oxide (NO) and citrulline. Citrulline can also be produced by condensation of ornithine with carbamoylphosphate (CP). A minor part of ornithine can be used for putrescine (PUTR), spermidine (SPMD), and spermine (SPM) synthesis

together with urea from arginine by the arginase activity in enterocytes (Mouillé et al. 2004), can be exported in the portal vein and be used in the liver as an intermediate in the urea cycle (Lund and Wiggins 1986). A part of ornithine released from the amino precursors is converted to citrulline in enterocytes (Blachier et al. 1991) (Fig. 1.3). Then, citrulline is released in the portal vein, and passes through the liver without major uptake, and is then used for de novo synthesis of arginine in kidneys (Cynober 1994; Dhanakoti et al. 1990). In addition, a minor part of ornithine released from arginine and glutamine can be used by enterocytes and colonocytes for the stepwise production of the polyamines putrescine, spermidine and spermine (Fig. 1.3). These amino acid-derived compounds are necessary for intestinal epithelial cells mitosis (Ray et al. 2001). However, except in the neonatal period, the endogenous production of polyamines by enterocytes and colonocytes appears barely detectable in mammals (Blachier et al. 1992; Mouillé et al. 2004), and, since the polyamine circulating concentration are below micromolar concentrations (Bartos et al. 1977), the enterocyte and colonocyte polyamine content depends almost exclusively on the polyamines in the luminal contents (Kumagai and Johnson 1988; Osborne and Seidel 1990), either from dietary or microbiota origin (detailed below) (Bardocz 1993; Blachier et al. 1991) (Fig. 1.3).

Arginine, apart from being a precursor of ornithine, is also a precursor of nitric oxide (NO) and citrulline in both enterocytes and colonocytes (Blachier et al. 2011, 1991, 1993; M'Rabet-Touil et al. 1993) (Fig. 1.3). The production of NO by the enterocytes appears to be involved in the protection of the gastrointestinal mucosa (Stark and Szurszewski 1992; Miller et al. 1993; Quintero and Guth 1992; Konturek et al. 1992; MacKendrick et al. 1993), the regulation of the intestinal motility (Calignano et al. 1992; Hata et al. 1990), and the modulation of the intestinal epithelial per-

meability (Kubes 1992, 1993). Although a limited amount of NO may play a protective role during active intestinal mucosal inflammation (Dijkstra et al. 1998; Perner and Rask-Madsen 1999; Guslandi, 1998), numerous studies reported increased production of NO in colon of patients suffering from ulcerative colitis and Crohn's disease (Guihot et al. 2000; Lundberg et al. 1994; Boughton-Smith et al. 1993; Rachmilewitz et al. 1995; Singer et al. 1996; Leonard et al. 1998; Mc Laughlan et al. 1997; Zhang et al. 1998). Excessive NO, by itself or through reactions with oxygen species (e.g. leading to the production of the oxidant peroxynitrite) is likely to play a role in the genesis of the colonic mucosa lesions as observed in inflammatory bowel diseases (Beckman and Koppenol 1996; Banan et al. 2001; Kubes and McCafferty 2000).

1.2.2 Branched-Chain Amino Acid Metabolism in Intestinal Absorptive Cells

Regarding the metabolic fate of branched-chain amino acids (i.e. leucine, isoleucine, and valine), it has been determined in the pig model that 32% of leucine in the diet is extracted by the portal-drained viscera in the first pass, with 21% of the extracted leucine being utilized for the intestinal mucosa protein synthesis (Stoll et al. 1998), the rest of leucine being presumably catabolized. Overall, 44% of total branched-chain amino acids are extracted by first-pass splanchnic metabolism in neonatal piglets (Elango et al. 2002). The catabolism of the branched-chain amino acids in enterocytes appears to imply extensive transamination and decarboxylation (Chen et al. 2009). In contrast, other essential amino acids (i.e. histidine, lysine, methionine, phenylalanine, threonine, and tryptophan) that are used for protein synthesis in the intestinal mucosa are apparently little catabolized in enterocytes (Chen et al. 2009). Metabolism of essential amino acids in colonic epithelial cells remains to be determined.

1.2.3 Sulfur-Containing Amino Acid Metabolism in Intestinal Absorptive Cells

As indicated above, cysteine is a precursor for the synthesis of glutathione, and it has been determined that between 30% and 50% of the total utilization of this amino acid by the body is devoted to the overall glutathione synthesis (Fukagawa et al. 1996; Malmezat et al. 1998). If we consider the utilization of sulfur-containing amino acids in enterocytes, the net portal balance for methionine represents as much as 48% of intake in piglets, suggesting that a relatively large part of the dietary methionine is consumed by the portal-drained viscera for protein synthesis and catabolism (Stoll et al. 1998). More precisely, the piglet gastrointestinal tract consumes approximately 20% of the dietary methionine (Riedijk et al. 2007). Cysteine, fed enterally or parenterally appears effective for sparing dietary methionine (Shoveller et al. 2003). In the neonatal piglet model, sulfur-containing amino acid deficiency results in small intestine atrophy with lower goblet cells and lower glutathione intestinal content (Bauchart-Thevret et al. 2009). These effects were associated with upregulation of the intestinal methionine cycle activity. Furthermore, in young pig, the gastrointestinal tract appears to be a site for whole-body transmethylation and transsulfuration, these two metabolic pathways being responsible for a majority of methionine utilization by the gastrointestinal tract (Riedijk et al. 2007). However, as previously said, despite large utilization by the intestine, methionine is little catabolized in enterocytes suggesting that this amino acid may be substantially consumed in other cells of the portal-drained viscera, and/or by the intestinal microbiota.

1.2.4 Threonine Metabolism in Intestinal Absorptive Cells

As noted above, the metabolic capacity of enterocytes for threonine catabolism is close to the limit of detection (Chen et al. 2009). The intestinal mucins are glycoproteins very rich in threonine

(Fogg et al. 1996). In a model of experimental colitis, the intestinal inflammation increases the gastrointestinal uptake of threonine and mucin synthesis (Rémond et al. 2009). Dietary threonine extraction by the small intestine is likely to reduce threonine availability for other tissues when mammals are fed a diet marginally deficient in threonine (Hamard et al. 2009). Interestingly, a moderate threonine deficiency was responsible for an alteration of the intestinal functionality in terms of paracellular permeability (Hamard et al. 2010). The high rate of utilization by the intestinal mucosa appears largely due to the incorporation of this amino acid in the proteins of the mucosa, notably in the proteins secreted by the mucous (goblet) cells (Schaart et al. 2005).

1.2.5 Lysine and Phenylalanine Metabolism in Intestinal Absorptive Cells

In the piglet model, when expressed as a percentage of the enteral tracer input, it has been determined that the first-pass metabolism of lysine is substantial, averaging 35% (Stoll et al. 1998). However, only 18% of what is used in the first-pass metabolism is recovered in the intestinal mucosa proteins. This may be due to lysine utilization by the microbiota. However, there are also evidences in favor of *de novo* synthesis of lysine by the intestinal microbiota (Torrallardona et al. 1996; Backes et al. 2002). However, the net result of lysine production and utilization by the intestinal microbiota in different contexts remains to be determined (Davila et al. 2013). Interestingly, it has been shown that dietary lysine used by the portal-drained viscera is driven by its luminal bioavailability; and this utilization is stimulated immediately after meal ingestion (Bos et al. 2003).

In the piglet model, when expressed as a percentage of the enteral tracer, a marked first pass metabolism of phenylalanine is measured averaging 35%, with 18% of what is used in the total first-pass metabolism being recovered in mucosal proteins (Stoll et al. 1998).

1.3 Regulatory Roles of Amino Acids in Endocrine and Intestinal Barrier Functions

1.3.1 Amino Acids and Enteroendocrine Function

Enteroendocrine cells are one type of polarized differentiated intestinal epithelial cells. These cells that are the hormone-producing cells of the intestine, represent not more than 1% of the intestinal epithelial cells, and are present all along the gastro-intestinal tract, where they are located in the intestinal villi, but also in the crypts (Janssen and Depoortere 2013). The hormones secreted by the entero-endocrine cells present a broad spectrum of physiological effects. For instance, the effects of cholecystokinin (CCK) include stimulation of endocrine pancreas secretion (Hermansen 1984), intestinal motility (Meyer et al. 1989), regulation of gastric emptying (Liddle et al. 1986), and food intake (Lo et al. 2014). A wide range of luminal compounds, such as nutrients (Furness et al. 2013), bacterial metabolites (e.g. short-chain fatty acids (Christiansen et al. 2018)), and microbial components (Bugunovic et al. 2007; Lebrun et al. 2017) are able to stimulate the expression and the secretion of gut enterohormones. Among these compounds, protein hydrolysates and amino acids are known to stimulate the release of CCK through numerous type of receptors (Choi et al. 2007). The aromatic amino acids phenylalanine and tryptophan have been identified as the most effective for increasing CCK release (Hira et al. 2008; Liou et al. 2011; Wang et al. 2011). The taste receptor T1R1 and T1R3 expressed in CCK-secreting cells have been shown to be implicated in the CCK secretion in response to phenylalanine, leucine, and glutamate (Daly et al. 2013). In the small intestine, amino acids sensing by enteroendocrine cells via G protein-coupled receptors (GPCR) such as calcium-sensing receptor (CaSR) and GPR142 induces the releases of hormones such as glucagon-like peptide 1 (GLP1) (Gribble and Reimann 2016).

1.3.2 Amino Acids and Intestinal Barrier Function

The intestinal mucosa is a physicochemical and immunological barrier against luminal antigens and enteric pathogens. Besides their role as building blocks for protein synthesis, amino acids regulate critical functions of the intestinal barrier such as epithelial permeability, tight junction formation, antimicrobial peptides secretion, mucus production and innate immune responses (Vidal-Lletjos et al. 2017; Coëffier et al. 2010). Herein, we summarize the main effects on the gut barrier of amino acids individually or in combination (Fig. 1.4).

Tryptophan plays a key role in mucosal homeostasis, as exemplified by the detrimental effects of a tryptophan deficient diet in a colitis mouse model, notably through the down regulation of multiple antimicrobial peptides in a mammalian target of rapamycin (mTOR)-dependent manner (Hashimoto et al. 2012). Beneficial effects of dietary tryptophan supplementation were observed in a porcine model of colitis, with a reduction of intestinal permeability and of pro-inflammatory cytokine production (Kim et al. 2010). In a mouse model of colitis, tryptophan supplementation also prevented intestinal inflammation through activation of the aryl hydrocarbon receptor (AhR) (Islam et al. 2017). Indeed, several tryptophan catabolites produced by the gut microbiota (detailed bellow) activate the AhR pathway that is a master regulator of the gut barrier function (Agus et al. 2018). Tryptophan supplementation also upregulated the gene expression of AhR target genes and downregulated the expression of interleukin 8 in piglets (Liang et al. 2018). Moreover, some of the beneficial effects of tryptophan for gut health might be related to its metabolism by epithelial indoleamine 2,3 oxygenase (IDO) that produces the immune regulator metabolite kynurenine (Agus et al. 2018).

The beneficial effects of glutamine for mucosal homeostasis have been demonstrated by numerous studies. Glutamine limits intestinal inflammation by downregulating the production of cytokines by immune (macrophages, lymphocytes) and epithelial cells notably through nuclear factor-κB (Nf-κB) pathway inhibition (Achamrah et al. 2017). Additionally, glutamine prevents oxidative stress by regulating intracellular glutathione (Coëffier et al. 2010). Glutamine also regulates the epithelial permeability through an upregulation of the

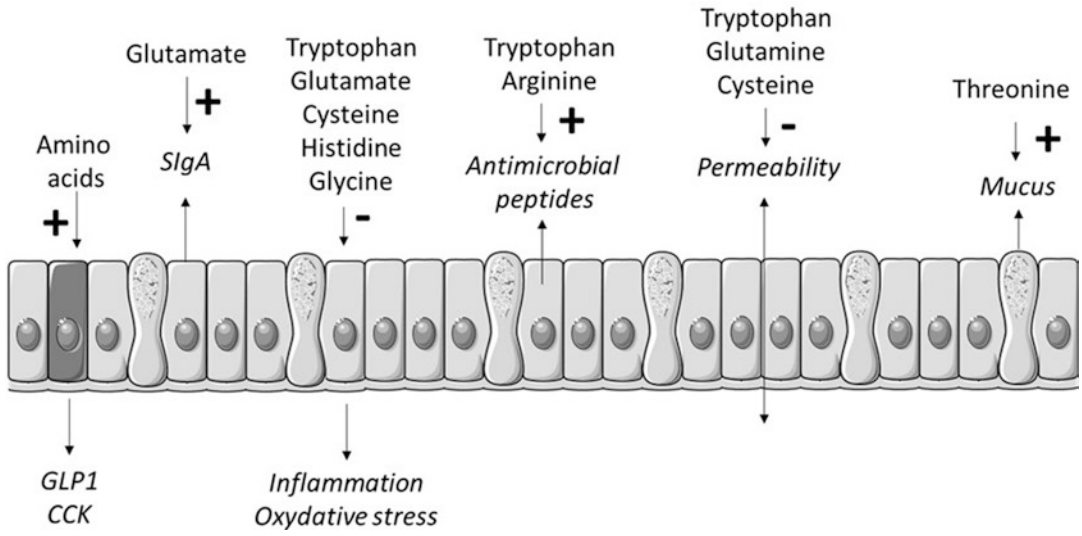

Fig. 1.4 Main effects of amino acids on the intestinal epithelium
The effects of amino acids (either positive or negative) are indicated in regards (left to right) to hormone secretion, innate immune functions, mucosal inflammation, antimicrobial peptide secretion, epithelial permeability, and mucus secretion

expression of tight junction proteins such as occludin, claudin-1, zonula occludens-1 (Achamrah et al. 2017). Glutamine supplementation in mice upregulated the expression of Toll-Like receptor 4 (*Tlr4)* and pro-inflammatory cytokines in the ileum, while in the jejunum, glutamine upregulated the expression of *Mucin 4* and of several antimicrobial peptides (Ren et al. 2014a). These results suggest that the effects of glutamine supplementation on immune responses are dependent of the intestinal segment considered. It remains to be investigated whether this activation of immune response in the mucosa, depending on the overall context, is beneficial or not for gut health. Another study in mice showed that glutamine supplementation increased the number of immunoglobulin A (IgA)+plasma cells, upregulated the expression of the polymeric immunoglobulin receptor (*Pigr*), and increased IgA secretion in intestine of mice (Wu et al. 2016). These effects were associated with an upregulation of the expression of genes involved in plasma cells maturation (TGF-B proteins, Th2 cytokines, BAFF and APRIL), but were not observed after an antibiotic treatment, suggesting a potential involvement of the microbiota in the regulation of IgA secretion by glutamate.

Endotoxemia and sepsis have been shown to represent situations of markedly impaired glutamine metabolism in intestine. Endotoxemia that is defined as the presence of endotoxin in blood may result from a transfer of a pathological amount of endotoxin (also called bacterial lipopolysaccharide, LPS) from the intestinal lumen to the bloodstream due to impaired gut selective barrier function. Major endotoxemia can lead to sepsis that is characterized by a whole-body inflammatory state (Tsiotou et al. 2005). Sepsis shock can lead to multiple organ dysfunction syndrome (Nardi et al. 2013; Venkatesh et al. 2013). In critically ill patients, the gastrointestinal tract is believed to play a central role in the pathogenesis of septic shock (Hassoun et al. 2001; Swank and Deitch 1996). Indeed, increased gut permeability and bacterial translocation play an active role in multiple organ failure by inducing a vicious cycle of increased intestinal permeability, leading to increased transfer of luminal compounds in the bloodstream (Deitch et al. 1987; Hassoun et al. 2001). In septic patients, the sodium-dependent glutamine transport is decreased in both jejunum and ileon (Salloum et al. 1991). Furthermore, gut glutamine and oxygen consumption are markedly diminished in such patients (Souba et al. 1990). In rats receiving an intraperitoneal injection of LPS, transport measurement indicated a decreased activity in the jejunum of the sodium-dependent glutamine uptake and glutaminase activity (Salloum et al. 1991; Souba et al. 1990; Haque et al. 1997), suggesting less glutamine being available for enterocyte metabolism. In the model of sepsis provoked experimentally by caecal ligation and puncture, the capacity of enterocytes for oxidation and the intestinal mucosa glutaminase activity are decreased (Ardawi et al. 1990), with a concomitant negative nitrogen balance. Although the mechanisms underlying decreased glutamine metabolism in enterocytes in septic animals remain unclear, interleukin-1 (IL-1) has been shown to act as a mediator of the alterations in gut glutamine in endotoxemia and sepsis (Augsten et al. 1991; Mester et al. 1993). However, it is worth noting that the effect of endotoxemia and sepsis on glutamine metabolism in the intestinal mucosa is likely unspecific since absorption of several other amino acids, including leucine, proline, glutamate and arginine, are also affected (Salloum et al. 1991; Abad et al. 2001; Gardiner et al. 1995; Sodeyama et al. 1993). Indeed, following endotoxemia, almost all circulating amino acids are markedly decreased suggesting a marked decrease of the intestinal functions, notably the function of absorption (Boutry et al. 2012), even if the associated anorexia may contribute to the decrease of amino acid concentration in blood.

Several other amino acids showed protective effects for the intestinal mucosa when tested individually. Arginine can modulate the intestinal immune response through regulation of nitric oxide production, polyamine synthesis or by upregulating the expression of antimicrobial peptides (Coëffier et al. 2010). In mice, arginine supplementation upregulated the expression of *Tlr4*, pro-inflammatory cytokines, and antimicrobial peptides in the ileum (Ren et al. 2014b). Here again, the consequences for gut health of these latter modifications remained to be deter-

mined, but could represent, depending on the context, either a reinforcement of the innate immunity or a detrimental inflammatory response. In a pig model of colitis, cysteine supplementation reduced intestinal permeability and mucosal inflammation (Kim et al. 2009). Histidine reduced histologic damages and pro-inflammatory cytokines levels in a mouse model of colitis, (Andou et al. 2009). Glycine is able to reduce the myeloperoxidase activity and pro-inflammatory cytokines in the colonic mucosa in a rodent model of colitis (Tsune et al. 2003).

The mucus layers that are part of the intestinal barrier function protect the intestinal epithelium from luminal aggression (Birchenough et al. 2015). Intestinal goblet cells, the cells responsible for mucus secretion, are polarized differentiated epithelial cells. Their density increases from the duodenum to the colon and this increase parallels the increase in the number of bacteria (Deplancke and Gaskins 2001). In the piglet model, an adequate dietary threonine consumption appears critical for the production of the intestinal mucus, and parenteral threonine supply can ameliorate different signs of threonine deficiency (Law et al. 2007). Under pathological conditions such as ileitis, threonine requirement is presumably increased to participate in the maintenance and/or the recovery of intestinal morphology and physiology (Mao et al. 2011).

Lastly, some studies reported beneficial effects of amino acids mixtures on mucosal healing after an inflammatory episode. In a rat model of colitis, dietary supplementation with amino acids highly represented in mucins (threonine, cysteine, proline and serine) increased intestinal mucus production and thus favored mucosal healing (Faure et al. 2006). In another rodent study, dietary supplementation after colitis induction with a mixture of three amino acids (glutamate, methionine and threonine) improved mucosal healing while it did not improved inflammatory parameters (Liu et al. 2013). The beneficial effects of this amino acid mixture might be related to their involvement in energy metabolism, and glutathione and mucus synthesis.

In summary, several amino acid (mainly tryptophan and glutamine) play a critical role in the maintenance of intestinal barrier. Although direct effects of amino acids are involved in their beneficial role on the mucosa, it has been recently hypothesized that the gut microbiota could mediate some of the health effects of amino acids.

1.4 Amino Acid Metabolism by the Intestinal Microbiota and Functional Implications

Although the microbiota is not abundant in the proximal part of the small intestine, and the intestinal transit is relatively rapid there (Schippa and Conte 2014; Dinning 2016), some recent data suggest that the microbiota could play a role in the utilization and production of amino acids, even if, as said above, the balance between both processes is not fully understood (Portune et al. 2016).

The situation is further complicated by the fact that a part of both endogenous and dietary proteins present in the small intestine luminal content can be transferred to the large intestine through the ileocaecal junction (Gibson et al. 1976). In the large intestine, the microbiota, thanks to its protease and peptidase activities, degrade these undigested or not fully digested proteins in peptides and amino acids (Portune et al. 2016). Amino acids are not believed to be absorbed by the large intestine epithelium to any significant extent, except during a short period following birth (Fuller 2012; van der Wielen et al. 2017). Then, amino acids are used by the intestinal microbiota for its own protein synthesis, and for utilization in catabolic pathways that generates numerous metabolic end products (Libao-Mercado et al. 2009, Dai et al. 2010, Blachier et al. 2007). Some of these metabolites are absorbed through the colonic epithelium, and during this process can be further metabolized giving rise to the production of co-metabolites (i.e. bacterial metabolites that are modified by the host (Rajani and Jia 2018)). Some of these amino acid-derived bacterial metabolites and co-metabolites have been shown to exert both beneficial and deleterious effects, depending on their chemical structure

and concentrations, on the intestinal epithelial cells (Blachier et al. 2017).

1.4.1 Relevance for Gut Health of Bacterial Metabolites Produced from Amino Acids

Bacterial metabolites are key molecular intermediates between the microbiota and its host. From a quantitative point of view, complex carbohydrate (mostly dietary fibers) degradation is the most important metabolic activity of the gut microbiota, releasing the short chain fatty acids (SCFA) acetate (C2), propionate (C3) and butyrate (C4) (O'Keefe 2016). SCFA are generally considered beneficial for gut health since they contribute to epithelial energy metabolism and promote the intestinal barrier function (Koh et al. 2016). Although quantitatively less important, the metabolic output of amino acid degradation by the gut microbiota is much more diverse (summarized in Table 1.1). Bacterial catabolism of amino acids releases in the intestinal lumen ammonia, SCFA, branched chain fatty acids, hydrogen sulfide (H_2S), amines, polyamines, phenolic and indolic compounds, and compounds known as neurotransmitters. Strikingly, the greatest diversity of metabolites is observed for aromatic amino acid-derived bacterial metabolites.

Some bacterial metabolites produced by the microbiota from amino acids have been identified as metabolic troublemakers (Fig. 1.5). Although at low concentration (i.e. <40 μM), the cysteine-derived bacterial metabolite H_2S is used as an energy substrate by colonocytes and provides ATP (Goubern et al. 2007), at a higher concentration, H_2S inhibits mitochondrial respiration and inhibits SCFA oxidation, leading to an impairment of epithelial energy metabolism (Blachier et al. 2019). Similarly, the tyrosine-derived bacterial metabolite *p*-cresol impairs colonocyte mitochondrial metabolism, an effect associated with reactive oxygen species (ROS) production (Andriamihaja et al. 2015). Last, the bacterial deamination product ammonia inhibits SCFA oxidation and oxygen consumption by colonocytes (Andriamihaja et al. 2010). Collectively, these data show that some bacterial metabolites derived from amino acids degradation can severely impair energy supply in the intestinal epithelium when they reach high concentrations.

Several bacterial metabolites produced by the gut microbiota from amino acids degradation were

Table 1.1 Main metabolites produced by the gut microbiota from amino acids

Category	AA precursor	Bacterial metabolites
Branched AA	Leucine	Isovalerate
	Valine	Isobutyrate, 2-methylbutamine
	Isoleucine	2-methylbutyrate
Aromatic AA	Tryptophan	Indole, indole-3-pyruvate, indole-3-lactate, indole-3-propionate, indole-3-acrylic acid,indole-3-acetate, indole-3-aldehyde, tryptamine, skatole, serotonin
	Tyrosine	4-hydroxyphenyllactate, 4-hydroxyphenylpyruvate, 4-hdyroxyphenylpropionate, 4-hydroxyphenylacetate, 4-hydroxybenzoate, 4-ethylphenol, p-cresol, phenol, dopamine
	Phenylalanine	Phenylpyruvate, phenyllactate, phenylacetate, 3-phenylpropionate, benzoate, phenylethylamine
Sulfur containing AA	Cysteine	Hydrogen sulfide
Other AA	Proline	Valerate
	Glycine	Methylamine, acetate
	Ornithine	Agmatine, putrescine, spermidine, spermine, GABA
	Lysine	Cadaverine, acetate, butyrate
	Arginine	Putrescine, spermidine, spermine, GABA
	Alanine	Ethylamine, acetate, propionate
	Histidine	Histamine
	Threonine	Acetate, propionate
	Glutamate	Acetate, butyrate
	Aspartate	Acetate
AA deamination		Ammonia

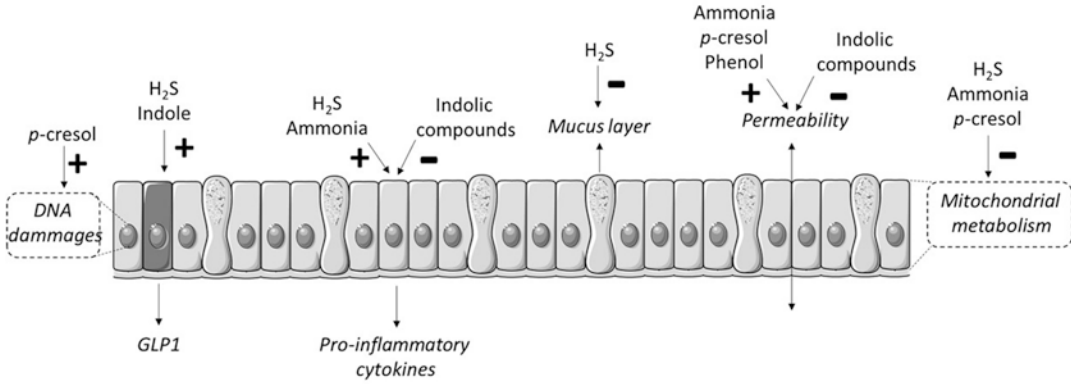

Fig. 1.5 Effects of amino acid-derived bacterial metabolites on the intestinal epithelium
The effects (either positive or negative) are indicated in regards (left to right) to DNA damages, hormone secretion, production of pro-inflammatory cytokines, mucus layer integrity, epithelial permeability, and mitochondrial energy production

found to disrupt intestinal barrier and induce inflammation (Fig. 1.5). H_2S in excess may disrupt the mucus layer through disulfur bond reduction, leading to an increased exposure of the epithelial cells to toxic luminal compounds, such as heme (Ijssennagger et al. 2015). Moreover, this metabolite upregulates pro-inflammatory genes expression in epithelial cells in a model of intra-colonic instillation in rats (Beaumont et al. 2016). The tyrosine-derived bacterial metabolites phenol and *p*-cresol increase epithelial permeability *in vitro* (Hughes et al. 2008; Wong et al. 2016). Ammonia also increases epithelial permeability and induces the expression of the tumor necrosis factor α (Villore Tudela et al. 2015). In contrast, other metabolites produced from amino acids exert protective effects for mucosal homeostasis. A mixture of branched chain fatty acids (isovalerate and isobutyrate), respectively derived from leucine and valine, dose dependently prevented the disruption of the epithelial barrier *in vitro* (Boudry et al. 2013). Interestingly, disruption of the epithelial barrier induced by *p*-cresol was prevented by 3-phenylpropionate and 3-(3-hydroxyphenyl)propionate, these bacterial metabolites being produced notably from phenylalanine or tyrosine degradation (Wong et al. 2016). The histidine and arginine-derived bacterial metabolites histamine, putrescine and spermine dose dependently inhibited interleukin-18 secretion by mice colon explants (Levy et al. 2015). Importantly, gut microbiota derived metabolites produced from tryptophan recently emerged as major regulators of intestinal gut barrier homeostasis (Agus et al. 2018, Roager and Licht 2018). Indole, the main tryptophan derived bacterial metabolite (Jin et al. 2014), strengthen the epithelial barrier *in vitro* (Bansal et al. 2010) and prevents colitis-associated mucosal damages *in vivo* (Shimada, et al. 2013). However, in order to establish the beneficial vs. deleterious effects of increased production of indole by the gut microbiota, it appears important to consider that indoxyl sulfate, a co-metabolite derived from indole is considered to contribute to renal disease progression (Ellis et al. 2018; Leong and Sirich 2016; Tan et al. 2017; Ramezani and Raj 2014).

Indole-3-acrylic acid, produced from tryptophan by *Peptostreptococcus* species, alleviates intestinal inflammation and upregulates *Mucin* 2 gene expression (Wlodarska et al. 2017). Indole-3-propionate, produced from tryptophan by *Clostridium* species, reduced intestinal permeability and inflammation through pregame X receptor (PXR) (Venkatesh et al. 2014; Dodd et al. 2017). Indole-3-aldehyde, produced from tryptophan by *Lactobacillus* species, regulates mucosal immunity through interleukin-22 production and AhR (Zelante et al. 2013). Together, the protective effects of bacterial metabolites derived from tryptophan on gut health might con-

tribute to the beneficial effects of tryptophan supplementation observed in colitis models (Hashimoto et al. 2012; Kim et al. 2010).

Interestingly, several recent reports suggest that bacterial amino acid derived metabolites could regulate the endocrine function of the gut (Fig. 1.5). The tryptophan bacterial catabolite indole regulates the secretion of glucagon-like peptide 1 (GLP-1) by enteroendocrine cells *in vitro* (Chimerel et al. 2014). Similarly, hydrogen sulfide stimulated GLP-1 secretion by L-cells (Pichette et al. 2017). If confirmed *in vivo,* these results would indicate that amino acid derived bacterial metabolites represent new compounds that could be targeted for the control of intestinal hormones secretion.

Some bacterial metabolites produced from amino acids have been shown to alter DNA integrity (Fig. 1.5). This topic is in our opinion of particular importance as long-term exposure of colonic crypt stem cells to excessive DNA-damaging agents is likely to increase the risk of unrepaired DNA lesions in these cells (Gill and Rowland 2002). To make a long and complicated story short, the cancer stem cell hypothesis propose that crypt stem cells are the cells at the origin of intestinal cancer (Vermeulen et al. 2008; Barker et al. 2009). However, whether a colorectal cancer stem cell is a transformed descendent of a normal intestinal stem cell, or whether differentiated cells can acquire a cancer stem cell phenotype upon transformation remains unknown (Yousefi et al. 2017). The mechanisms for genetic changes in colorectal cancer and their interactions with the environmental risk factors are difficult to unravel. However, recent studies have begun to better clarify how the intestinal microbiota can generate genomic changes in colorectal cancer, for instance through toxin production, metabolite synthesis, reactive species production etc. (Wang et al. 2017). Among the luminal compounds that have been identified as able to alter DNA integrity in mitochondria and nuclei, several of them appears to be bacterial metabolites derived from amino acids. The bacterial metabolite *p*-cresol that is produced from tyrosine has been shown to be genotoxic upon human colonocytes in a dose-dependent manner without any cytotoxic effects (Andriamihaja et al. 2015). Phenol that is also produced by the intestinal microbiota from tyrosine, appears to be a precursor of the mutagenic compounds p-diazoquinone after reacting with nitrite (Kikugawa and Kato 1988). Hydrogen sulfide that is a bacterial metabolite produced from cysteine was reported to be able to alter DNA integrity in intestinal colonic epithelial cells (Attene-Ramos et al. 2010). However, these results were not confirmed in further experiments, likely because of different experimental design. Indeed, using both *in vivo* colonic intraluminal instillation in rats and longer-term culture of human colonic epithelial cells with millimolar concentrations of the H_2S donor NaHS, no effect of this agent on DNA integrity was detected using the sensitive gamma H2AX genotoxicity test (Beaumont et al. 2016).

1.4.2 Effects of Amino Acid Supplementation on Microbiota Composition and Metabolic Activity

Although the effects of dietary protein on the gut microbiota composition are well described (Blachier et al. 2019), only few studies investigated the impact of individual amino acid supplementation on the microbiota composition and its metabolic activity. In weaned piglets, dietary tryptophan supplementation increased large intestine microbiome α-diversity and the abundance of the SCFA producers *Prevotella* and *Roseburia* while it reduced the abundance of *Clostrium* species and *Enterobacter* (Liang et al. 2018). Moreover, in this study tryptophan supplementation increased the colonic concentration of propionate, indole 3-acetate and tryptamine (Liang et al. 2018). In a pilot study with Humans, glutamine supplementation for 14 days decreased the abundance of the *Actinobacteria*, *Firmicutes*, *Dialister* and *Dorea* (de Souza et al. 2015). In mice, glutamine supplementation reduced the abundance of *Firmicutes* and increased *Bifidobacterium* and *Streptococcus* in the jejunum while it decreased the abundance of *Firmicutes*,

Streptococcus and *Lactobacillus* in the ileum, suggesting intestinal segments-specific effects (Ren et al. 2014a). Arginine supplementation in mice decreased the *Firmicutes* to *Bacteroidetes* ratio in the jejunum and ileum (Ren et al. 2014b). Proline supplementation during gestation in mini-pigs reduced the abundance of *Prevotella* and SCFA concentration in the proximal colon (Ji et al. 2018).

1.5 Conclusion

Amino acid metabolism by the epithelial cells and by the gut microbiota is involved in key processes of intestinal homeostasis such as epithelium renewal, gut hormone secretion, gut barrier function and immune regulations. Therefore, an adequate supply of amino acids from dietary proteins (both in quality and quantity) to epithelial cells and intestinal bacteria is a pivotal determinant of intestinal health and functions. Intestinal bacteria produce numerous metabolites from amino acids, and, although several of these metabolites have been shown to impact the intestinal epithelium metabolism and functions either in a beneficial or deleterious way, further works are required to understand better how these compounds, individually or in mixture, impact the intestinal mucosa in different situations including healthy and inflammatory states. In addition, other important questions remain unanswered, such as the relevance of essential amino acids utilization and production by bacteria for host nutrition, as well as the consequences of the modifications of the gut microbiota composition for gut health. An improved understanding of the host-microbiota crosstalk in amino acid metabolism is essential to refine the nutritional recommendations for amino acid and dietary protein intake in relationship with the maintenance or recovery of intestinal health.

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2 Amino Acid Metabolism in the Liver: Nutritional and Physiological Significance

Yongqing Hou, Shengdi Hu, Xinyu Li, Wenliang He, and Guoyao Wu

Abstract

The liver plays a central role in amino acid (AA) metabolism in humans and other animals. In all mammals, this organ synthesizes many AAs (including glutamate, glutamine, alanine, aspartate, asparagine, glycine, serine, and homoarginine), glucose, and glutathione (a major antioxidant). Similar biochemical reactions occur in the liver of birds except for those for arginine and glutamine hydrolysis, proline oxidation, and gluconeogenesis from AAs. In contrast to mammals and birds, the liver of fish has high rates of glutamate and glutamine oxidation for ATP production. In most animals (except for cats and possibly some of the other carnivores), the liver produces taurine from methionine or cysteine. However, the activity of this pathway is limited in human infants (particularly preterm infants) and is also low in adult humans as compared with rats, birds and livestock species (e.g., pigs, cattle and sheep). The liver exhibits metabolic zonation and intracellular compartmentation for ureagenesis, uric acid synthesis, and gluconeogenesis, as well as AA degradation and syntheses. Capitalizing on these extensive bases of knowledge, dietary supplementation with functional AAs (e.g., methionine, *N*-acetylcysteine, and glycine) to humans and other animals can alleviate or prevent oxidative stress and damage in the liver. Because liver diseases are common problems in humans and farm animals (including fish), much research is warranted to further both basic and applied research on hepatic AA metabolism and functions.

Keywords

Liver · Amino acids · Metabolism · Nutrition · Humans · Animals

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2.1 Introduction

The liver plays a central role in the digestion, metabolism, transport, and storage of nutrients, as well as detoxification, immunity and health (Treyer and Müsch 2013). In this organ, amino

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acids (AAs) serve as the building blocks of proteins (including such transport proteins as albumin, lipoproteins, transferrin, and retinol-binding protein); the regulators of intracellular protein turnover (protein synthesis and proteolysis); conjugators with bile acids; substrates for the syntheses of glutathione (the most abundant low-molecular-weight antioxidant in cells), taurine (essential for retinal, cardiac and skeletal muscle functions), glucose, lipids, and anti-inflammatory molecules; and protectors against toxic xenobiotics and pathogenic microorganisms (Hou et al. 2015a; Wu 2018). For example, the liver of a healthy human adult releases about 20 g albumin per day (Maxwell et al. 1990). Moreover, the liver is a major site for the metabolism of lipoproteins, such as very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL; Perez-Matos et al. 2019). Thus, in humans and other animals, abnormal metabolism of AAs in the liver results in many diseases [including edema, hepatic encephalopathy, fatty liver, hepatic injury, hepatic cirrhosis (scarring), and liver failure (a life-threatening condition)] and increases risk for liver cancer (Holm et al. 1999; Lee and Kim 2019). In all animals (including mammals, birds, fish, and shrimp), liver dysfunction also reduces their food intake, growth, and development (Wu 2020a). This issue is critical for the production of farm animals worldwide, because feed efficiency is a major factor affecting economic returns and sustainability. Furthermore, in mammalian fetuses and avian embryos, where the production of erythrocytes occurs in their livers, spleens and bone marrows, this physiological process is stimulated by fetal/embryonic liver-derived erythropoietin (Palis 2014). Therefore, research on hepatic AA metabolism has both medical and agricultural significance, and is the focus of the current work.

2.2 Anatomy of the Liver

Based on gross anatomy, the liver has four lobes: left, right, caudate, and quadrate. In animals (bears, cats, cattle, channel catfish, chickens, dogs, geese, goats, guinea pigs, hawks, humans, mice, monkeys, owls, pigs, rabbits, sheep, and zebrafish) that have a gallbladder, the liver is closely connected with the gallbladder (Oldham-Ott and Gilloteaux 1997). The latter stores bile (a mixture of water, bile salts, cholesterol and bilirubin) produced by the liver, and then releases the bile into the duodenum in response to a feeding-induced surge of cholecystokinin (a peptide secreted by enteroendocrine cells in the small intestine) in plasma (Liddle 1995). In contrast, some mammals (i.e., horses, deer, rats, seals, and laminoids), birds (e.g., pigeons, parrots and doves), certain fish (e.g., lampreys), and all invertebrates lack a gallbladder (Oldham-Ott and Gilloteaux 1997). In animals without a gallbladder, bile flows directly from the liver into the lumen of the duodenum through the bile duct. As the bile flows through the bile ducts, its composition is modified by the addition of a bicarbonate secretion from ductal epithelial cells in response to a surge of the duodenum-derived secretin.

The liver has a high rate of oxidative metabolism and, therefore, needs a large amount of oxygen. This is met by a high rate of blood flow into this organ. The liver accounts for only 2.5% of body weight (BW) but receives 20–25% of the cardiac blood output (Lautt 2010). In a healthy 70-kg human with a 1.75 kg liver, the total blood flow into the liver is 30 ml/min per kg BW or 120 ml/min per 100 g liver, with ~70–75% and 25–30% of the blood being supplied by the portal veil and the hepatic artery, respectively. Water- and lipid-soluble nutrients that are carried within the portal vein and the hepatic artery enter the liver for extraction by hepatocytes and other types of cells (Wu 2018). The nutrients that bypass the liver without uptake and metabolites released from the liver enter the inferior vena cava for utilization in extrahepatic tissues or for excretion (Lautt and Greenway 1987).

The functional unit of the liver is the hepatic acinus, which contains the terminal branches of the portal vein, hepatic arteries, and bile ducts. There are about 100,000 acini in the human liver (Lautt and Greenway 1987). Blood from the portal venule and blood from the hepatic arteriole enter hepatic sinusoids (the capillaries in the liver with

discontinuous basement membrane), where nutrients and gases are exchanged with hepatocytes and associated cells. The porous sinusoidal endothelium of the liver is permeable to molecules as large as albumin, but not to cells (e.g., red blood cells, platelets, and monocytes) (Kent and Stylianou 2011). Components of the blood plasma, including small molecules (e.g., AAs, glucose, and ammonia) and macromolecules (e.g., albumin, LDL and HDL) within the hepatic sinusoid cross its endothelium through the endothelial cells (for small molecules) and the fenestrae (porous sites between endothelial cells; for macromolecules) into the space of Disse (a location in the liver between a hepatocyte and a sinusoid; Fig. 2.1). Subsequently, these substances cross the basolateral (sinusoidal) membrane of the hepatocyte into the cell, as reported for humans, pigs, rodents, and zebrafish (Nedredal et al. 2003; Cheng et al. 2019). Their uptake into the hepatocyte is mediated through specific transporters for AAs or glucose, receptor-mediated endocytosis for albumin, LDL receptor-mediated endocytosis for LDL, and scavenger receptor class B member 1 (SR-B1)-mediated endocytosis for HDL (Kent and Stylianou 2011). Likewise, AAs, glucose, proteins (e.g., albumin, VLDL, and HDL), and some conjugated bilirubin are released by the hepatocyte across its basolateral membrane into the space of Disse, and then enter (a) the hepatic sinusoid through the fenestrae in its endothelium, and (b) the hepatic lymphatic system through its lym-

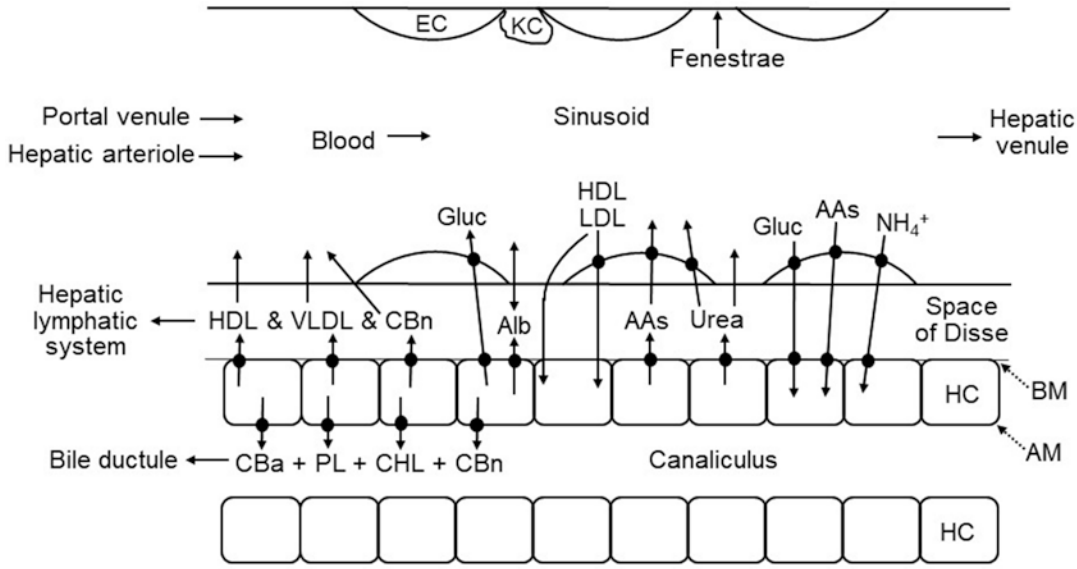

Fig. 2.1 The hepatic acinus (the functional unit of the liver) consists of the terminal branches of the portal vein and hepatic arteries for exchanges of nutrients and gases with hepatocytes and associated cells. Small molecules (e.g., AAs, glucose, and ammonia) and macromolecules (e.g., albumin, LDL and HDL) within the hepatic sinusoid (the capillaries in the liver) cross its endothelium through the endothelial cells (for small molecules) and the fenestrae (for macromolecules) into the space of Disse. Subsequently, these substances cross the basolateral (sinusoidal) membrane of the hepatocyte into the cell. Likewise, AAs, glucose, proteins (e.g., albumin, VLDL, and HDL), and some conjugated bilirubin are released by the hepatocyte across its basolateral membrane into the space of Disse, and then enter (**a**) the hepatic sinusoid through the fenestrae in its endothelium and (**b**) the hepatic lymphatic system. In contrast, glycine- or taurine-conjugated bile acids, phospholipids (e.g., phosphatidylcholine and phosphatidylethanolamine), unesterified cholesterol, and some conjugated bilirubin (a metabolite of heme) are released by the hepatocyte across its apical (canalicular) membrane into the canaliculus formed from adjacent hepatocytes. The bile flows into bile ductules and then bile ducts. The solid circle denotes a transporter, receptor-mediated endocytosis, or receptor-mediated exocytosis. *AAs* amino acids, *Alb* albumin, *AM* apical membrane, *BM* basolateral membrane, *CBa* conjugated bile acids, *CBn* conjugated bilirubin, *CHO* cholesterol (unesterified), *EC* endothelial cell, *HDL* high-density lipoprotein, *KC* Kupffer cell (macrophage in liver), *Gluc*, glucose, *HC* hepatocyte, *LDL* low-density lipoprotein, *PL* phospholipids, *VLDL* very low-density lipoprotein

phatic capillaries (Tanaka and Iwakiri 2016). The non-diffusion discharge of these substances at the basolateral membrane of the hepatocyte is mediated through specific transporters for AAs and glucose, the neonatal crystallizable fragment receptor (FcRn)-mediated exocytosis for albumin, and receptor-mediated exocytosis for VLDL and HDL, and multidrug resistance protein-3 for conjugated bilirubin (Treyer and Müsch 2013; Keppler 2014; Pyzik et al. 2017). In contrast, glycine- or taurine-conjugated bile acids, phospholipids (e.g., phosphatidylcholine and phosphatidylethanolamine), unesterified cholesterol, and some conjugated bilirubin (a metabolite of heme) are released by the hepatocyte across its apical (canalicular) membrane into the canaliculus (a duct-like structure) formed from adjacent hepatocytes (Gissen and Arias 2015). The bile salt export pump, ATP-binding cassette (ABC) transporters (particularly ABC-B4), and multidrug resistance protein-2 are responsible for the canalicular efflux of bile salts, phospholipids, and conjugated bilirubin, respectively (Keppler 2014; Khabou et al. 2017), whereas ABC-G5/ABC-G8 as a dimer (Brown and Yu 2009) and SR-B1 (Wiersma et al. 2009) transport unesterified cholesterol from the hepatocyte across its apical membrane into the canaliculus. The bile (e.g., 400–800 ml per day in an adult human) flows into bile ductules and then bile ducts. Thus, the polar structure of the hepatocyte is essential for its physiological function.

Based on oxygen supply and metabolism, the hepatic acinus is divided into three zones (Wu 2018). Zone I (periportal hepatocytes) is nearest to the venules of the portal vein and the arterioles of the hepatic artery and is the most oxygenated. Zone III (perivenous hepatocytes), which is farthest from the microvasculature of the entering blood vessels, is around the hepatic central vein and is poorly oxygenated. Zone II (midzone) is the transition zone located between zones I and III. Hepatocytes in zones I, II and III represent ~80%, 10–15%, and 5–10% of total hepatocytes, respectively, and have very different metabolic patterns (Häussinger et al. 1992; Schleicher et al. 2015). Examples are given in Table 2.1. This concept of metabolic zonation helps us understand how AAs are metabolized in the liver.

Table 2.1 Primary localization of metabolic pathways in periportal or perivenous hepatocytes of the liver[a]

Periportal hepatocytes	Perivenous hepatocytes
Amino acid (AA) metabolism	Amino acid (AA) metabolism
AA uptake and degradation (except glutamate, aspartate and histidine)	Glutamate and α-ketoglutarate uptake[b]
	Ornithine aminotransferase
Glutaminase[b]	Glutamine synthetase[b]
Histidine degradation[b]	Aspartate uptake
Urea cycle	Glutathione synthesis
Lipid metabolism	Lipid metabolism
ATP-citrate lyase	Bile acid synthesis[b]
Cholesterol synthesis	Esterification of free fatty acids
Fatty acid oxidation	Fatty acid synthesis
Ketogenesis[c]	Triacylglycerol synthesis
Hepatic lipase	Very-low-density-lipoprotein synthesis
Glucose metabolism	Glucose metabolism
Glucose release	Glucose uptake
Gluconeogenesis	Glycolysis
Glycogen degradation to glucose	Glycogen degradation to pyruvate
Glycogen synthesis from lactate & AAs	Glycogen synthesis from glucose
Other pathways	Other pathways
Carbonic anhydrase V	Carbonic anhydrase II and III
Oxidative energy metabolism	Xenobiotic metabolism and detoxification

Adapted from Wu (2018)

[a]Except for those pathways indicated with a superscript letter b, most pathways are unequally distributed along the acinus (metabolic zonation), with highest activities in the indicated hepatocytes

[b]Exclusively located in the indicated hepatocytes

[c]This pathway is highly active in most animals during starvation but is limited in pigs

2.3 Amino Acid Transporters and Uptake by the Liver

Hepatocytes express AA transport systems A, N, ASC, L, T, X^-_{AG}, y^+, and Orn/Cit exchanger, with the letters denoting their substrate specificity (Table 2.2). The liver extracts most AAs from the

Table 2.2 Amino acid (AA) transporters in the mammalian liver

Protein name	Gene name	Transport system	Transport mechanism	Major AA	Consequences of defects
EAAT3/EAAC1	*SLC1A1*	X^-_{AG}	Na^+	L-Glu, D/L-Asp, L-Cys	Metabolic disorders
EAAT2/GLT1	*SLC1A2*	X^-_{AG}	Na^+	L-Glu, D/L-Asp	Metabolic disorders
ASCT1	*SLC1A4*	ASC	Antiporter	L-Ala, L-Ser, L-Cys	Metabolic disorders
rBAT	*SLC3A1*	HC-HAAT	Exchanger	Neutral and basic AAs (heterodimer with SLC7A9)	Cystinuria
4F2hc	*SLC3A2*	HC-HAAT	Exchanger	Neutral AAs (heterodimer with SLC7A5-8 and SLC7A10-11)	Metabolic disorders
GlyT1	*SLC6A9*	Gly	Na^+/Cl^-	Gly	NKHG
CAT-1	*SLC7A1*	y^+	Uniporter	Basic AAs	Hyptertension
CAT-2	*SLC7A2*	y^+	Uniporter	Basic AAs	Intestinal inflammation
LAT2	*SLC7A8*	L	Antiporter	Large neutral AAs	Metabolic disorders
TAT1 (MCT10)	SLC16A10	T	Facilitated	Aromatic AAs (Phe, Tyr, Trp)	Metabolic disorders
VGLUT3	*SLC17A8*	VGT	Cl^-/uniporter	L-Glu	Metabolic disorders
ORC2	*SLC25A2*	Orn/Cit carrier	H^+/antiporter	Mit L-Orn/L-Cit exchange	HHH syndrome, UCD
AGC2	*SLC25A13*	Asp/Glu carrier	H^+/antiporter	L-Asp, L-Glu	Type-2 citrullinemia
ORC1/ORNT1	*SLC25A15*	Orn/Cit carrier	H^+/antiporter	Mit L-Orn/L-Cit exchange	HHH syndrome
GC1	*SLC25A22*	Glu carrier	H^+-coupled; OH^-/antiporter	L-Glu	Metabolic disorders
MGT	*SLC25A38*	Gly	Uniporter	Gly (Mit influx)	Anemia
SNAT2	*SLC38A2*	A	Na^+	Ala-preferring, neutral AAs	Metabolic disorders
SNAT3	*SLC38A3*	N	Na^+	L-Gln, L-Asn, L-His, L-Ala	Metabolic disorders
SNAT4	*SLC38A4*	A	Na^+	Ala-preferring, neutral AAs	Metabolic disorders
SNAT5	*SLC38A5*	N	Na^+	L-Gln, L-Asn, L-His, L-Ala	Metabolic disorders
SNAT7	*SLC38A7*	N	Na^+	L-Gln, L-Asn, L-His, L-Ala	Metabolic disorders
LAT3	*SLC43A1*	L	Uniporter	Large neutral AAs	Metabolic disorders
Cystinosin	*SLC66A4*	LCT	H^+-coupled	Efflux of Cys from lysosome	Metabolic disorders

Adapted from Kandasamy et al. (2018) and Wu (2013)

HC-HAAT heavy chain of heteromeric amino acid transporter, *HH* hyperornithinemia-hyperammonemia-homocitrullinuria, *LCT* lysosomal cystine transporter, *MGT* mitochondrial glycine transporter, *Mit* mitochondrial, *NKHG* non-ketotic hyperglycemia, *rBAT* related to $b^{o,+}$ amino acid transporters, *UCD* urea-cycle defect, *VGT* vesicular glutamate transporter *EAAC1* excitatory amino-acid carrier 1; *GLT1* glutamate transporter 1

blood in the portal vein and hepatic artery at different rates but cannot take up some AAs from the blood. For example, mammalian hepatocytes lack transporters for citrulline and have a limited ability to extract arginine, lysine, ornithine and histidine from the blood, whereas periportal hepatocytes do not take up glutamate or aspartate (Wu 2013). Defects of AA transporters result in metabolic disorders (including diseases) in humans and other animals (Kandasamy et al. 2018).

Using the pig as an animal model, Kristensen and Wu (2012) characterized the pattern of AA uptake by the liver in vivo. In the 60-kg growing pigs fed a 16%-crude protein diet, the liver has the net uptake of basic and neutral proteinogenic AAs from the portal vein at different proportions of their net portal-vein fluxes (Table 2.3). Generally, the rates of hepatic uptake of AAs in decreasing order are: small neutral AAs > large neutral AAs > BCAAs = basic AAs. Based on the portal and hepatic fluxes of AAs, proteinogenic AAs in the diet are classified into three groups. The first group of AAs are those AAs that are absorbed from the small intestine into the portal vein and taken up by the liver at ≥17% of their net portal fluxes: alanine, asparagine, cysteine, glycine, methionine, phenylalanine, proline, serine, tryptophan, and tyrosine. The second group of AAs are those AAs that are absorbed from the small intestine into the portal vein and taken up by the liver at ≤10% of their net portal fluxes: arginine, histidine, isoleucine, leucine, lysine, threonine, and valine. The third group of AAs are those AAs that exhibit little entry from the small-intestinal lumen into the portal vein: aspartate and glutamate. The 60-kg pig liver receives little dietary aspartate and glutamate from the portal vein as noted previously, but takes up 230 mg glutamine/kg body weight from the portal vein (ultimately the diet and arterial blood), and releases 1260 mg glutamate and 10.8 mg aspartate per kg body weight as a result of hepatic AA metabolism. Among all AAs, the percentages of hepatic uptakes of Gly and Cys from the portal vein are greatest in growing pigs, indicating the active metabolism of these two AAs in the liver. The low uptake of branched-chain AAs and basic AAs helps to maximize the availability of these AAs in the diet for utilization by extra-hepatic tissues.

Table 2.3 Net uptake of amino acids (AAs) from the portal vein or the net release of AAs by the liver of 60-kg pigs fed a 16%-crude protein diet

Group 1 AAs		Group 2 AAs		Group 3 AAs	
AA	Net uptake from the PV by the liver	AA	Net uptake from the PV by the liver	AA	Net release by the liver
Ala	52%	Arg	8%	Asp	10.8 mg/kg BW
Asn	56%	His	9%	Glu	1260 mg/kg BW
Cys	70%	Ile	9%		
Gln	230 mg/kg BW	Leu	9%		
Gly	73%	Lys	10%		
Met	17%	Thr	10%		
Phe	51%	Val	10%		
Pro	40%				
Ser	28%				
Trp	35%				
Tyr	54%				

Adapted from Wu (2018). The 60-kg pig was fed every 8 h (3 times daily) a 16%-crude protein diet. The daily feed intake of the pig was 3.6% of its body weight. Values in the table are the percentage (%) of the portal vein flux, unless specified otherwise for aspartate, glutamate, and glutamine

(+) denotes net uptake, and (−) net release

BW body weight, *PV* portal vein

Because the surgical technique to cannulate the portal vein is invasive and risky, little information is available about the uptake of AAs by the human liver. However, the analysis of arterial – hepatic venous differences in AAs revealed that the human splanchnic bed (the portal-drained viscera plus liver) extracts a large amount of alanine (likely occurring in the liver) and glutamine (likely occurring in the small intestine) but relatively a very small amount of arginine, lysine and ornithine, while releasing a significant amount of citrulline (likely occurring in the small intestine) (Felig 1975). Van de Poll et al. (2007) reported that 92% of citrulline and 88% of arginine reaching the liver from the hepatic artery and portal vein were not extracted by the liver of fasting patients with colorectal metastases. Similar

results were obtained from studies with patients undergoing a pylorus-preserving pancreaticoduodenectomy (Neis et al. 2017).

2.4 Amino Acid Metabolism in the Liver

The liver is the major organ to degrade AAs, although its ability to catabolize branched-chain AAs is limited in mammals and birds (Brosnan 2003). Interestingly, in humans and other terrestrial animals consuming optimal amounts of AAs, only a small amount of AAs is degraded in their livers. For example, in 60-kg pigs fed a 16%-crude protein diet, all the ammonia absorbed into the portal blood is removed during its single passage through the liver, and this organ takes up a small amount of ammonia released from peripheral tissues (Kristensen and Wu 2012). These authors have also reported that 95% of the urea-N released from the liver is accounted for by the hepatic uptake of ammonia from the portal vein and the hepatic artery in 60-kg pigs. In other words, only 5% of the urea-N released by the liver is provided from hepatic AA degradation when the pigs are fed a balanced diet. This is consistent with a low rate of hepatic gluconeogenesis in fed pigs (Wu 2018).

2.4.1 Gluconeogenesis

In mammals, the liver is a major organ to synthesize glucose from substrates with ≥3 carbons during the post-prandial period, such as glucogenic AAs (e.g., alanine, asparagine, glutamine, serine and phenylalanine), fructose, lactate, pyruvate and glycerol via the gluconeogenesis pathway in mammals, including humans and pigs (Wu 2018). This metabolic pathway also requires ATP and NADH. In fasting mammals, the liver and kidneys contribute to almost equal amounts of glucose to the blood to meet the requirement for glucose (e.g., 125 g/day in a 70-kg human or 74.4 mg/h per kg BW) by the brain, red blood cells, immunocytes, eyes, kidneys, and other cell types (Jungas et al. 1992; Møller et al. 2000). In 30-kg overnight-fasted pigs, the whole-body production of glucose is 133 mg/h per kg BW (Iozzo et al. 2006). For comparison, the rates of glucose synthesis in nonpregnant, pregnant, and lactating fed sheep are 87, 110, and 218 mg/h per kg BW, respectively (Wu 2018). In nonruminant mammals, alanine is the principal AA extracted by the liver, accounting for 25% and 33% of total hepatic AA extraction, respectively, in the post-absorptive and fed state (Brosnan et al. 2001). In gestating sheep, serine is likely the major glucogenic substrate in the liver (Kwon et al. 2003).

Hepatic gluconeogenesis is regulated by hormones as well as the availability of substrates, ATP, NADH and fructose-2,6-bisphophate. Catecholamines, glucagon and glucocorticoids stimulate hepatic synthesis of glucose from AAs and other substrates (e.g., lactate and glycerol) via: (a) activating the cAMP-dependent signaling decreasing the intracellular concentration of fructose-2,6-bisphophate, as occurring during intensive exercise (the short-term mechanism); (b) enhancing the long-term expression of key genes related to the pathway [e.g., phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase)], as occurring in diabetes and fasting (the long-term mechanisms); and (3) acting on extrahepatic tissues (e.g., skeletal muscle and white adipose tissue) to increase the release of glucogenic substrates (both short- and long-term mechanisms; Hatting et al. 2018). In contrast, insulin inhibits this hepatic metabolic pathway through the opposite modes of actions. In addition to these mechanisms, the oxidation of fatty acids to provide ATP plays a crucial role in hepatic glucose synthesis, as demonstrated by hypoglycemia in infants lacking carnitine palmitoyltransferase-I, a key enzyme responsible for the transfer of long-chain acyl-CoA from the cytosol to the mitochondrion for biological oxidation (Collins et al. 2010). Furthermore, whether or not AAs are used for glucose synthesis in hepatocytes depends on the intracellular location of PEPCK. For example, in the liver of chickens, PEPCK is exclusively localized in the mitochondrion and, therefore, the catabolism of AAs in the cells does not provide the cytosol with NADH that is required for the conversion of 1,3-bisphosphoglycerate into glyceraldehyde

3-phosphate (Watford 1985). Therefore, the liver of fed or fasted chickens cannot synthesize glucose from any AA.

In obese and type-II diabetic subjects, excessive production of glucose by their livers contributes to hyperglycemia and metabolic syndrome. Thus, the production of glucose by the liver must be suppressed in these patients. In contrast, hepatic gluconeogenesis is vital for the survival and growth of newborns because this pathway provides at least 50% of glucose needed by suckling neonates (including humans, pigs and calves) because the milk of mammals generally does not contain sufficient lactose or glucose (Wu 2018). Therefore, the same pathway can be either beneficial or detrimental, depending on the physiological or pathological state of the subjects.

2.4.2 Glutamine and Glutamate Metabolism

The liver synthesizes and degrades both glutamine and glutamate in a compartment-specific manner (Brosnan 2003; Hou and Wu 2018). In mammals, periportal hepatocytes express phosphate-activated glutaminase (a mitochondrial enzyme) that hydrolyzes glutamine into glutamate and ammonia. The latter is detoxified locally via the urea cycle. Liver-type glutaminase absolutely requires NH_3 for activation, has high K_m for glutamine and high affinity for phosphate, and is not affected by low glutamate concentration. The glutamine-derived glutamate is taken up by perivenous hepatocytes via cytosolic glutamine synthetase to regenerate glutamine. This constitutes an intercellular glutamine-glutamate cycle in the liver. Thus, the absence of an arterial-venous difference in the concentration of glutamine across the liver does not necessarily indicate the absence of its hepatic metabolism, and may be associated with high rates of both glutamine hydrolysis in periportal hepatocytes and glutamate amination in perivenous hepatocytes. In the rat perfused with ≤1 mM glutamine, there is no net utilization of this AA (Curthoys and Watford 1995). The inter-cellular glutamine-glutamate cycle plays an important role in: (a) scavenging ammonia by the high-affinity glutamine synthetase to maintain low concentrations of ammonia in plasma; and (b) adjusting ammonia flux into either urea or glutamine according to the needs for regulation of the acid-base balance. For example, at normal pH, there is no release of glutamine by the liver. However, at pH < 7.4, the hydrolysis of glutamine into glutamate and ammonia is decreased and the synthesis of glutamine from glutamate and ammonia is increased, thereby resulting in the release of glutamine from the liver.

The plasma concentration of glutamine (~1 mM) in birds is much greater than that (~0.25 to 0.5 mM) in mammals (Wu 2013). In the avian liver, phosphate-activated glutaminase is absent but glutamine synthetase is present in the mitochondria of hepatocytes to generate glutamine as an intermediate in uric acid synthesis (Watford and Wu 2005; Wu et al. 1998). This ensures the successful detoxification of ammonia that is derived from AA catabolism. Whether the avian liver extracts glutamine from the blood depends on the nutritional state. For example, in the fed state, the liver of chickens does not take up glutamine from the arterial blood (Tinker et al. 1986). Thus, the high concentration of glutamine in the liver of fed poultry results primarily from its endogenous synthesis of glutamine from glutamate and ammonia, and glutamine can be considered as the sink of both the nitrogen and carbon atoms of AAs (Hou et al. 2015b). In the fasting state, the chicken liver actively takes up glutamine from the arterial blood, and the extracted amount is the highest (the same order of ranking as alanine) among all AAs (Tinker et al. 1986). The physiological significance of this process is largely unknown, but it may help to regulate hepatic nutrient metabolism, such as inhibiting proteolysis, AA degradation, and glycogenesis to spare protein, AAs and glucose.

The livers of zebrafish, hybrid-striped bass, and largemouth bass actively oxidize both glutamine and glutamate to provide ATP (Jia et al. 2017; Li and Wu 2019). Phosphate-activated glutaminase (a mitochondrial enzyme) hydrolyzes glutamine into ammonia and glutamate, whereas glutamine transaminases L and K (cytosolic and mitochondrial enzymes) converts glutamine into α-ketoglutaramate, which is deaminated by

ω-amidase into ammonia plus α-KG. Both glutamate dehydrogenase (a mitochondrial enzyme) and glutamate transaminases (cytosolic and mitochondrial enzymes) convert glutamate into α-KG plus either ammonia (in the case of the former) or alanine/aspartate (in the case of the latter). These enzymes are expressed in the liver (possibly periportal hepatocytes) of the fish. The hepatocytes of fish, such as elasmobranch fish and holocephalan elephant fish, also express glutamine synthetase (a mitochondrial enzyme) to synthesize glutamine from ammonia and glutamate (Anderson 2001), and this reaction likely occurs in both periportal and perivenous hepatocytes.

In mammals, birds and fish, glutamine participates in many vital physiological processes, including: the syntheses of uric acid in the liver, aminosugars, nucleic acids, and NAD (Wu 2013). Of particular note, glutamine:fructose-6-phosphate transaminase (a cytosolic enzyme) catalyzes the formation of glutamate from glutamine in the liver. In this reaction, glutamine donates the amide group for the synthesis of UDP-*N*-acetylglucosamine, which is a precursor for the formation of all macromolecules containing amino sugars (including membrane hormone receptors and heparin) (Häussinger and Schliess 2007). Thus, the hexosamine-synthetic pathway is essential to the growth, development, and function of hepatocytes, as well as the structure of the extracellular matrix in the liver. For the oxidation of glutamate in the liver, it must be deaminated into α-ketoglutarate by either glutamate dehydrogenase (a mitochondrial enzyme) or glutamate transaminases (cytosolic and mitochondrial enzymes), followed by the sequential conversion of α-ketoglutarate into succinate, malate, pyruvate and acetyl-CoA (Wu 2013).

2.4.3 Arginine, Proline and Ornithine

Periportal hepatocytes extract a small amount of arginine from the hepatic artery and portal vein, as noted previously. The mammalian liver contains a particularly high activity of arginase I (a cytosolic enzyme) in periportal hepatocytes to hydrolyze arginine into ornithine and urea. The resulting ornithine enters the mitochondrion in exchange for the export of citrulline from the mitochondrion into the cytosol via the Cit/Orn exchanger. This helps to facilitate the hepatic urea cycle for the removal of ammonia. Because periportal hepatocytes lack ornithine aminotransferase (OAT, a mitochondrial enzyme; Kuo et al. 1991), they release most of the resultant ornithine, which is subsequently taken up by perivenous hepatocytes that express OAT (O'sullivan et al. 1998). Thus, in perivenous hepatocytes, ornithine is metabolized into pyrroline-5-carboxylate (P5C; O'sullivan et al. 1998), which is reduced by P5C reductase [a cytosolic enzyme that is enriched in periportal hepatocytes but is also present at a much lower activity in perivenous hepatocytes (Pink 2002)] to generate proline. In addition, perivenous hepatocytes (which also express arginase activity in the cytosol) extract a small amount of arginine from the blood for the production of proline via OAT and P5C reductase (Pink 2002).

The mammalian liver takes up a large amount of proline and metabolizes this AA via proline oxidase (a mitochondrial enzyme) in a cell-specific manner. Specifically, periportal hepatocytes express a low activity of proline oxidase that converts a small amount of proline into P5C (Pink 2002). This reaction may help to regulate glucose metabolism via the pentose cycle activity and the transport of cytosolic NADPH into the mitochondrion (Wu 2013). Because these cells lack OAT, the proline-derived P5C is not further metabolized locally. Thus, it appears that perivenous hepatocytes are the major site for proline catabolism in the liver. The activity of proline oxidase was considered to be the highest in the liver among mammalian tissues (including the small intestine and kidney) (Valle and Simell 1995). However, we found that the activity of proline oxidase in the porcine liver was only 58%, 34%, and 14% of that in the porcine kidney, intact jejunum, and jejunal mucosa, respectively (Wu et al. 1997). This is consistent with the finding that proline oxidase is enriched in perivenous hepatocytes that account for only a small percentage of cells in the liver (Pink 2002).

The avian liver lacks arginase and proline oxidase but contains a low activity of OAT (Furukawa et al. 2018). Thus, compared with the mammalian liver, metabolism of arginine, proline and ornithine via these enzymes in the avian liver is absent or very limited. This helps to explain why the concentration of ornithine in the plasma of chickens is nearly not detectable (only about 1 nmol/ml), in comparison with that in the plasma of pigs (50-100 nmol/ml) (Wu et al. 1997). In chickens, a significant amount of arginine may be utilized for the hepatic production of creatine, because their livers express both arginine:glycine amidinotransferase and guanidinoacetate *N*-methyltransferase for converting arginine and glycine into creatine (Zhu and Evans 2001). This is likely important for the rapid growth of skeletal muscle in broilers.

The livers of elasmobranch fish and holocephalan elephant fish are capable of synthesizing urea from ammonia as a mechanism for its detoxification (Anderson 2001). Ureotelic terrestrial vertebrates express carbamoylphosphate synthetase (CPS)-I to convert ammonia and bicarbonate into carbamoylphosphate in the mitochondria of periportal hepatocytes. In contrast, the livers of ureotelic fish contain CPS-III (a mitochondrial enzyme), which uses glutamine rather than ammonia as the nitrogenous substrate and depends on *N*-acetylglutamate as an allosteric activator. For comparison, CPS-II (a cytosolic enzyme for pyrimidine synthesis) also uses glutamine as the nitrogenous substrate but does not depend on *N*-acetylglutamate for catalytic activity. Thus, in the fish, the synthesis of glutamine from ammonia and glutamate by the mitochondrial glutamine synthetase is the first step in the conversion of ammonia into urea. This is analogous to avian livers, which synthesize glutamine from ammonia and glutamate by the mitochondrial glutamine synthetase as the initial reaction of a metabolic pathway for the detoxification of ammonia as uric acid (Wu 2018). Most teleost fish excrete 5–20% of nitrogen as urea, but a greater value (30%) has been reported for largemouth bass. This is consistent with an exceedingly high activity of arginase in the liver of largemouth bass to hydrolyze arginine into urea plus ornithine. Because the liver of largemouth bass lacks proline oxidase (our unpublished work), arginase is the only source of ornithine that is generated within this organ.

2.4.4 Sulfur AA Metabolism

Methionine, cysteine, and taurine are called sulfur AAs, because they contain a sulfur atom. Animal-source foods provide taurine and generally contain more methionine and cysteine than plant-source foods (Hou et al. 2019; Li and Wu 2020). In humans and other animals, the liver is the only organ that can completely metabolize methionine and cysteine into CO_2 (Wu 2013). All animals can convert methionine into cysteine via the cytosolic transsulfuration pathway but lack enzymes to recycle cysteine into methionine. Thus, dietary provision of cysteine can spare the requirements of animals for dietary methionine. The transsulfuration pathway also generates *S*-adenosylmethionine (the major donor of the methyl group required for biochemical reactions such as polyamine and creatine syntheses) from methionine (Brosnan and Brosnan 2007). In subjects with a defect of the transsulfuration pathway, cysteine must be included in diets, because the hepatic syntheses of proteins, glutathione (a major antioxidant), taurine (in most species), and other biologically active substances (e.g., H_2S) depends on cysteine. In humans and other animals (including fish), hepatic dysfunction reduces the ability of the liver to synthesize glutathione for local function and release into the blood, contributing to systemic oxidative stress and the darkening of tissues such as the heart and skin due to the presence of oxidized molecules (e.g., protein and lipids). Under conditions of oxidative stress, exogenous administration of cysteine in the form of *N*-acetylcysteine to humans and animals is beneficial for improving metabolic profiles and health (including liver health). For example, dietary supplementation with *N*-acetylcysteine alleviates liver injury in lipopolysaccharide-challenged piglets (Yi et al. 2014), as reported for humans with drug- or oxidative stress-induced liver injury (Wu et al. 2004).

There are species differences in the hepatic synthesis of taurine from cysteine. Humans and most other animals can convert cysteine into taurine in the liver at various rates. However, this metabolic pathway is negligible in some animals (e.g., cats) due to a very low activity of hepatic cysteine dioxygenase and cysteinesulfinate decarboxylase (Rogers and Morris 1979). Thus, taurine must be included in diets for cats to prevent retinal degeneration and cardiomyopathy. Human infants, particularly preterm infants, have a limited ability to synthesize taurine despite an adequate provision of its precursors in diets (Geggel et al. 1985), as reported for young or adult cats. In adult humans, the rate of taurine synthesis is exceedingly low in comparison with rats, because the activity of hepatic cysteinesulfinate decarboxylase (a key enzyme in taurine synthesis) is about 3 orders of magnitude lower in the young and adult men than that in rats (Sturman 1993). Compared with livestock (e.g., cattle, pigs, and sheep) and poultry (e.g., chickens and ducks), adult humans also have a very low ability to synthesize taurine. Thus, adult humans who consume only plant-source foods (which contain no taurine) but no animal products are at increased risk for taurine deficiency (Rogerson 2017), because the precursors of taurine (methionine and cysteine) are present at low concentrations in most proteins of plant origin (e.g., corn, potato, rice, wheat, and vegetables) (Hou et al. 2019). Although dogs are also carnivores like cats, dogs can synthesize sufficient taurine from cysteine when fed diets containing adequate methionine or cysteine. However, a significant decrease in plasma taurine concentration occurs in dogs fed a protein-restricted diet (e.g., 10% protein, dry matter basis) due to a reduced availability of both methionine and cysteine (Sanderson et al. 2001).

Taurine plays major roles in physiology and nutrition. First, this AA is used to conjugate bile acids to form bile salts in the liver that facilitate intestinal absorption of dietary lipids (including lipid-soluble vitamins) and eliminate cholesterol in bile via the fecal route. While humans and many other animals can use both taurine and glycine to conjugate bile acids, cats and dogs are only able to utilize taurine to conjugate bile acids (Sturman 1993), indicating a unique role of taurine in the nutrition and metabolism of the companion animals. Second, taurine is a major antioxidant, anti-inflammatory, antiapoptotic factor, and a physiological stabilizer of cell membranes. Third, taurine regulates Ca^{2+} signaling, fluid homeostasis in cells, retinal photoreceptor activity, osmoregulation; nerve and muscle conduction networks, and neurological development. Thus, humans, cats and possibly some other animals (e.g., carnivorous fish) have requirements for dietary taurine.

2.4.5 Metabolism of Aromatic AAs

Phenylalanine, tyrosine, and tryptophan are aromatic AAs, because they contain an aromatic ring. In humans and other animals, the liver is the only organ that can completely metabolize these three AAs into CO_2 (Wu 2013). All animals can convert phenylalanine into tyrosine via the cytosolic tetrahydrobiopterin (BH4)-dependent phenylalanine hydroxylase, but lack enzymes to recycle tyrosine into phenylalanine. Thus, dietary provision of tyrosine can spare the requirements of animals for dietary phenylalanine. The hydroxylation of phenylalanine competes with NO synthase for BH4, thereby influencing blood flow and other NO-dependent signaling pathways. In subjects with a deficiency of BH4 or phenylalanine hydroxylase, tyrosine must be included in diets, because the cell-specific syntheses of proteins, thyroid hormones, dopamines, catecholamines, and melanin in the body depends on tyrosine. In these patients, dietary intake of protein (particularly phenylalanine) must be controlled tightly to prevent a pathologically high concentration of phenylalanine in plasma. Of note, BH4 is essential as a cofactor of tyrosine hydroxylase in the metabolic pathway of converting tyrosine into dopamines, catecholamines, and melanin. Likewise, tryptophan hydroxylase requires BH4 for the production of serotonin, melatonin, and related metabolites from tryptophan. Humans and other animals (including fish) with hepatic dysfunction because of viral, bacte-

rial and parasite infections or inadequate nutrition have an impaired ability to degrade and remove: (a) the black-color products (e.g., melanin and homogentisate) of phenylalanine and tyrosine catabolism by melanocytes and the liver; and (b) melatonin (a product of tryptophan) generated primarily by the pineal gland. The tyrosine metabolites are accumulated in the skin, directly resulting in black skin syndrome. In addition, melatonin can interfere with the metabolism and function of melanocytes of the skin (Kim et al. 2015) and, therefore, may also play a role in the onset of black skin syndrome.

2.4.6 Glycine and Serine Metabolism

The liver is the most active organ in the interconversion of glycine and serine through vitamin B_6-dependent serine hydroxymethyltransferase (SHMT). This reaction plays an important role in intracellular one-carbon metabolism (Stover and Schirch 1990). There are two isoforms of SHMT: SHMT1 (cytosolic) and SHMT2 (mitochondrial). Due to the compartmentation of metabolic pathways, SHMT1 is involved in the syntheses of purines and thymidylate, whereas SHMT2 contributes to the production of formate from serine (Brosnan et al. 2015). The mitochondria and cytosol of hepatocytes readily convert serine and tetrahydrofolate into glycine and N^5,N^{10}-methylene tetrahydrofolate. Thus, oral administration of serine to humans and other animals markedly increases the concentration of glycine in the plasma. In contrast, animals have a limited ability to convert glycine into serine due to a low concentration of N^5,N^{10}-methylene tetrahydrofolate or other methyl group donors such as S-adenosylmethionine, betaine and choline in the cells. Note that in the mitochondria, the oxidation of glycine into ammonia plus CO_2 with the concomitant formation of N^5,N^{10}-methylene tetrahydrofolate from tetrahydrofolate is coupled with the conversion of glycine into serine. Therefore, oral administration of glycine to humans and other animals only slightly augments the concentration of serine in the plasma (Wang et al. 2013). Nonetheless, dietary supplementation with glycine can alleviate oxidative stress and hepatic injury in response to various pathological conditions, such as infections, sepsis, and drug toxicity (Wang et al. 2013). Besides serine, 4-hydroxyproline is another major substrate for the synthesis of glycine through hydroxyproline oxidase (Wu et al. 2019). This pathway is nutritionally important for the provision of glycine to milk-fed neonates, because milk is deficient in glycine but contains a large amount of 4-hydroxyproline and its peptides. Because all plant-source foods contain a low content of glycine (Hou et al. 2019) and cannot provide adequate glycine for mammals (including humans), vegans are at high risk for glycine deficiency. This will result in metabolic abnormalities, including anemia, oxidative stress, and impaired protein synthesis.

2.4.7 Branched-Chain Amino Acid (BCAA) Metabolism

In the liver of mammals, the degradation of BCAAs is limited due to a low activity of BCAA transaminase, as noted previously (Brosnan 2003). However, hepatocytes actively decarboxylate branched-chain α-keto acids (released primarily from skeletal muscle) into acyl-CoA, which is used either for ketogenesis (in most animals) or gluconeogenesis (for valine and isoleucine). In the livers of zebrafish, hybrid-striped bass, and largemouth bass, leucine transamination and decarboxylation are active but their rates are much lower than those for glutamate or glutamine oxidation (Jia et al. 2017; Li and Wu 2019). This illustrates species-dependent differences in hepatic BCAA catabolism. Thus, the liver participates in the inter-organ metabolism of BCAAs and produces ketone bodies (in most animals) or glucose in response to physiological needs.

In patients with liver cirrhosis (the scarring of the liver) or hepatic encephalopathy (brain dysfunction as a result of severe liver disease), the plasma concentrations of BCAAs decrease but those of aromatic AAs increase (Campollo et al. 1992; Holeček 2018). Under those conditions, the hepatic urea cycle is impaired, resulting in: (a) hyperglycemia that stimulates BCAA trans-

amination and oxidation in red and white skeletal muscles (Holecek et al. 2011); and (b) decreases in the catabolism of aromatic AAs by the liver. This is an adaptation of the living individuals to the liver diseases because the transamination of BCAAs with α-KG generates glutamate, which undergoes amination with ammonia to form glutamine in tissues (e.g., skeletal muscle and brain) as a mechanism to remove ammonia from the blood. There have been suggestions that dietary supplementation with BCAAs may promote anabolic pathways and remove ammonia, therefore alleviating syndromes of liver cirrhosis (the scarring of the liver) or hepatic encephalopathy (Tajiri and Shimizu 2013). However, there is no consensus regarding the efficacy of such a nutritional treatment for the liver disorders (Milan 2018). At present, hyperammonemia is managed by restricting dietary protein intake and administering benzoate or phenylbutyrate to remove ammonia. Benzoate acts through conjugating with glycine to form hippurate, whereas phenylbutyrate is converted by mitochondrial β-oxidation into phenylacetyl-CoA that is conjugated with glutamine to generate phenylacetylglutamine (Wu 2013). Both hippurate and phenylacetylglutamine are excreted in the urine.

2.4.8 Homoarginine Synthesis from Arginine and Lysine

The liver and kidneys of humans and other animals (e.g., rats and pigs) synthesize L-homoarginine and ornithine from arginine and lysine via a putative mitochondrial enzyme called arginine:glycine amidinotransferase (AGAT; Hou et al. 2016; Tsikas and Wu 2015). This enzyme catalyzes the transfer of the amidino group from L-arginine to L-lysine to form homoarginine. The concentrations of homoarginine in the plasma and liver of rats are about 2 and 115 μM, respectively (Hou et al. 2015b). Less than 0.025% and <0.045% of ingested arginine is metabolized to homoarginine in growing pigs and adult rats, respectively (Hou et al. 2016; Wu et al. 2016). At present, little is known about homoarginine synthesis in birds, fish or shrimp (Li et al. 2020). The function of this arginine metabolite in the liver is unknown, but as an analogue of arginine may play a role in regulating the synthesis of NO and creatine by hepatocytes and kidneys.

2.4.9 Creatine Synthesis from Arginine, Glycine and Methionine

Creatine plays an important in the energy metabolism of the brain and skeletal muscle via creatine kinase (that converts creatine and ATP into creatine phosphate and ADP), as well as antioxidative reactions in multiple types of cells and tissues (Wu 2020b). Under physiological conditions, creatine and creatine phosphate are spontaneously converted to creatinine, which is excreted in the urine. The loss of creatine plus creatine phosphate is about 1.7% of the total body creatine pool or 2.04 g creatine/day in a 70-kg healthy adult human that contains 120 g of total creatine in the form of free creatine and creatine phosphate (Brosnan and Brosnan 2007). Because the total pool of creatine in the body is constant, the lost creatine must be replaced from either diets or de novo synthesis (da Silva et al. 2009). Humans consuming a typical meat-containing Western diet obtain about one-half of their creatine through de novo synthesis and one-half from diets (Brosnan and Brosnan 2007). In contrast, vegetarians, who do not ingest animal-source foods, obtain very little dietary creatine and must depend on endogenous synthesis for creatine provision. These subjects have a high risk for creatine deficiency (Wu 2020b).

Studies with the rat model have established that creatine synthesis from arginine, glycine and methionine in animals involves the metabolism of AAs among different organs, primarily the kidneys, liver, and pancreas (Wu and Morris 1998). This metabolic pathway involves: (1) arginine:glycine amidinotransferase (AGAT, a mitochondrial enzyme located primarily in the renal tubules and pancreas, and to a much lesser extent in the liver, brain and other organs), and (2) guanidinoacetate *N*-methyltransferase (GAMT, a cytosolic enzyme located primarily in

the liver and pancreas and, to a much lesser extent, the kidneys and brain). AGAT converts arginine and glycine into ornithine and guanidinoacetate (glycocyamine). The latter is methylated by GAMT to creatine in the presence of *S*-adenosylmethionine (a metabolite of methionine). This reaction maintains the circulating guanidinoacetate at a low concentration to protect the injury of the brain, because elevated levels of guanidinoacetate result in neurotoxicity (Brosnan and Brosnan 2007). It is unlikely that in mammals, the liver contributes a significant fraction of guanidinoacetate production in the whole body, because the hepatic uptake of arginine is low and the cytosolic arginine is rapidly hydrolysed by arginase I to urea and ornithine. In rats, the kidney is the principal site of guanidinoacetate production, although the pancreas may provide a physiologically significant amount of guanidinoacetate to the liver. This notion is supported by the following lines of evidence, First, the concentrations of arginine in the livers of humans and rats are exceedingly low [about 15 μM (Barle et al. 1996) and 60 μM (Dohm et al. 1981), respectively]. Second, rat hepatocytes synthesize creatine from guanidinoacetate but not arginine, glycine plus methionine (da Silva et al. 2009). Third, the renal output of guanidinoacetate in rats fed a creatine-free diet is equal to the renal excretion of creatinine (the product of the nonenzymatic degradation of creatine and creatine phosphate) when expressed as mmol/day (Edison et al. 2007). In humans, the renal production of guanidinoacetate represents only 20% of the daily loss of creatinine (Edison et al. 2007), suggesting that most of guanidinoacetate must be synthesized in other tissues, possibly including the pancreas, small intestine, and the brain. Thus, there are species-differences in creatine synthesis among animals. At present, little is known about this synthetic pathway in farm animals (including pigs, cattle, sheep, poultry, fish, and shrimp) (Li et al. 2020).

Studies with rats have demonstrated that de novo synthesis of creatine is regulated primarily via changes in the expression of AGAT in the kidneys. Renal expression of this enzyme is induced by growth hormone but is strongly inhibited by dietary intake of creatine (Guthmiller et al. 1994). Consistent with such a finding, Edison et al. (2007) reported that dietary supplementation with creatine (4 g/kg diet; similar to the content of creatine in red meat for human diets) to adult rats decreased renal AGAT activity by 86% and plasma guanidinoacetate concentration by 70%, in comparison with control rats fed a creatine-free diet. Similar regulatory mechanisms occur in humans, as the ingestion of creatine by young adults [20 g creatine monohydrate per day for the first week (loading phase) and 5 g/day for 19 subsequent week (maintenance phase)] reduced plasma guanidinoacetate levels by 50% after creatine loading and by 30% during the maintenance phase (Derave et al. 2004). This finding is consistent with the downregulation of renal AGAT expression.

2.5 Conclusion and Perspectives

In mammals (including humans, pigs and rats), the liver is the only organ to degrade all AAs taken up from the blood except for BCAAs, and plays an active role in the syntheses of glutamate, glutamine, alanine, aspartate, asparagine, glycine, serine, proline, homoarginine, and glucose. In addition, the mammalian liver converts phenylalanine into tyrosine, as well as methionine into cysteine, but cannot recycle tyrosine into phenylalanine or cysteine into methionine. Similar findings have been reported for the liver of birds except that it lacks arginase, phosphate-activated glutaminase, proline oxidase, and gluconeogenesis from AAs. The liver of fish extensively catabolizes glutamate and glutamine and, to a much lesser extent, BCAAs, for ATP production (Li et al. 2020). Most animals (except for cats and possibly some of the other carnivores) can synthesize taurine from cysteine in hepatocytes. Ammonia generated from hepatic AA degradation is converted into urea and uric acid in the livers of mammals and birds, respectively. In teleost fish, most of the ammonia produced from AA catabolism in their hepatocytes is released into the living environment, and some of the ammonia is converted into urea via the urea

cycle analogous to that in mammals except that CPS-III catalyzes the formation of carbamoylphosphtae from glutamine and bicarbonate. Dietary supplementation with functional AAs (e.g., methionine, *N*-acetylcysteine and glycine) is beneficial for alleviating or preventing oxidative stress and damage in the liver. Although the mammalian liver synthesizes homoarginine, the physiological functions of this metabolite are unknown. Furthermore, little is known about the zonation of glutamate, glutamine, serine, glycine, arginine, ornithine and proline metabolism in the livers of aquatic animals. Filling in this knowledge gap is expected to elucidate the mechanisms responsible for the beneficial effects of functional AAs on improving hepatic structure and function.

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Amino Acids in Circulatory Function and Health

3

William Durante

Abstract

Cardiovascular disease is the major cause of global mortality and disability. Abundant evidence indicates that amino acids play a fundamental role in cardiovascular physiology and pathology. Decades of research established the importance of L-arginine in promoting vascular health through the generation of the gas nitric oxide. More recently, L-glutamine, L-tryptophan, and L-cysteine have also been shown to modulate vascular function via the formation of a myriad of metabolites, including a number of gases (ammonia, carbon monoxide, hydrogen sulfide, and sulfur dioxide). These amino acids and their metabolites preserve vascular homeostasis by regulating critical cellular processes including proliferation, migration, differentiation, apoptosis, contractility, and senescence. Furthermore, they exert potent anti-inflammatory and antioxidant effects in the circulation, and block the accumulation of lipids within the arterial wall. They also mitigate known risk factors for cardiovascular disease, including hypertension, hyperlipidemia, obesity, and diabetes. However, in some instances, the metabolism of these amino acids through discrete pathways yields compounds that fosters vascular disease. While supplementation with amino acid monotherapy targeting the deficiency has ameliorated arterial disease in many animal models, this approach has been less successful in the clinic. A more robust approach combining amino acid supplementation with antioxidants, anti-inflammatory agents, and/or specific amino acid enzymatic pathway inhibitors may prove more successful. Alternatively, supplementation with amino acid-derived metabolites rather than the parent molecule may elicit beneficial effects while bypassing potentially harmful pathways of metabolism. Finally, there is an emerging recognition that circulating levels of multiple amino acids are perturbed in vascular disease and that a more holistic approach that targets all these amino acid derangements is required to restore circulatory function in diseased blood vessels.

Keywords

Amino acids · L-Arginine · L-Glutamine · L-Cysteine · L-Tryptophan · Endothelium · Vascular smooth muscle cells · Atherosclerosis · Vascular disease

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3.1 Introduction

Cardiovascular disease is the leading cause of morbidity and mortality, accounting for 31% of all deaths worldwide (Benjamin et al. 2017). Advancing age, genetic factors, hypertension, hypercholesterolemia, insulin resistance, obesity, diabetes, and lifestyle choices such as smoking, diet, and sedentarism are major risk factors for cardiovascular disease. While the age-adjusted mortality rate for cardiovascular disease has declined in industrialized countries due to societal changes, improvements in risk factors, and medical care, the aging population and the continuing epidemic of obesity and type 2 diabetes mellitus (T2DM) threatens to reverse this achievement, highlighting the need for new treatment modalities that target this disease.

Atherosclerosis is the most common underlying cause of cardiovascular disease. It is an inflammatory disease that is distinguished by the progressive accumulation of lipids and inflammatory cells within the intima of arteries (Tabas et al. 2015). The disease is initiated by the subendothelial retention of low density lipoproteins (LDLs) in focal regions of arteries where blood flow is disturbed. Turbulent flow in these areas triggers endothelial cell (EC) dysfunction which facilitates the entry of lipoproteins into the susceptible region. Various modifications of the trapped lipoproteins, including the formation of oxidized LDL (oxLDL), leads to the expression of adhesion receptors and chemokine production by ECs resulting in the recruitment of immune cells to the forming lesion. The modified lipoproteins also leads to the proliferation and migration of vascular smooth muscle cells (SMCs) into the intima. Infiltrating monocytes differentiate into macrophages and scavenger receptor-mediated uptake of lipoproteins by macrophages leads to foam cell formation. Eventual foam cell death generates a core region in the plaque comprised of necrotic and apoptotic cells, extracellular material, and cholesterol.

The adaptive arm of the immune response also contributes in the pathogenesis of atherosclerosis (Durante 2011). T cells present in the plaque, adjacent adventitia, or in the draining lymph node undergo activation following interaction with antigen presenting cells, which process and present local antigens. Owing to a favorable cytokine environment, a T helper 1 (T_H1) cell response usually predominates leading to the production of tumor necrosis factor-α (TNFα) which accelerates lesion formation by stimulating the expression of inflammatory cytokines, adhesion molecules, proteolytic enzymes, and thrombotic mediators. In contrast, T regulatory (T_{Reg}) cells interfere with the inflammatory actions of T_H1 cells. In many instances inflammation within the plaque resolves leading to the formation of a thick fibrous cap that provides a protective barrier between circulating platelets and the prothrombotic material in the plaque (Libby 2008). However, non-resolving inflammation leads to the formation of a plaque with a large lipid-filled necrotic core and a vulnerable thin fibrous cap. Subsequent rupture of the compromised plaque by hemodynamic forces within the vessel wall or loss of ECs leads to abrupt thrombotic occlusion of the artery that precipitates acute clinical events such myocardial infarction, stroke, and end-organ failure.

Growing evidence indicates that amino acids play an instrumental role in cardiovascular physiology and pathology. While all amino acid are important for protein synthesis, a subset of amino acids are intimately associated with vascular health. Decades of research established the importance of L-arginine (Arg) in regulating vascular and immune cell function and atherosclerosis through the production of the gas nitric oxide (NO). Recent clinical studies have also identified circulating levels of L-glutamine (Gln) and L-glutamate (Glu) as key predictors of cardiovascular disease while experimental work reveals fundamental mechanisms by which glutaminolysis modulates vascular cell function and pulmonary arterial hypertension. More recently, the complex actions of L-tryptophan (Trp) and its metabolites on vascular cell function and atherosclerosis have been revealed. In addition, ongoing studies are documenting the importance of a panoply of L-cysteine (Cys)-derived metabolites in maintaining vascular homeostasis. In this review, we will describe the metabolism and

function of these four key amino acids in the circulation, and highlight potential therapeutic approaches that target these amino acids in vascular disease.

3.2 L-Arginine

Arg is a cationic, semi-essential amino acid that participates in numerous physiological processes (see Wu and Morris 1988; Durante 2001). Arg metabolism is regulated both through the expression of the cationic amino acid transporters of the solute carrier 7 (SLC7) family and by the enzymes responsible for its catabolism (Fig. 3.1). The discovery in the late 1980s that Arg is oxidized to NO and L-citrulline (Cit) by NO synthase (NOS) spurred considerable interest in this amino acid within the cardiovascular community. Besides serving as a substrate for NOS, Arg plays an important structural and functional role by assisting in the intracellular assembly of the dimeric form of NOS and ensuring the proper coupling between the oxidative and reductive domains of the enzyme. In this respect, lack of Arg or the obligatory cofactor tetrahydrobiopterin leads to the uncoupling of the enzyme and the production of superoxide rather than NO. NOS-derived Cit is subsequently converted back to Arg by the sequential action of argininosuccinate synthetase and argininosuccinate lyase. The significance of this recycling mechanism is illustrated by the decrease in NO synthesis and increase in blood pressure in argininosuccinate lyase-deficient rodents and humans (Erez et al. 2011).

There are three distinct isoforms of NOS: neuronal NOS (NOS1), inducible NOS (NOS2), and endothelial NOS (NOS3) (see Forstermann and Sessa 2012). While NOS1-derived NO is involved in synaptic plasticity and acts as a neurotransmitter for the central control of blood pressure, NOS2-derived NO plays a role in host defense and contributes to the pathophysiology of inflam-

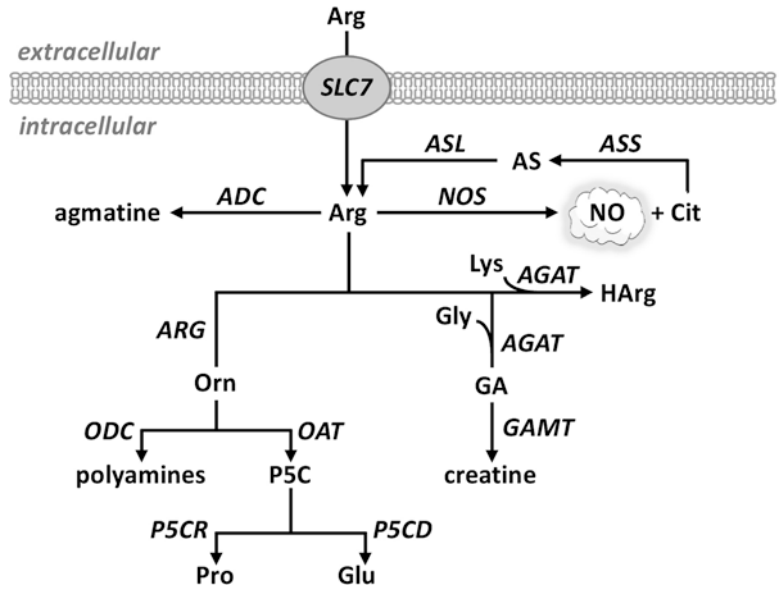

Fig. 3.1 Overview of L-arginine transport and metabolism. L-Arginine is transported into cells by the SLC7 family of transporters and metabolized via four discrete enzymatic pathways. *ADC* arginine decarboxylase, *AGAT* L-arginine:glycine amidinotransferase, *Arg* L-arginine, *ARG* arginase, *ASL* argininosuccinate lyase, *AS* argininosuccinate, *ASS* argininosuccinate synthetase, *Cit* L-citrulline, *GA* guanidinoacetate, *GAMT* guanidinoacetate N-methyltransferase, *Gly* glycine, *Glu* L-glutamate, *HArg* L-homoarginine, *Lys* L-lysine, *NO* nitric oxide, *NOS* nitric oxide synthase, *Orn* L-ornithine, *ODC* ornithine decarboxylase, *OAT* ornithine aminotransferase, *Pro* L-proline, *P5C* pyrroline-5-carboxylate, *P5CR* pyrroline-5-carboxylate reductase, *P5CD* pyrroline-5-carboxylate dehydrogenase, *SLC7* solute carrier family 7

matory diseases. In contrast, the release of NO by NOS3 performs a pivotal task in preserving vascular health. EC-derived NO regulates blood flow and blood pressure by inhibiting arterial tone. In addition, NO exerts a potent antithrombotic effect by hindering platelet aggregation and adhesion and limits intimal thickening by blocking SMC proliferation, migration, and collagen synthesis. The generation of NO by ECs also retards inflammation by inhibiting the expression of adhesion molecules, the production of inflammatory cytokines and chemokines, and the recruitment, infiltration and activation of leukocytes within the vessel wall. Conversely, a NO deficiency results in endothelial dysfunction that is characterized by increased EC apoptosis, impaired endothelium-dependent vasodilation, and EC activation, which collectively contributes to the development of vascular disease. Consistent with this notion, loss of function polymorphisms in the NOS3 gene are associated with a significantly higher risk of ischemic heart disease while gain of function polymorphisms are correlated with decreased blood pressure and reduced risk of coronary heart disease, peripheral artery disease, and stroke (Casas et al. 2004; Emdin et al. 2018). Similarly, mice with NOS3 deficiency exhibit hypertension and aggravated atherosclerosis (Chen et al. 2001).

Arg methylation is a common post-translational modification that is catalyzed by protein arginine methyl transferases. Subsequent proteolysis of proteins containing methylated Arg results in the liberation of asymmetric dimethylarginines (ADMA), symmetric dimethylarginine (SDMA), and N^G-monomethyl-L-arginine (L-NMMA). Elevated plasma concentrations of ADMA and L-NMMA are strong risk factors for cardiovascular disease (Liu et al. 2018), and high serum ADMA levels are associated with an increased risk of microvascular and macrovascular disease in T2DM (Ganz et al. 2017). The robust association between ADMA/L-NMMA and cardiovascular disease likely reflect the ability of these methylated compounds to antagonize NO synthesis. ADMA and L-NMMA, but not SDMA, are competitive inhibitors of NOS3. In addition, they both enhance NOS3 uncoupling leading to the production of superoxide, which further reduces NO bioavailability. ADMA and L-NMMA may also block NOS3 function by competing with Arg for uptake by the SLC7 transporters.

Vascular cells also express the enzyme arginase that hydrolyzes Arg to ornithine and urea. There are two distinct isoforms of arginase, arginase I and II: arginase I is a cytosolic enzyme that is highly expressed in the liver where it catalyzes the final step of the urea cycle, while arginase II is a cytosolic enzyme that is widely found outside the liver. The presence of arginase has been documented in a multitude of blood vessels and both arginase isoforms have been detected in ECs (Li et al. 2016) and SMCs (Durante 2013). The arginase product L-ornithine is further metabolized by ornithine decarboxylase to the polyamine putrescine which forms the successive polyamines spermine and spermidine. Vascular cell proliferation is associated with elevations in polyamine production and inhibition of polyamine synthesis negates cell growth (Durante et al. 1998; Li et al. 2002). L-Ornithine is also converted to pyrroline-5-carboxylate by ornithine aminotransferase that is further catabolized by pyrroline-5-carboxylate reductase to L-proline, a key precursor needed for the synthesis of many structural proteins, including collagen (Durante et al. 2000, 2001). Alternatively, L-ornithine is metabolized by pyrroline-5-carboxylate dehydrogenase to Glu.

Numerous studies show that arginase limits endothelial NO production by competing with NOS3 for substrate Arg (see Durante et al. 2007; Pernow and Jung 2013; Caldwell et al. 2018). Arginase-mediated impairments in NO bioavailability and endothelial function have been demonstrated in patients and/or animals with diabetes, hypercholesterolemia, atherosclerosis, hypertension, heart failure, aging, and sickle cell disease. Moreover, we recently reported that vascular arginase activity is upregulated in obese Zucker rats and that it impairs NO-mediated arteriolar vasodilation in these animals (Johnson et al. 2015). In a follow-up study, we identified an age-dependent increase in arginase in blood vessels and plasma of obese Zucker rats that is paralleled by a decline in plasma Arg and NO (Peyton et al.

2018a). Moreover, chronic administration of an arginase inhibitor to pre-hypertensive animals prevents the rise in blood pressure and this is associated with increases in blood Arg and NO. The ability of arginase to promote hypertension has also been documented in animal models of diabetes, insulin-resistance, sickle cell anemia, and essential hypertension.

Arginase also plays a role in atherosclerosis. Increases in arginase II expression and activity are observed in atherosclerotic blood vessels (Ryoo et al. 2008). Strikingly, pharmacological inhibition of arginase or deletion of arginase II in apolipoprotein E (apoE)-deficient mice reduces plaque burden, SMC senescence and apoptosis, macrophage content, oxidative stress, and inflammatory cytokine expression within the atherosclerotic lesion, while improving EC dysfunction and arterial stiffness (Ryoo et al. 2008; Ming et al. 2012; Xiong et al. 2013). Moreover, adoptive transfer experiments indicate that fewer donor arginase II-deficient monocytes than arginase II-replete monocytes enter the plaque of apoE-null mice. Conversely, overexpression of arginase II in ECs promotes endothelial dysfunction and atherosclerosis in apoE-deficient animals (Vaisman et al. 2012). Surprisingly, an atheroprotective role for arginase I has been detected. Arginase I expression is elevated in macrophages derived from rabbits with a low predisposition to atherosclerosis compared to macrophages obtained from animals with a high proclivity for the disease (Teupser et al. 2006). In addition, intraplaque gene transfer of arginase I attenuates macrophage infiltration and inflammation, whereas local silencing of arginase I enhances these responses (Wang et al. 2011a). Thus, the regulatory role of arginase in atherosclerosis is intricate, isoform-dependent, and requires further study.

Diminished global Arg bioavailability is associated with the development of coronary artery disease and heightened long-term risk for major adverse cardiovascular events, including death, myocardial infarction, and stroke (Tang et al. 2009). Low serum Arg also predicts the evolution of microvascular disease in patients with T2DM (Ganz et al. 2017), while a reduction in Arg availability is independently associated with pulmonary hypertension and increased mortality in patients with sickle cell disease (Morris et al. 2005). Preclinical studies have also noted a decrease in plasma Arg in animal models of obesity, diabetes, and sickle cell disease (Pieper and Peltier 1995; Romero et al. 2002; Johnson et al. 2015). Moreover, a plethora of small clinical and experimental studies indicate that enteral or parenteral administration of Arg corrects endothelial dysfunction associated with coronary artery disease, peripheral artery disease, hyperlipidemia, smoking, hypertension, sickle cell anemia, diabetes, and obesity (see Wu and Meininger 2000; Wu et al. 2009). Alternatively, restoration of circulating and tissue levels of Arg by Cit administration also improves NO bioavailability and endothelium-dependent vasodilation in numerous maladies (Allerton et al. 2018). In fact, owing to a more favorable pharmacokinetic profile, supplemental Cit is more efficient than Arg in increasing systemic Arg availability (Schwedhelm et al. 2008). In addition, dietary supplementation with Arg retards the progression of atherosclerosis in high cholesterol-fed rabbits (Boger et al. 2007; Cooke et al. 1992). Arg administration also normalizes blood pressure in obese rats and elicits a preferential vasodepressor action in obese hypertensive patients relative to lean normotensive subjects (Johnson et al. 2015; Castejon et al. 2002). Furthermore, Arg feeding influences vascular remodeling. Short-term Arg/Cit supplementation reduces arterial stiffness in middle-aged men and in patients with kidney disease (Ochiai et al. 2012; Annavarajula et al. 2012). Arg administration also reduces intimal thickening after arterial injury or vein grafting in rodents (McNamara et al. 1993; Hamon et al. 1994; Okazaki et al. 1997). The vascular benefits associated with dietary Arg supplementation are largely due to an increase in NO bioavailability; however, other mechanisms may also be operative (Wu and Meininger 2000).

The beneficial effects of Arg supplementation are not universally observed. A recent meta-analysis found that flow-mediated dilation and NO levels are not improved by oral supplementation of Arg in patients with cardiovascular or metabolic disease (Rodriques-Krause et al.

2019). Moreover, long-term clinical studies indicate that Arg administration does not improve vascular function when added to standard therapy in patients with coronary or peripheral arterial disease (Blum et al. 2000; Wilson et al. 2007). Beyond failure, Arg therapy is associated with higher post-infarction mortality in patients with acute coronary syndrome and may worsen endothelial function in patients with peripheral artery disease (Schulman et al. 2006; Wilson et al. 2007). These findings are in-line with a Mendelian randomization study proposing that high Arg levels are associated with a higher risk of ischemic heart disease (Au Yeung et al. 2016). However, they are at odds with studies demonstrating the safety of dietary supplementation with Arg in healthy humans and animals (Yang et al. 2015; Wu et al. 2016; McNeal et al. 2018), raising the possibility that the detrimental effects of Arg are disease-specific. In this respect, the adverse effects of Arg may be related to NOS3 uncoupling due to the oxidation of tetrahydrobiopterin and/or channeling of Arg through harmful pathways such as arginase or NOS2, which are upregulated in atherosclerosis. In addition, chronic exposure to Arg accelerates EC senescence providing another mechanism by which Arg may exacerbate vascular disease (Scalera et al. 2009).

Arg is also metabolized by the enzyme L-arginine:glycine amidinotransferase (AGAT) which catalyzes the transfer of the amidino group from Arg to L-glycine (Gly) to produce guanidinoacetate. Subsequently, guanidinoacetate N-methyl transferase (GAMT) methylates guanidinoacetate to creatine. However, AGAT also utilizes lysine to generate L-homoarginine (HArg). Clinical studies indicate that low plasma concentrations of HArg independently predicts mortality from cardiovascular disease, whereas high plasma concentrations are independently associated with reduced mortality in stroke patients (Marz et al. 2010; Pilz et al. 2011; Choe et al. 2013). Genome-wide association studies identified AGAT as the key enzyme for HArg synthesis in humans (Choe et al. 2013). Furthermore, AGAT-null mice have low plasma HArg levels and demonstrate increased morbidity in an experimental model of stroke that is rectified by HArg feeding. Conversely, GAMT-deficient mice have higher plasma HArg concentrations and smaller experimental strokes, suggesting that HArg is causally involved in the pathophysiology of stroke. Oral supplementation with HArg for up to 4 weeks also elevates circulating HArg levels in human subjects without any adverse effects, supporting the implementation of larger prospective studies investigating the effect of HArg administration in patients with cardiovascular disease (Atzler et al. 2016). The mechanism underlying the protective actions of HArg remain unknown but may be related to the ability of HArg to promote NO synthesis by serving as a NOS substrate or arginase inhibitor. Finally, Arg may also be metabolized by Arg decarboxylase to agmatine. Further studies are needed to confirm the presence and functional significance of this pathway in the vasculature.

3.3 L-Glutamine

Gln is a conditionally essential amino acid that is indispensable during stages of rapid growth or in pathological states such as trauma, sepsis, and critical illness (Xi et al. 2011). Gln is a necessary nitrogen donor for the *de novo* synthesis of purines, pyrimidines, nucleotides, and glucosamine (Fig. 3.2). However, a majority of Gln is metabolized to Glu and ammonia (NH_3) by the mitochondrial enzyme, glutaminase (GLS). There are two isoforms of GLS, GLS1 and GLS2, but GLS1 is preferentially expressed in the vasculature (Bertero et al. 2016; Peyton et al. 2018b). The GLS product Glu is then catabolized by Glu dehydrogenase and/or aminotransferases to α-ketoglutarate which enters the tricarboxylic acid (TCA) cycle for ATP synthesis or as an anaplerotic source of carbon for the production of lipids and non-essential amino acids, satisfying both the energetic and macromolecular requirements of cells. Furthermore, Glu is used for the synthesis of the antioxidant glutathione (GSH).

Cross-sectional clinical studies indicate that plasma Gln or the Gln:Glu ratio is inversely associated with higher body mass index, blood

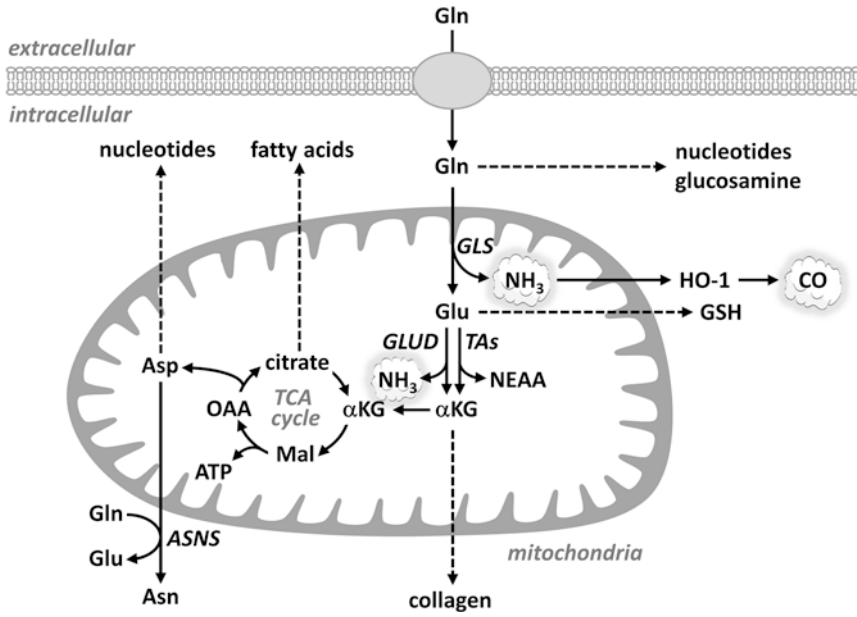

Fig. 3.2 Overview of L-glutamine transport and metabolism. L-Glutamine is transported into cells by various transporters and preferentially metabolized to L-glutamate and ammonia by the mitochondrial enzyme glutaminase. L-Glutamine and L-glutamate can be converted to a number of important molecules. *Asn* L-asparagine, *ASNS* asparagine synthetase, *Asp* L-aspartate, *ATP* adenosine triphosphate, *CO* carbon monoxide, *Gln* L-glutamine, *GLS* glutaminase, *Glu* L-glutamate, *GLUD* glutamate dehydrogenase, *GSH* glutathione, *HO-1* heme oxygenase-1, *αKG* α-ketoglutarate, *Mal* L-malate, NH_3 ammonia, *OAA* oxaloacetate, *TAs* transaminases, *TCA* tricarboxylic acid, *NEAA* nonessential amino acids

pressure, triglycerides, and insulin resistance, whereas plasma Glu levels are associated with adverse metabolic parameters (Newgard et al. 2009; Wurtz et al. 2012; Cheng et al. 2012). In a prospective study, the circulating Gln:Glu ratio was associated with diminished risk for incident cardiovascular disease, while plasma Glu was linked with increased risk (Zheng et al. 2016). Interestingly, small clinical trials found that Gln supplementation enhances myocardial repair and improves risk factors in patients with coronary heart disease (Khogali et al. 2002; Lomivorotov et al. 2011; Sufit et al. 2012). In addition, perioperative oral Gln administration reduces cardiac damage after coronary revascularization (Chavez-Tostado et al. 2017). Moreover, a large prospective study found that dietary Gln intake is inversely related to the risk of cardiovascular mortality, independent of other dietary or lifestyle factors (Ma et al. 2018). Collectively, these studies highlight on important protective role for Gln against cardiovascular disease.

The mechanisms by which Gln promotes vascular health are not fully known. However, experimental studies show that Gln protects ECs against oxidative and inflammatory stress (Hinshaw and Burger 1990; Hsu et al. 2006). In addition, Gln supplementation corrects endothelium-dependent relaxation in L-NMMA-treated animals and lowers blood pressure in mice fed a high-fat diet (Cheng et al. 2012; Addabbo et al. 2013). The metabolism of Gln by GLS1 also prevents EC senescence and is critical for redox homeostasis through the production of GSH (Unterluggauer et al. 2008; Kim et al. 2017; Huang et al. 2017). Furthermore, the selective loss of GLS1 in ECs results in impaired vessel sprouting in mouse models of angiogenesis, suggesting a key role for this enzyme in blood vessel formation (Kim et al. 2017; Huang et al. 2017). More recently, we reported that GLS1 stimulates

EC proliferation and migration by fueling the TCA cycle (Peyton et al. 2018b). Interestingly, we also found that Gln-derived NH_3 is a major driver of heme oxygenase-1 (HO-1) gene expression in ECs (Liu et al. 2017). This is noteworthy since HO-1 is responsible for generating carbon monoxide (CO) from heme (Durante et al. 2006). Much like NO, CO exerts many salutary effects in the circulation. Indeed, we recently documented that NH_3 protects against EC death via the HO-1-mediated generation of CO (Liu et al. 2017). Moreover, the ability of Gln to prevent tissue injury following ischemia-reperfusion may also be HO-1 dependent (Korthuis and Durante 2005). Thus, the NH_3-HO-1-CO signaling axis represents a unique mechanism by which Gln preserves vascular health.

However, increases in glutaminolysis may have undesirable effects. For example, vascular stiffening stimulates the hyperproliferation of pulmonary ECs and SMCs due to the induction of GLS1 (Bertero et al. 2016). GLS1 inhibition or knockdown prevents stiffness-induced cellular hyperproliferation, which is restored by the addition of Glu. Pulmonary artery stiffness and GLS1 expression is increased in the rat monocrotaline model of pulmonary hypertension and Gln measured in isolated pulmonary vascular cells is decreased, suggestive of anaplerotic flux through the TCA cycle. Interestingly, glutaminolysis also fosters fibrosis by stimulating collagen translation via the α-ketoglutarate-mediated activation of mammalian target of rapamycin, setting up a vicious cycle of extracellular matrix stiffening, glutaminolysis, and hyperproliferation (Ge et al. 2018). GLS1 inhibition interrupts this cycle and ameliorates pulmonary hypertension in rats. Similar patterns of Glu and GLS1 expression are noted in rhesus macaques with simian immunodeficiency virus–associated pulmonary hypertension and in lung samples from human patients with pulmonary hypertension. Furthermore, lower blood Gln:Glu ratios are detected in patients who had higher pulmonary arterial pressure, further implicating glutaminolysis in pulmonary arterial hypertension.

GLS1 may also modulate vascular cell function by interacting with NOS. An early report identified a GLS-initiated pathway for Arg synthesis from Gln in macrophages and monocytes (Murphy and Newsholme 1998). This study found that the addition of Gln to macrophages stimulates a significant increase in NO synthesis, while pharmacological inhibition of GLS1 decreases NO generation. In contrast, Gln inhibits the production of NO in cultured ECs (Sessa et al. 1990). However, the inhibitory effect of Gln on NO synthesis does not involve GLS1, as inhibition of this enzyme has no effect of NOS3 expression or activity (Huang et al. 2017). Instead, this inhibitory effect is mediated by the metabolism of Gln by L-glutamine:fructose-6-phosphate aminotransferase to glucosamine, which inhibits pentose cycle activity and lowers NADPH levels that are required for NOS activity (Wu et al. 2001).

3.4 L-Tryptophan

Trp is an essential amino acid that is metabolized via two major pathways (Fig. 3.3). Trp hydroxylase mediates the conversion of 5% of Trp to 5′-hydroxytrytophan, which is then transformed by amino acid decarboxylase to serotonin, an important neurotransmitter and modulator of platelet aggregation and blood pressure. However, a large majority (95%) of Trp is metabolized by the kynurenine (Kyn) pathway. Here, Trp is catabolized by tryptophan 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase (IDO) to N-formylkynurenine. These enzymes form the rate-limiting reaction within the Kyn pathway, with TDO governing basal Trp flux while IDO controls Trp movement through this pathway under disease conditions. There are two isoforms of IDO: IDO1 is widely expressed, inducible, and contributes to a majority of Trp degradation, while IDO2 is constitutively expressed in specific tissues. Once formed, N-formylkynurenine is degraded to Kyn by Kyn formamidase. Kyn is further metabolized to kynurenic acid or anthranilic acid by the actions of kynurenic amino transferases or kynurenase, respectively. Alternatively, Kyn is converted to 3′-hydroxykynurenic acid by kynurenine-3-monooxygenase

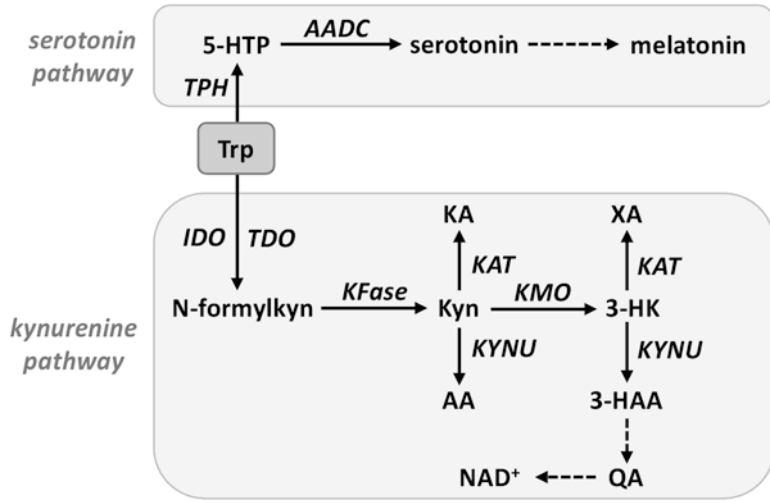

Fig. 3.3 Overview of L-tryptophan metabolism. L-Tryptophan is metabolized by the serotonin or kynurenine pathway. *AA* anthranilic acid, *ADC* amino acid decarboxylase, *Gly* glycine, *3-HAA* 3-hydroxyanthranilic acid, *3-HK* 3-hydroxykynurenine, *IDO* indoleamine 2,3-dioxygenase, *KA* kynurenine acid, *KAT* kynurenine aminotransferase, *KFase* N-formyl-kynurenine formamidase, *5-HTP* 5-hydroxytryptophan, *KMO* kynurenine hydroxylase monooxygenase, *Kyn* kynurenine, *KYNU* kynureninase, *NAD*$^+$ nicotinamide adenine dinucleotide, *N-formylKyn* N-formylkynurenine, *QA* quinolinic acid, *TDO* tryptophan 2,3-dioxygenase, *Trp* L-tryptophan, *TPH* tryptophan hydrolase

which is further metabolized into 3-hydroxyanthranilic acid (3-HAA), and ultimately to quinolinic acid and nicotinamide adenine dinucleotide.

Clinical studies indicate that Trp metabolism via the Kyn pathway is associated with cardiovascular disease. The plasma Kyn:Trp ratio, a surrogate for IDO activity, is increased in patients with coronary artery disease and correlates with established cardiovascular risk factors, including body mass index, LDL, triglycerides, and waist circumference (Wirleitner et al. 2003; Pertovaara et al. 2007). Elevated IDO expression is also observed in the macrophage-rich cores of advanced atherosclerotic plaques in human subjects (Niinisalo et al. 2010). In addition, blood IDO activity is positively associated with increases in carotid artery intima-media thickness, an early marker of atherosclerosis (Niinisalo et al., 2008). Elevated levels of plasma kynurenines including kynurenic acid, 3-hydroxykynurenic acid, anthranilic acid, and 3-HAA also predict the risk of acute myocardial infarction in patients with stable angina pectoris (Pedersen et al. 2015). Furthermore, high kynurenic acid concentrations in plaques are associated with an unstable phenotype whereas undetectable or low levels of this acid are seen in stable plaques, indicating a role for Trp metabolites in modulating plaque stability (Metghalchi et al. 2015).

Experimental studies suggest a role for the Kyn pathway in atherosclerosis. Pharmacological inhibition of IDO results in a significant increase in atherosclerosis in apoE-deficient mice that is paralleled by an increase in the expression of adhesion molecules and chemokines, and accumulation of macrophages within the plaque (Polyzos et al. 2015). Moreover, the acceleration in atherosclerosis and inflammation is reversed by the exogenous administration of 3-HAA. Similarly, genetic deletion of IDO1 in apoE-deficient mice exacerbates atherosclerosis at 15 weeks of age by downregulating the expression of the anti-inflammatory cytokine, interleukin-10 (IL-10) (Cole et al. 2015). Although IDO1 deletion does not alter circulating cholesterol levels, it significantly increases the infiltration of macrophages and

T-cells into the atherosclerotic plaque. Moreover, loss of IDO results in an unstable plaque at 30 weeks of age that is predisposed to rupture. Interestingly, addition of 3,4-dimethoxycinnamoylanthranilic acid, an orally active synthetic derivative of 3-HAA, but not Kyn, increases IL-10 production by splenic B cells and decreases inflammatory cytokine production by human atheroma cell cultures. These results suggest the IDO1-derived 3-HAA protects against atherosclerosis. This notion is further supported by work showing that IDO1-expressing aortic dendritic cells block atherosclerosis in mice by dampening vascular inflammation via the induction of T_{Reg} cells (Yun et al. 2016) and by another study demonstrating that 3-HAA reduces lesion size in LDL receptor-deficient mice by lowering plasma lipids and T-cell-mediated inflammation in plaques (Zhang et al. 2012a). However, in direct opposition, one study found that IDO1-deficiency inhibits the development of atherosclerosis in LDL receptor-knockout mice via an upregulation of IL-10 (Metghalchi et al. 2015). The reason for these conflicting reports is not known but may be related to possible differences in the genetic background of animals. Clearly, additional studies are needed to elucidate the role of IDO in atherogenesis.

The Kyn pathway is also associated with endothelial dysfunction. Infusion of mice with angiotensin II augments IDO expression and 3′-hydroxykynurenic acid content in ECs and this is accompanied by increases in oxidative stress, EC apoptosis, and impaired endothelium-dependent relaxation (Wang et al. 2014). However, these actions of angiotensin II are suppressed in IDO1-deficient mice. Mechanistically, it was determined that angiotensin II-enhanced 3-hydroxykynurenine production stimulates the generation of superoxide by NAD(P)H oxidase leading to EC apoptosis and dysfunction. Interestingly, knockout of IDO1 also averts vascular SMC apoptosis and the development of abdominal aortic aneurysms in angiotensin II-treated LDL receptor-deficient mice, further incriminating IDO1 in the pathological actions of angiotensin II (Metghalchi et al. 2018). In addition, kynurenic acid triggers the adhesion of neutrophils and leukocytes to vascular endothelium via the activation of G protein-coupled receptor 35, suggesting that this acid may lead to the recruitment of leukocytes into the vessel wall (Barth et al. 2009).

The metabolism of Trp also plays an important role in blood pressure regulation. In a seminal study, Wang et al. (2010) reported that both Trp and Kyn dilate pre-constricted blood vessels; however, the dilating response to Trp requires IDO activity and an intact endothelium whereas the response to Kyn is IDO- and endothelium-independent. In addition, they showed that Kyn-induced arterial relaxation is mediated by the activation of soluble guanylate cyclase and adenylate cyclase, and that intravenous infusion of Kyn decreases blood pressure in spontaneously hypertensive mice. They also found that IDO is expressed in ECs of resistance arteries and contributes to the decrease in blood pressure in mouse models of inflammation, and suggested that IDO-derived Kyn is a novel mediator of hypotension in human sepsis. Circulating Kyn levels are elevated in hypertensive versus normotensive patients (Pedersen et al. 2015), and plasma Kyn as well as kynurenic acid, 3-hydroxykynurenic acid, and anthranilic acid are increased in renovascular hypertensive rats, suggesting that activation of the Kyn pathway functions in a compensatory manner to mitigate hypertension. Furthermore, injection of kynurenic acid into the rostral ventrolateral medulla results in a pronounced drop in arterial blood pressure in spontaneously hypertensive rats, indicating that multiple IDO-derived products possess anti-hypertensive properties (Ito et al. 2000). Lastly, IDO-deletion exaggerates pulmonary hypertension, right ventricular hypertrophy, and pulmonary arterial thickening in mice exposed to hypoxia (Xiao et al. 2013). In contrast, augmented pulmonary endothelial IDO expression attenuates the development of pulmonary hypertension and its associated pathologies in rodent models of pulmonary hypertension.

3.5 L-Cysteine

Cys is a semi-essential amino acid that is metabolized to a number of important molecules (Yin et al. 2016). Cys is transformed to GSH via two successive enzymatic reactions (Fig. 3.4). Cys and Glu are first coupled by glutamate cysteine ligase to form γ-glutamylcysteine, which along with Gly is converted by GSH synthase to GSH; an endogenous antioxidant that directly neutralizes free radicals and reactive oxygen species (ROS). Cys is also oxidized by cysteine dioxygenase to cysteine sulfinate. This product is further metabolized by cysteine sulfinate decarboxylase to hypotaurine, which is subsequently oxidized to taurine. Alternatively, cysteine sulfinate is transaminated by aspartate aminotransferase (AAT) to β-sulfinylpyruvate which spontaneously decomposes to SO_2. Finally, H_2S is produced enzymatically from Cys by cystathionine β-synthase and cystathionine γ-lyase (CSE), or by the combined action of Cys amino transferase and 3-mercaptopyruvate sulfurtransferase. Minor amounts of H_2S may be oxidized to SO_2.

Several Cys metabolites exert favorable effects in the circulation. In particular, a high intake of taurine is associated with a reduced risk of cardiovascular disease (Yamori et al. 2001). Furthermore, subjects with higher levels of urinary taurine excretion have lower body mass, blood pressure, and plasma total cholesterol levels. Experimental studies indicate that taurine prevents the development of atherosclerosis in animals (Murakami et al. 2002). Several mechanisms account for the anti-atherosclerotic effect of taurine. Taurine blocks arterial lipid accumulation and the elevation of LDL in animals fed a high-fat/high-cholesterol diet. Taurine supplementation also decreases serum and aortic levels of lipid peroxides in these animals. In addition, taurine protects against EC apoptosis and inflammation induced by oxLDL, and normalizes endothelium-dependent vasodilation in mice fed a high cholesterol diet (Kamata et al. 1996; Ulrich-Merzenich et al. 2007). Taurine also inhibits the proliferation of vascular SMCs and subsequent neointima formation in injured carotid arteries of rats by downregulating ROS production (Murakami et al. 2010).

The signaling gas H_2S has also emerged as a critical regulator of vascular homeostasis. It stimulates blood flow by dilating blood vessels and inhibiting platelet aggregation. H_2S also elicits potent antioxidant, anti-inflammatory, anti-apoptotic, and angiogenic responses. Many of its biological actions occur by targeting proteins for sulfhydration, where sulfur is added to the thiol groups of specific cysteine residues to form a persulfide. This posttranslational modification alters protein function and has been shown to upregulate numerous protective signaling pathways (Meng et al. 2018). In addition, H_2S promotes the NOS3 signaling pathway by enhancing the phosphorylation and activation of the enzyme, stabilizing the enzyme in its dimeric form, and

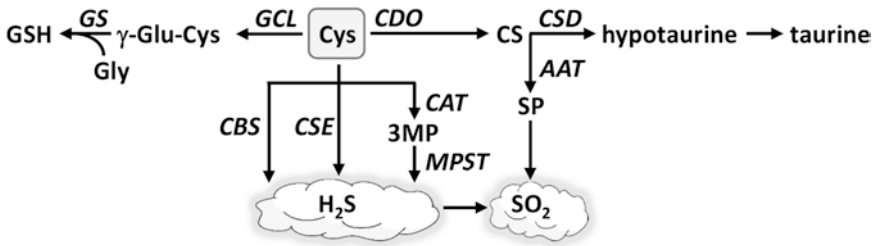

Fig. 3.4 Overview of L-cysteine metabolism to glutathione, taurine, hydrogen sulfide, and sulfur dioxide. *AAT* aspartate aminotransferase, *CAT* cysteine amino transferase, *CBS* cystathionine β-synthase, *CDO* cysteine dioxygenase, *CS* cysteine sulfonate, *CSD* cysteine sulfonate decarboxylate, *CSE* cystathionine γ-lyase, *GCL* glutamate cysteine ligase, *Cys* L-cysteine, *γ-Glu-Cys* γ-glutamylcysteine, *GS* glutathione synthase, *GSH* glutathione, *H_2S* hydrogen sulfide, *3MP* 3-mercaptopyruvate, *MPST* 3-mercaptopyruvate sulfurtransferase, *SO_2* sulfur dioxide, *SP* β-sulfinylpyruvate

inhibiting the activity of phosphodiesterase (Wang et al. 2017).

While all three enzymatic pathways have been identified in vascular cells, CSE is the major regulator of H_2S in the vasculature. Considerable evidence indicates that CSE plays a protective role in atherosclerosis. CSE knockout mice have low circulating levels of H_2S and when fed a high-fat diet develop increased fatty streak formation, oxidative stress, adhesion molecule expression, and aortic intimal proliferation relative to wild-type animals (Mani et al. 2013). Similarly, inhibition or deletion of CSE exacerbates inflammation and atherosclerosis in apoE-knockout mice (Zhang et al. 2012b; Ford et al. 2013). In all instances, restoration of H_2S levels following the administration of a H_2S donor reduces plaque size as well as the formation of inflammatory cytokines and superoxide in these animals (Liu et al. 2013). H_2S administration also improves endothelium-dependent vascular relaxation and circulating levels of NO in apoE-deficient mice (Lin et al. 2016). A deficiency of H_2S is also observed in humans with atherosclerosis. Diminished plasma H_2S levels are detected in chronic hemodialysis patients with accelerated atherosclerosis and in patients with severe coronary artery disease (Gao et al. 2015; Wang et al. 2017). Defective H_2S production has also been observed in patients with diabetes (Jain et al. 2010), and a recent study reported reduced CSE expression and H_2S levels in patients with abdominal aortic aneurysms (Gomez et al. 2016). Thus, oral supplementation with H_2S donors may serve as a therapeutic approach to treat a number of vascular pathologies.

Studies in our laboratory established an important protective role for H_2S in ischemia-reperfusion injury (Zuidema et al. 2011). Preconditioning of animals with H_2S prior to an ischemic insult of the murine small intestine causes post-capillary venules to shift to an anti-inflammatory phenotype such that these vessels fail to support increases in post-ischemic leukocyte rolling and adhesion. H_2S also induces HO-1 activity in this setting, and pharmacological blockade or genetic deletion of HO-1 prevents the anti-adhesive actions of antecedent H_2S, implicating HO-1 in this process. H_2S also defends against ischemia-reperfusion injury in other tissues via both HO-1-dependent and independent mechanisms (Wu et al. 2015). By dilating resistance arteries, H_2S also contributes to the maintenance of arterial blood pressure (Kanagy et al. 2017). Pharmacological inhibition of H_2S synthesis elevates blood pressure in rats while global deletion of CSE results in age-dependent increases in blood pressure in mice. Contrarily, administration of H_2S donors reduces blood pressure in animal models of hypertension.

There is some evidence that SO_2 also serves as a signaling molecule in the circulation. Endogenously derived or exogenously administered SO_2 relaxes blood vessels in the rat. The mechanisms underlying this response are complex involving ion channels and cyclic nucleotides (Huang et al. 2016). In addition, SO_2 and its derivatives decreases arterial hypertension in rats, and lowers mean pulmonary arterial blood pressure and improves structural pulmonary arterial remodeling by blocking collagen deposition, pulmonary arterial SMC proliferation, and inflammation in rodents with pulmonary hypertension (Jin et al. 2008; Sun et al. 2010). SO_2 administration also diminishes atherosclerosis and reduces myocardial infarct size following ischemia-reperfusion in rodents (Li et al. 2011; Wang et al. 2011b).

3.6 Summary

There is an emerging appreciation for the role of amino acids in vascular health. Amino acids serve as precursors for the synthesis of a remarkable array of molecules that impact vascular function. Of particular importance is the metabolism of amino acids to signaling gases such as NO, CO, NH_3, H_2S, and SO_2 which underlie many of the cardioprotective actions of amino acids. These gases along with other amino acid metabolites play a central role in preserving vascular homeostasis by regulating critical cellular processes such as proliferation, migration, differentiation, apoptosis, contractility, and senescence. In addition, amino acids and their

metabolites exert potent anti-inflammatory and antioxidant effects in the circulation and block the accumulation of lipid within the vessel wall. Moreover, they mitigate known risk factors for cardiovascular disease, including hypertension, hyperlipidemia, obesity, and diabetes. However, in some instances, the metabolism of amino acids through certain pathways may yield compounds that promote vascular disease.

Preclinical and clinical studies have identified deficiencies in circulating levels of amino acids in a number of vascular pathologies. While supplementation with amino acid monotherapy targeting the deficiency has successful ameliorated arterial disease in many animal models, mixed results have been reported in the clinic. In such situations combination therapy should be considered. In the case of Arg deficiency, co-administration of Arg and Cit results in a more rapid increase in plasma Arg levels and marked augmentation of NO availability than supplementation of the single amino acid alone (Morita et al. 2014). Combining amino acid supplementation with antioxidants and anti-inflammatory agents to better combat the oxidative and inflammatory environment of atherosclerosis should also be considered. In addition, the use of specific pharmacological inhibitors may further optimize amino acid supplementation strategies by channeling amino acids towards beneficial metabolic pathways. For example, the use of arginase or NOS2 inhibitors may increase the efficacy of Arg and/or Cit supplementation by directing Arg to NOS3 while avoiding the potential harmful arginase and NOS2 pathways. Alternatively, one may bypass dangerous pathways of metabolism by administering specific amino acid metabolites with known protective actions rather than the parent molecule. Particularly attractive is the administration of amino acid-derived gases or synthetic analogues of amino acid metabolites. A number of gas-releasing molecules and stable amino acid derivatives have been developed and proven safe and efficacious in various experimental models, and are currently undergoing clinical testing (Cole et al. 2015; Ismailova et al. 2018; Li et al. 2018). Finally, there is a growing realization that circulating levels of multiple amino acids may be perturbed in patients with vascular disease. Thus, a more holistic approach that targets all these amino acid derangements may be needed to fully restore circulatory function in these patients.

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4 Epithelial Dysfunction in Lung Diseases: Effects of Amino Acids and Potential Mechanisms

Jingqing Chen, Yuhang Jin, Ying Yang, Zhenlong Wu, and Guoyao Wu

Abstract

Lung diseases affect millions of individuals all over the world. Various environmental factors, such as toxins, chemical pollutants, detergents, viruses, bacteria, microbial dysbiosis, and allergens, contribute to the development of respiratory disorders. Exposure to these factors activates stress responses in host cells and disrupt lung homeostasis, therefore leading to dysfunctional epithelial barriers. Despite significant advances in therapeutic treatments for lung diseases in the last two decades, novel interventional targets are imperative, considering the side effects and limited efficacy in patients treated with currently available drugs. Nutrients, such as amino acids (e.g., arginine, glutamine, glycine, proline, taurine, and tryptophan), peptides, and bioactive molecules, have attracted more and more attention due to their abilities to reduce oxidative stress, inhibit apoptosis, and regulate immune responses, thereby improving epithelial barriers. In this review, we summarize recent advances in amino acid metabolism in the lungs, as well as multifaceted functions of amino acids in attenuating inflammatory lung diseases based on data from studies with both human patients and animal models. The underlying mechanisms for the effects of physiological amino acids are likely complex and involve cell signaling, gene expression, and anti-oxidative reactions. The beneficial effects of amino acids are expected to improve the respiratory health and well-being of humans and other animals. Because viruses (e.g., coronavirus) and environmental pollutants (e.g., PM2.5 particles) induce severe damage to the lungs, it is important to determine whether dietary supplementation or intravenous administration of individual functional amino acids (e.g., arginine-HCl, citrulline, N-acetylcysteine, glutamine, glycine, proline and tryptophan) or their combinations to affected subjects may alleviate injury and dysfunction in this vital organ.

Keywords

Amino acids · Lung dysfunction · Barrier integrity · Inflammatory response · Signaling pathways

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Abbreviations

CaMKK2	calcium/calmodulin-dependent kinase 2
COPD	chronic obstructive pulmonary disease
IL-6	interleukin 6
Keap1	Kelch-like ECH associated protein 1
LPS	lipopolysaccharide
NF-κB	nuclear factor kappa B
NLR	Nod-like receptors
NO	nitric oxide
Nrf2	nuclear factor erythroid 2-related factor
PAR	protease-activated receptors
PRRs	pathogen recognition receptors
RLR	RIG-I-like receptors
ROS	reactive oxygen species
Th	T helper
TJs	tight junctions
TLR	Toll-like receptors
TNF-α	tumor necrosis factor alpha
ZO	zonulae occludens

4.1 Introduction

The lungs are the foundational organs of the respiratory system, whose most important function is to facilitate gas exchange between the environment and the bloodstream (Zhang et al. 2018). In this process called respiration, oxygen in the ambient air enters the blood, whereas CO_2 (a product of nutrient metabolism) leaves the blood. Structurally, the bronchial monolayer epithelium in the respiratory system is responsible for preserving airway homeostasis in the lungs. Disruption of the barrier integrity by various stimuli, such as toxins, chemical pollutants, detergents, viruses, bacteria, microbial dysbiosis, and allergens, can contribute to the development of lung diseases (Budden et al. 2017; Georas and Rezaee 2014). Biochemically, these endogenous or exogenous risk factors activate an abnormal inflammatory response in the respiratory system, leading to the accumulation of reactive oxygen species (ROS), the breakdown of the epithelial integrity in the airways and alveoli, and ultimately reduced airflow capacity and lung dysfunction (Agusti and Hogg 2019; Guo and Ward 2007; Lang et al. 2002; Rahman et al. 2005; Zhang et al. 2018).

Despite significant advances in the pathogenesis of lung diseases in the last two decades, currently clinical therapies for lung diseases are largely dependent on the application of bronchodilators or glucocorticoids to improve airflow and ameliorate clinic symptoms in patients (Atto et al. 2019; Boskabadi et al. 2018; Bream-Rouwenhorst et al. 2008; Grainge and Rice 2010). Growing evidence has shown that the impairment of epithelial barrier in the alveoli is a critical step for the initiation and development of lung diseases (Steelant 2020). In our recent study, we found that dietary supplementation with L-arginine or glycine reduced immune cell infiltration, decreased mRNA levels for inflammatory cytokines and chemokines, and decreased the apoptosis of alveolar cells in LPS-challenged mice (Ma et al. 2019). These findings provide a new nutritional strategy to ameliorate lung injury through oral administration of functional amino acids. In this article, we reviewed recent progress in epithelial barrier biology and pathobiology related to lung disease. We also discussed potential mechanisms responsible for the beneficial effects of some amino acids in human patients and animal models.

4.2 Respiratory Barrier Integrity

The lung is made up of dozens of cell types and has evolved architecturally into a series of branching airways and alveoli to support an efficient permeable transfer of oxygen and carbon dioxide for the respiratory system and the whole body (Warheit-Niemi et al. 2019). An appropriate function of the lung is predominantly dependent on the alveolar epithelial cells, one of the predominant cells in the respiratory tract (Fig. 4.1). Under physiological conditions, the intracellular homeostasis and the normal function of the lungs are maintained through nutrient metabolism and

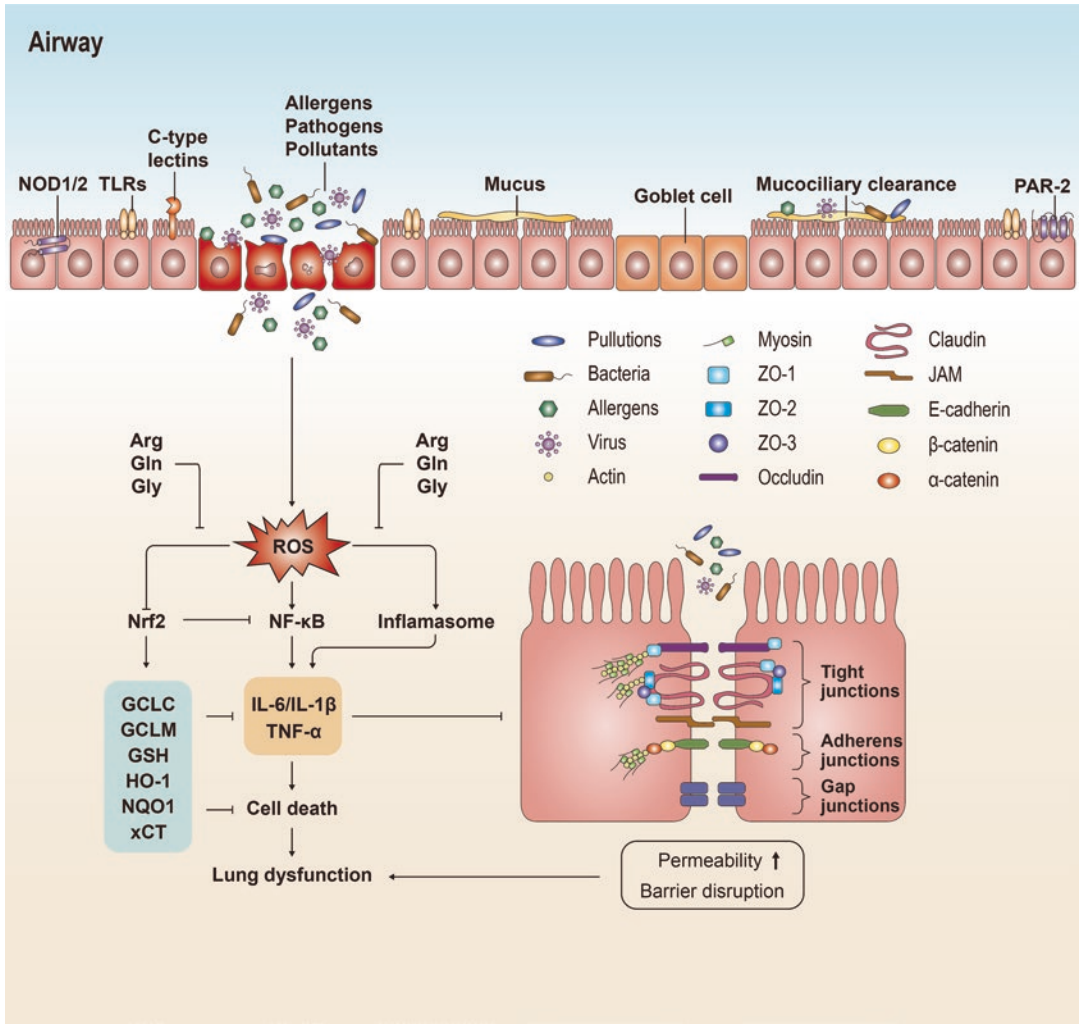

Fig. 4.1 Functional amino acids activate Nrf2 survival signaling, while inhibiting NRLP3 inflammasome to alleviate lung injury. Air pollutants, cigarette smoke and bacterial or viral infections cause oxidative stress and inflammation in the lungs. Nrf2 activation induces the expression of cytoprotective genes to counteract the toxic effect of *ROS* and inhibits the transcription of proinflammatory cytokines, especially in macrophages, to reduce the recruitment of inflammatory cells into the lungs. Increased levels of ROS reduce Nrf2 and activate inflammasome, leading to the upregulation of IL-1β, IL-6, and TNF-α expression as well as the death of lung epithelial cells and consequently emphysema. Functional amino acids such as Arg, Gln, Gly activate the Nrf2 survival signaling to increase the expression of anti-oxidative genes possibly through modulating the NLRP3 inflammasome to inhibit the pro-inflammatory factors, thus improving the lung epithelial barrier. Arg, L-arginine; *Gly,* glycine; Gln, L-glutamine; *IL,* interleukin; *ROS,* reactive oxygen species; *Nrf2,* nuclear factor erythroid 2-related factor 2

its regulation. However, following a severe or prolonged insult or injury to the alveolar epithelial cells, over-activation of immune responses leads to the accumulation of ROS, increased infiltration by immunocytes, and impaired function of the lungs (Kosmider et al. 2011; Steelant 2020).

The neighboring alveolar epithelial cell of the respiratory airway are held together by tight junctions (TJs) and adherens junctions, therefore forming a physical barrier against external particles and a first line of defense of the mucosal immunity (Georas and Rezaee 2014; Lambrecht and Hammad 2014). Similar to TJ proteins in the gas-

trointestinal tract, the claudin family proteins, occludin, and scaffolding proteins, such as zonulae occludens (ZO)-1, ZO-2, ZO-3, multi-PDZ domain protein 1, and others, have been identified as main components of the TJ complexes in the lungs (Sugita et al. 2018). A decrease in the abundance of TJ proteins and an increase in the epithelial permeability have been observed in multiply lung diseases, such as asthma, chronic obstructive pulmonary disease (COPD), and allergic lung inflammation (Georas and Rezaee 2014; Lambrecht and Hammad 2014; Tatsuta et al. 2019). Disruption of TJs with a leaking epithelium allows a close contact of airway lumen contents (including pathogens, allergens, and toxins) with immune cells, triggering the activation of inflammatory responses (Mattila et al. 2011; Wang et al. 2019; Wieczfinska et al. 2015). In addition, the respiratory fluid, mucus, surfactant proteins, and the motility of cilia are critical for the mucosal barrier function and the innate immune response by promoting the elimination of luminal contents. If this defense system is impaired or cannot be repaired, epithelial damage and lung dysfunction will occur (Price and Sisson 2019). Consistently, therapeutic molecules with an ability to regulate the abundances of TJ proteins and surfactant proteins, as well as the secretion of the mucins have been reported to improve mucosal barrier integrity and lung function in human patients and animal models (Boskabadi et al. 2018; Kouis et al. 2018; Ma et al. 2019; Marudamuthu et al. 2019).

4.3 Amino Acid Metabolism in the Lungs

Amino acids enter the lungs from the blood circulation via various sodium-dependent and independent transporters (Table 4.1). There is no *de novo* synthesis of citrulline, arginine, cysteine, three branched-chain AAs (BCAAs; isoleucine, leucine and valine), methionine, phenylalanine, taurine, threonine, tryptophan, tyrosine, histidine, or lysine in the lungs (Wu 2013). In addition, the lungs lack: (a) the transsulfuration pathway for converting methionine into cysteine (Berggren et al. 1984); (b) phenylalanine hydroxylase for converting phenylalanine into tyrosine (McGee et al. 1972); and (c) enzymes for converting taurine, threonine, tryptophan, tyrosine, histidine, or lysine into pyruvate, acetyl-CoA or the intermediates of the Krebs cycle (Wu 2013). However, the lungs can synthesize: (a) arginine from citrulline via argininosuccinate synthase and lyase (Wu and Morris Jr. 1998); (b) ornithine and proline from arginine via arginase-II, ornithine aminotransferase, and pyrroline-5-carboxylate reductase; (c) alanine, glutamate and glutamine from BCAAs plus α-ketoglutarate (α-KG) via BCAA transaminase, glutamate transaminase, and glutamine synthetase (Souba et al. 1990); and (d) glutamate and aspartate from glutamine via phosphate-activated glutaminase, glutamate: pyruvate transaminase (alanine transaminase), and glutamate: oxaloacetate transaminase (aspartate transaminase); and (e) ornithine from proline via proline oxidase and ornithine aminotransferase (mitochondrial enzymes; Wu et al. 1997). These synthetic reactions also serve as metabolic pathways for the catabolism of arginine, alanine, glutamate, glutamine, aspartate, and proline in the lungs. In addition, serine and glycine are interconvertible in this organ through the action of serine hydroxymethyltransferase (present in both the cytosol and mitochondria), which plays an important role in both pulmonary health and the treatment of lung cancer (Amelio et al. 2014). Furthermore, in all cell types of the lungs (including macrophages, endothelial cells, epithelial cells, and smooth muscle cells), nitric oxide (NO; a major vasodilator and signaling molecule) and citrulline are generated from arginine by NO synthase (Folkerts et al. 2001), whereas ornithine decarboxylase decarboxylates ornithine to form putrescine (Hoet and Nemery 2000). The latter is converted into spermidine and spermine by decarboxylated 5-adenosylmethionine-dpendent spermidine synthase and spermine synthase (cytosolic enzymes), respectively. The polyamines (putrescine, spermidine and spermine) are essential for DNA and protein syntheses and, therefore, play an important role in pulmonary health and diseases (including lung cancer; Agostinelli et al. 2020). During

Table 4.1 Amino acid (AA) transporters in the mammalian lungs

Protein name	Gene name	Transport system	Transport mechanism	Major AA	Consequences of defects
EAAT3/EAAC1	*SLC1A1*	X^-_{AG}	Na^+	L-Glu, D/L-Asp, L-Cys	Metabolic disorders
ASCT1	*SLC1A4*	ASC	Antiporter	L-Ala, L-Ser, L-Cys	Metabolic disorders
ASCT2	*SLC1A5*	ASC	Antiporter	L-Ala, L-Ser, L-Cys	Metabolic disorders
rBAT	*SLC3A1*	HC-HAAT	Exchanger	Neutral and basic AAs	Cystinuria
				(heterodimer with SLC7A9)	
4F2hc	*SLC3A2*	HC-HAAT	Exchanger	Neutral AAs	Metabolic disorders
				(heterodimer with SLC7A5-8	
				and SLC7A10–11)	
GlyT1	*SLC6A9*	Gly	Na^+/Cl	Gly	NKHG
$ATB^{0,+}$	SLC6A14	Gly	Na^+/Cl	Neutral AAs and cationic AAs	Obesity; abnormal
					Food intake
CAT-1	*SLC7A1*	y^+	Uniporter	Basic AAs	Hyptertension
CAT-2	*SLC7A2*	y^+	Uniporter	Basic AAs	Inflammation
ORC1/ORNT1	*SLC25A15*	Orn/Cit carrier	H^+/antiporter	Mit L-Orn/L-Cit exchange	HHH syndrome
GC2	*SLC25A18*	Glu carrier	H^+-coupled;	L-Glu	Metabolic disorders
			OH^-/antiporter		
Not assigned	*SLC38A10*	A	Na^+	L-Gln, L- Ala	Metabolic disorders
LAT3	*SLC43A1*	L	Uniporter	Large neutral AAs	Metabolic disorders
Cystinosin	*SLC66A4*	LCT	H^+-coupled	Efflux of Cys from lysosome	Metabolic disorders

Adapted from Kanai and Hediger (1992), Kandasamy et al. (2018) and Wu (2013)
HC-HAAT heavy chain of heteromeric amino acid transporter, *HHH* hyperornithinemia-hyperammonemia-homocitrullinuria, *LCT* lysosomal cystine transporter, *MGT* mitochondrial glycine transporter, *it* mitochondrial, *NKHG* non-ketotic hyperglycemia, *rBAT* related to $b^{o,+}$ amino acid transporters

sepsis or LPS infection, the lungs export an increased amount of glutamine by inducing the expression of glutamine synthetase at the transcriptional level partially mediated by glucocorticoid hormones (Lukaszewicz et al. 1997).

4.4 Functional Amino Acids, a Paradigm Shift in Protein Nutrition

Besides serving as building blocks for proteins, which are the most fundamental component in tissues, amino acids have enormous physiological importance, such as the synthesis of low molecular-weight substances (e.g., nitric oxide, polyamines, creatine, carnosine, dopamine, serotonin, and glutathione), regulate metabolism and immune response, and maintain intestinal barrier function (Wu 2013; Wu et al. 2014). Based on studies on the fundamental effects of amino acids, as well as their metabolism and biological functions, a new concept of "functional amino acid" has been proposed (Hou and Wu 2017). In contrast to the traditional classification of nutritionally essential or non-essential amino acids, which were defined based on the criterion of growth or nitrogen balance, the con-

cept of "functional amino acids" is based on physiological functions of amino acids to improve survival, growth, development, lactation, reproduction, and health of humans and other animals (Wu 2014). This view advances our understating of amino acid nutrition and metabolism, as well as dietary requirements for amino acids to maintain various functions of cells and tissues under both physiological and pathological conditions. Accumulating evidence has shown that functional amino acids are critical for the regulation of protein synthesis, gene expression, immune response, intestinal barrier function, and cellular fate decision (Hou et al. 2015; Wu et al. 2014).

4.5 Effects of Functional Amino Acids on Respiratory Barrier Function

The pulmonary epithelium is the predominant cells that prevent the entry of luminal contents into the blood circulation, while ensuring proper gas exchange. A relationship between low TJ protein abundance and high epithelial permeability has been reported for the gastrointestinal or respiratory epithelium of human patients and animal models (He et al. 2017). This observation supports a critical role of TJ proteins in epithelial integrity and prompts new search for molecules or compounds to reduce epithelial permeability and improve mucosal barrier function using both *in vivo* or *in vitro* models (Atto et al. 2019). A comparative study has identified differences in the metabolism of amino acids between patients with and without bacterial infection during the early stage of chronic obstructive pulmonary disease (COPD) (32). Specifically, COPD patients affected with bacterial infection had lung dysfunction, which was accompanied by decreased plasma levels of asparagine, citrulline, glutamine, histidine, methionine, serine, and threonine, compared with COPD patients without bacterial infection (Inoue and Ikeda 2019). This finding links lung injury with the abnormal metabolism of amino acids under pathological conditions.

L-Arginine (Arg), a product of glutamine (via the formation of glutamate) and proline metabolism via the intestinal-renal axis (Wu and Morris 1998), is a critical substrate for nitric oxide (NO) production by NOS, as noted previously. It has been shown that plasma Arg concentration is reduced in animal models of lung injury, including sheep (Murakami et al. 2007), rabbits (Chao et al. 2011; Yoshida et al. 1999), and rodents (Chu et al. 2005; Mabalirajan et al. 2010). This is likely due to increases in the expression of arginase-II in extrahepatic tissues and the leakage of hepatic arginase-I and extrahepatic arginase II into the blood, resulting in an excessive hydrolysis of arginine into ornithine plus urea. Supplementation with Arg leads to an increased bioavailability of Arg, which in turn, restores endothelial function, decreases inflammatory response, improves bronchial epithelial barrier, and mitochondrial dysfunction, therefore improving the lung function (Chao et al. 2011; Mabalirajan et al. 2010). L-Citrulline can be converted into L-argininosuccinate by argininosuccinate synthase, and L-argininosuccinate is subsequently converted into Arg by argininosuccinate lyase (Curis et al. 2005). These two enzymes are present in the lungs and other tissues in mammals and birds. Oral or intravenous administration of L-citrulline can enhance the circulating levels of Arg and systemic synthesis of NO, thereby attenuating hyperoxia-induced lung damage (Grisafi et al. 2012). Of particular note, dietary supplementation with arginine, which is safe for healthy adult humans (up to 30 g/day in divided doses; McNeal et al. 2018) as well as growing and adult pigs (up to 2% in the diet; Wu et al. 2016), prevents or alleviates pulmonary hypertension and injury in humans and farm animals under various pathological conditions (Wu 2020; Wu et al. 2000).

L-Glutamine (Gln) is the most abundant amino acid in the plasma of both humans and many other animals (Wang et al. 2015a). A critical function of Gln in maintaining intestinal mucosal barrier integrity has been described in various animal models (Jiao et al. 2015; Wu 2013; Wu et al. 2014). Depletion of plasma Gln is

associated with the impairment of intestinal barrier breakdown, which can be abolished by Gln supplementation in animals and human patients. We have investigated the physiological functions of amino acids in piglets, a well-known animal model for studying nutrition and metabolism. Our studies indicate that dietary supplementation with Gln, glutamate, or glycine attenuates weaning- or oxidative stress-induced epithelial barrier dysfunction by regulating the abundance and intracellular localization of TJ proteins (Fan et al. 2019; Wang et al. 2015b), as well as apoptosis (Fan et al. 2019; Jiao et al. 2015; Liu et al. 2018; Wang et al. 2014) and unfolded protein response (He et al. 2019) in piglets. Also, we found that Gln regulates the abundance of TJ proteins and intestinal barrier in a CaMKK2-dependent manner, thereby contributing to improvements in intestinal nutrient absorption and protein synthesis in weanling piglets (Wang et al. 2016; Wang et al. 2015b). It remains unknown whether these amino acids affect alveolar epithelium in virus- or endotoxin-challenged lungs.

We have conducted animal studies to address the foregoing issue. In our work, mice pretreated with aerosolized Arg, Gln, or glycine were exposed to aerosolized LPS to induce lung injury. We found that Arg or glycine pretreatment reduced LPS-induced collagen deposition, apoptosis of alveolar cells, decreased mRNA levels for inflammatory cytokines and chemokines, and reduced the accumulation of neutrophils and macrophages in the lung tissues of mice, thus contributing to an improved respiratory function (Ma et al. 2019). Gln administration reduced LPS-induced collagen deposition and inflammatory cytokines without affecting other parameters examined in the study (Ma et al. 2019). More studies are required to uncover underlying mechanisms responsible for these beneficial effects. In a previous study, Zhang et al. (2007) reported that Gln supplementation attenuated an LPS-induced increase in bronchoalveolar epithelial permeability and a concomitant decrease in the abundance of TJ proteins. The latter include occludin, zonula occludens (ZO)-1, and adherens junction protein E-cadherin. Clearly, increasing Gln availability protected the alveolar epithelium against barrier dysfunction and lung injury in rats (Zhang et al. 2007). These observations support the view that supplementation with functional amino acids, such as Arg, Gln, or glycine, may offer a novel nutritional strategy to reduce the deleterious effects of bacterial infection on alveolar function.

4.6 Effect of Amino Acid on Cellular Metabolic Programming and Lung Injury

Metabolic programming is one of the important mechanisms by which cellular responses are regulated under specific conditions (Vigeland et al. 2019). Dysregulation of metabolic reprogramming in lung diseases, such as asthma and COPD, might impair the innate function of immune cells (Michaeloudes et al. 2019). Gln utilization by lungs and other tissues increases under various stress conditions due to enhanced expression of mitochondrial phosphate-activated glutaminase, such that Gln becomes a conditional essential amino acid (Wilmore and Shabert 1998). Inhibition of glutamine metabolism with 6-diazo-5-oxo-L-norleucine that binds to glutamine-utilizing enzymes and transporters, accelerated recovery from LPS-induced acute lung injury, as shown by reduced immune cell infiltration and decreased protein levels for pro-inflammatory cytokines and chemokines (Vigeland et al. 2019). This metabolic programming is mainly due to the fact that immune cells, such as neutrophils, macrophages, and lymphocytes have a high metabolic rate and rely on Gln metabolism to support activation and immune response in response to stress or bacterial infection (Wang et al. 2015a). Also, a depletion of Gln in blood represents a riskf for poor treatment outcomes and is associated with increased mortality in critical illness (Oudemans-van Straaten et al. 2001). Consistently, Gln supplementation reduces abdominal sepsis, or LPS-induced lung injury in animals models (Lai et al. 2014). Consistently, a deficiency of ASCT2 (a transporter of Gln and small neutral amino acids) impaired the induc-

tion of T helper 1 (Th1) and Th17 cells, attenuated activation of mTORC1 signaling, and inflammatory T cell responses in mouse models, further substantiating a functional role of cellular programming on immunocyte activation and inflammatory responses (Nakaya et al. 2014). Gln enhances expression of AST2increases its availability in the cells to alleviate the adverse effects of general immune activation (Nakaya et al. 2014). In addition, Gln promotes tissue repair by enhancing the production of growth factors by immune cells. For example, EGF-like growth factor, amphiregulin (AREG), is a growth factor produced by macrophages, regulatory T cells, and type-2 innate lymphoid cells in the animal models of lung injury (Xu et al. 2016). Administration of an antibody to neutralize AREG has been reported to exacerbate LPS-induced lung injury (Ogata-Suetsugu et al. 2017; Xu et al. 2016). Inhibition of Gln metabolism with 6-diazo-5-oxo-L-norleucine increased the mRNA level of AREG in immune cells, which in turn promoted tissue repair and improved the function of the lungs in mice (Vigeland et al. 2019). These findings indicate a novel target for the prevention and treatment of lung injury by interfering with the metabolic programming of immunocytes. More studies are warranted to validate these effects in other inflammatory lung diseases and to elucidate the underlying molecular mechanisms.

The fibroblast is the most abundant mesenchymal cell responsible for producing the majority of extracellular matrix proteins (Nieweld and Summer 2019). Differentiation of lung fibroblasts into myofibroblasts leads to excessive deposition of extracellular matrix proteins, which impair the lung architecture and alveoli barrier function (Nalysnyk et al. 2012). However, metabolic requirements for myofibroblast activation and matrix production are largely unknown. In a recent study, using human lung fibroblasts as a model, Hamanaka et al. (2019) demonstrated that Gln-derived biosyntheses of glycine and proline through inter-organ metabolism are required for myofibroblast differentiation and collagen protein production in human lung fibroblasts (Hamanaka et al. 2019). This result has identified a novel metabolic reprogramming of fibroblasts in the development of pulmonary fibrosis. Both glycine and proline are necessary to maintain the extracellular matrix of lungs, and may also play an important role in their anti-oxidative reactions (Li and Wu 2018). It remains unknown how different host cells regulate their metabolism pathways in response to Gln depletion or supplementation in the body.

4.7 Effects of Amino Acids on NLRP3 Inflammasomes and Lung Diseases

The innate immune system acts as the first line of defense in response to environmental risk factors. A serial of pathogen recognition receptors (PRRs), such as Toll-like receptors (TLR), RIG-I-like receptors (RLR), protease-activated receptors (PAR), Nod-like receptors (NLR), C-type lectin receptors, sense the pathogens in contact with the airway epithelium (Hartl et al. 2018). Nod-like receptors (NLRs), including NOD1, NOD2, and NLRP3, are intracellular pattern recognition molecules that can detect microbial- and danger-associated molecular patterns.

NLRP3 inflammasomes is a multiprotein large-cytoplasmic complex that is composed of NLRP3, apoptosis-associated speck-like proteins (ASC), and pro-caspase-1. Activation of NLRP3 has emerged as an important regulator of lung diseases (Xu et al. 2018). Both LPS and ROS have been reported to activate the NLRP3 inflammasome and caspase-1, promote the cleavage and maturation of pro-interleukin (IL)-1β (biologically the most active cytokine in the lungs of patients), resulting in damage to lung tissue (Xu et al. 2018). The resistance of NLRP3-deficient mice to polymicrobial sepsis-induced lethality validates a critical role of NLRP3 in the pathogenesis of lung disease (Chen et al. 2019; Fukumoto et al. 2013; Lee et al. 2017; Liu et al. 2019). We found that glycine administration reduced the LPS-induced accumulation of neutrophils and macrophages, as well as inflammatory responses and collagen deposition in the lung tissues of mice (Ma et al. 2019). Further study showed that

LPS-induced upregulation of NLPP3 and IL-1β expression was reduced by glycine supplementation (Zhang et al. 2020), indicating a regulatory effect of glycine on decreasing NLRP3 inflammasomes. Considering that ROS are activators of NLRP3 but glycine alleviates ROS-induced cellular damage by promoting the synthesis of GSH (an endogenous antioxidant) in intestinal porcine epithelial cells (Wang et al. 2014), it is plausible that administration of glycine might modulate the NLRP3/IL-1β signaling, therefore improving the lung epithelial barrier in LPS-challenged mice.

4.8 Effects of Amino Acid on the Nrf2 Signaling and Lung Injury

The nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of genes implicated in protection against oxidative damage in the lungs and other tissues (Cho and Kleeberger 2020; Mizumura et al. 2020). Under normal conditions, Nrf2 is continuously degraded in a Kelch-like ECH associated protein (Keap)1-dependent manner through the proteasome pathway. However, in the presence of elevated concentrations of ROS or electrophiles, Nrf2 is stabilized due to the disruption of Keap1-mediated repression. This leads to the accumulation of NrF2 in the nucleus to activate the expression of anti-oxidative genes, such as catalytic and modulatory subunits of the GSH synthesizing enzyme glutamate-cysteine ligase (GCLC and GCLM), thioredoxin reductase 1, hemeoxygenase-1 (HO-1), NAD(P)H Quinone Dehydrogenase-1 (NQO-1), xCT, a subunit of cystine/glutamate transporter (Qian et al. 2018). Thus, NrF2 plays an important role in alleviating oxidative damage and improving the function of the lungs. Consequently, Nrf2-knockout mice are more susceptible to LPS-induced lung inflammation than their wild-type counterparts, as indicated by neutrophils infiltration, as well as the elevated levels of proinflammatory cytokines (tumor necrosis factor-α and interleukin-6) and chemokines (macrophage inflammatory protein 2 and magnesium-dependent phosphatase 1) (Li et al. 2017; Thimmulappa et al. 2006).

In critical illness or in response to severe stress, serum Gln level is decreased which is accompanied by a modest therapeutic outcome and an increased mortality (Oudemans-van Straaten et al. 2001). Gln supplementation promotes the expression of subunits of the GSH synthesizing enzyme, GCLC and GCLM, in a Nrf2-dependent manner, leading to increases in the synthesis and concentration of GSH and reduced cellular damage in intestinal and lung tissues (Venoji et al. 2015). In a recent study, de Oliveira et al. (2019) found that Gln treatment reduced myeloperoxidase activity, decreased inflammatory responses, and improved both the morphological alteration and function in the lungs of LPS-challenged mice. We also observed that functional amino acids (e.g., glycine) activated Nrf2 survival signaling, while inhibiting NRLP3 inflammasome (Zhang et al. 2020), indicating a regulatory effect of amino acids on the Nrf2 signaling pathway. Note that glycine is highly abundant in meat (e.g., beef; Wu et al. 2016) but is relatively deficient in all plant-source proteins (Hou et al. 2019; Li and Wu 2020). Therefore, antioxidant agents or functional foods that modulate Nrf2 would be expected to potentially therapeutic options to alleviate lung injury by enhancing intracellular concentrations of antioxidant molecules (including enzymes) in the alveolar epithelium.

4.9 Effects of Amino Acids on the Lung Microbiota and Lung Disease

Healthy lungs were traditionally believed to be sterile due to their effective antimicrobial defenses (Budden et al. 2017). This view was challenged by the isolation of bacteria from the respiratory tract of healthy individuals with the use of culture-independent approaches for microbial community profiling (Faner et al. 2017). Compared with the gastrointestinal tract where more than 100 trillion microorganisms reside, the lungs of humans and animals harbor 10^3–10^5 CFU/g of tissue (Remot et al. 2017), with *Bacteroides*, *Firmicutes* and *Proteobacteria* being the predominant phyla commonly observed

(He et al. 2017). There is a relatively much lower abundance of bacteria in the lungs than in the gastrointestinal tract. However, increasing evidence indicates that lung microbiota dysbiosis is a critical environmental factor that interacts with host cells and contributes to the pathogenesis of multiple lung diseases through various mechanisms (Dickson et al. 2013; He et al. 2017; Wu and Segal 2017). First, microbes play an important role in shaping the normal and pathologic immune responses in lungs (He et al. 2017). Deregulation of microbiota in the lungs may predispose humans and other animals to the development of respiratory disease, and has a significant impact on clinical outcomes of respiratory disorders (Segal et al. 2014). Also, the microflora in the lungs can translocate to the gastrointestinal tract and other tissues through blood circulation, therefore triggering inflammatory responses (Sze et al. 2014). Additionally, intestinal bacteria influence the composition and diversity of microbiota in the lungs, therefore forming a bidirectional gut-lung axis (Budden et al. 2017; He et al. 2017). More studies are required to uncover the complicated crosstalk between the lungs and gut, as well as their impacts on health.

It is known that the intestinal microbiota affects the physiology, metabolism, and immunity of the host through: (a) interactions with enterocytes and immune cells of the gastrointestinal tract; and (b) the production of bacterial metabolites. Studies with pigs have shown that most of the amino acids in the intestinal lumen can be utilized by intestinal bacteria for protein synthesis and catabolism at various rates (Dai et al. 2012a; Dai et al. 2011), therefore affecting the proportions of dietary amino acids entering the portal vein, as well as the availability of amino acids for various cells in the lungs and colonized bacteria. This may be a reason why the intestinal microbiota affects the development of lung diseases as observed in clinical patients and experimental animals (O'Dwyer et al. 2016). Of note, dietary supplementation with functional amino acids (e.g., Gln, Trp and Arg) has been reported to regulate the bacterial ecosystem of the gut and improve the intestinal health of animals (Dai et al. 2012b, 2013; Liang et al. 2019; Wang et al. 2020). To extend this observation, we conducted an experiment involving mice pretreated with aerosolized arginine, glutamine, or glycine before exposure to aerosolized LPS (13). Each of these three amino acids was found to have beneficial effects on reducing LPS-induced lung injury (Ma et al. 2019). Although arginine supplementation can stimulate the production of NO (a potential oxidant) by LPS-activated macrophages, other benefits of this amino acid may contribute to its role in alleviating the infiltration of neutrophils into the lung tissues and in improving alveolar integrity and function. More studies are needed to explore whether the beneficial effect of the functional amino acids is associated with microbiota alterations in the lungs of animals.

4.10 Conclusion and Perspectives

Studies from clinical patients and animal models have provided enormous amounts of data on the development to lung injury (including inflammatory cell infiltration and morphological alterations), as well as the cellular and molecular events underlying the pathogenesis. Recent studies have shown that certain amino acids are critically important nutrients for maintaining the integrity and the functionality of the lungs by promoting the syntheses of proteins, bioactive compounds, and nucleic acids, which are required for cell proliferation, immune response, and cellular homeostasis. Accumulating evidence indicates that depletion of amino acids or deregulation of amino acid metabolism is associated with the development of multiple lung diseases. Amino acid supplementation improves lung homeostasis by regulating mucosal barrier function, inhibiting apoptotic cell death, and restoring the integrity of epithelial barrier via multiple signaling pathways. However, there is a long way to translate the basic research into clinical applications for the treatment of patients with lung disease. First, lung injury is a complicated pathological process and multiple types of cells (including endothelial cells, epithelium, smooth muscle cells, and fibroblasts) contribute to the dysfunction of the lungs. It remains unknown how these cells respond to

amino acids under various physiological and pathological conditions. Second, amino acids have been reported to alleviate inflammatory responses and improve lung function. However, underlying mechanisms are not well defined and more studies are required to fulfill this gap of knowledge before their applications to clinical medicine. Third, most of our understanding and observations are based on studies with rodents. Despite a high degree of similarity in nutrition, physiology and immunology between mice and humans, it should be borne in mind that no animal models can fully mimic human disease. Thus, precautions should be taken not to simply extrapolate results from animals to humans. Also, data from various animal studies are not always consistent and sometimes are very different, due to differences in experimental designs, animal models, the duration and dosage of chemical exposure, methods for analysis, and the interpretation of results. Careful studies and a critical thinking are required to draw a convincing conclusion. Furthermore, because viruses [e.g., coronavirus (COVID-19, Shi et al. 2020) and influenza virus (Herold et al. 2015)] induce severe damage to the lungs, it is important to determine whether dietary supplementation or intravenous administration of individual functional amino acids (e.g., arginine-HCl, citrulline, N-acetylcysteine, glutamine, glycine, proline and tryptophan) or their combinations to affected subjects may ameliorate injury and dysfunction in this vital organ. Finally, because air pollution impairs the development of organs (particularly lungs; Wu et al. 2019), effects of environmental pollutants (e.g., $PM_{2.5}$ particles) on amino acid metabolism in the lungs and nutritional methods involving the use of amino acid supplementation to alleviate respiratory disorders should be investigated.

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5 Amino Acid Metabolism in the Kidneys: Nutritional and Physiological Significance

Xinyu Li, Shixuan Zheng, and Guoyao Wu

Abstract

The kidneys are developed from the intermediate mesoderm of the embryo. They are important for osmoregulation, regulation of acid-base balance, reabsorption of nutrients, and excretion of metabolites. In fish, the kidneys also serve as a *hematopoietic,* lymphoid and endocrine organ for the generation of red blood cells, the development of lymphocytes, and the production of hormones (e.g., glucocorticoids, catecholamines, and thyroid hormones). In humans and all animals, kidneys play a vital role in the metabolism and reabsorption of amino acids (AAs) and glucose. Specifically, this organ contributes to glucose synthesis from AAs, lactate and pyruvate via the gluconeogenesis pathway; regulates acid-base balance via inter-organ metabolism of glutamine; and synthesizes arginine, tyrosine, and glycine, respectively, from citrulline, phenylalanine, and 4-hydroxyproline. In mammals and birds, kidneys participate in creatine synthesis. Renal dysfunction adversely alters the concentrations of AAs in blood, while promoting muscle protein breakdown, inflammation, mitochondrial abnormalities, defects in the immune response, and cardiovascular diseases. Moderation of dietary AA intake has a protective and therapeutic effect on chronic kidney disease. Understanding the functions and metabolism of AAs in kidneys is essential for maintaining whole-body homeostasis, improving health and well-being, and preventing or treating renal metabolic diseases in humans and farm animals (including swine, poultry, ruminants, fish and shrimp).

Keywords

Kidney · Amino acids · Renal dysfunction · Animals · Human

5.1 Introduction

The main function of the kidney is to maintain the homeostasis of water, minerals and metabolites as well as acid-base balance, osmolality, and blood pressure in the body. This is achieved through filtering the blood supplied from the renal arteries, reabsorbing nutrients into the blood circulation, and excreting metabolites (e.g., ammonia, urea, homocysteine, ketone bodies, and methylarginines). The rate of blood flow through the kidneys is particularly high. For

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example, in humans, the kidneys receive about 20% of the blood pumped out of the heart and filter about 1.25 L of blood per minute (Kierszenbaum and Tres 2015). Kidneys also produce hormones that affect the function of other organs. As a result, well-functioning kidneys are essential to health and well-being. However, many individuals (e.g., 11.5% of the adult population in the United States) have chronic kidney disease and are at increased risk for cardiovascular events and progression to kidney failure (Tangri et al. 2011). According to the World Health Organization (WHO), globally 864,226 deaths (or 1.5% of deaths) were attributable to chronic kidney disease in 2012. Ranked the fourteenth in the list of leading causes of death, this disease accounts for 122 deaths per 1 million people (Webster et al. 2017).

Maintaining health and preventing disease outbreaks is vital to the economy and safety in animal production. This is because farm animals (including swine, poultry, cows, sheep, goats, fish, and shrimp) usually face numerous stresses, including high-density rearing, imbalance in nutrient intakes, heavy metal and non-metal containments, heat or cold environments, air and water pollution, and challenges by infectious agents (e.g., parasites, bacteria, fungi and viruses). Behavioral and physiological alterations will occur when affected animals cannot mount a successful response to one or more of these stresses (Blokhuis et al. 1998). For example, heat stress or metal toxicity can induce oxidative damage due to enhanced production of reactive oxygen species (ROS), mitochondrial dysfunction, and metabolic disorder, thereby compromising renal function (Pandey and Madhuri 2014; Belhadj Slimen et al. 2016). In fish, which lack bone marrow and lymph nodes, the kidneys serve as the hematopoietic organ and are a major lymphoid organ (Kum and Sekkin 2011). Renal dysfunction adversely affects immunity and increases susceptibility to disease, while decreasing feed intake and growth, thereby hindering animal production (Blokhuis et al. 1998; Anderson et al. 2011).

Most of amino acids (AAs) are catabolized by the kidneys via numerous pathways (including asparaginase, glutamate dehydrogenase, glutaminase, transaminases, and D-AA oxidases), and that filtered AAs are almost completely reabsorbed by the proximal convoluted tubule into the blood in normal physiological states (Levillain et al. 1997; Wu 2013a, b). The kidneys also play a vital role in the biosynthesis of some AAs and their derivatives. As a result, renal dysfunction will induce the deficiency of these AAs and their derivatives. Moreover, understanding the functions and metabolism of AAs in the kidneys is important for preventing and treating chronic renal diseases in both humans and farm animals. This review highlights the development and functions of the kidneys in different animals, as well as AA metabolism in this organ and its relationship with renal diseases.

5.2 Kidney Development and Structure

In all vertebrate species, the kidney originates from the intermediate mesoderm that is called the nephrogenic mesoderm (Cullen-McEwen et al. 2016). The embryonic development of the kidney is complex in that two or three different kidneys (depending on species) are formed in temporal and spatial sequences. In amniotes (birds, mammals, and reptiles), nephrogenesis begins at the embryonic stage and completes at birth. This process occurs through a series of successive phases: archinephros, pronephros, mesonephros, and metanephros (Cullen-McEwen et al. 2016). Archinephros occurs in the embryos as the simplest kind of excretory organ, which is nonfunctional in humans and other mammals. Then, three types of a more advanced kidney develop from the embryonic archinephros: the pronephros from its anterior section, the mesonephros from its middle section, and the metanephros from its hind section. The pronephros and mesonephros are generally transient embryonic kidneys that subsequently degenerate and have little or no function in adult mammals. However, the mesonephros is the functional kidney in fish and amphibians (Seely 2017). Generally, the metanephros develops when an outgrowth of the pri-

mary nephric duct (the ureteric bud or metanephric diverticulum) extends into the surrounding metanephric mesenchyme (Dressler 2006; Seely 2017). The metanephros is the adult kidney or the functional kidney in humans and other amniotes (Cullen-McEwen et al. 2016). The functional kidney has an extensively branched collecting duct system and a large number of nephrons (Cullen-McEwen et al. 2016; Denic et al. 2016). During postnatal growth, mammals increase their renal performance via increases in nephron size and glomerular filtration rate (Davidson 2014).

In contrast to mammals, fish form the pronephros and mesonephros, with the latter being the permanent adult kidney (Davidson 2014). Generally, fish embryos develop externally and are therefore exposed to environmental pressures from water and salts, and the pronephric kidneys play a vital role in osmoregulation. In zebrafish, the functional larval pronephros consists of two nephrons with glomeruli fused at the embryo midline just ventral to the dorsal aorta (Drummond 2005). At the juvenile stage, the mesonephros kidney is formed, which consists of hundreds of nephrons that branch distally (Davidson 2014). Unlike the mammalian metanephric kidneys, the mesonephric kidneys of fish continue to add new nephrons as their body mass increases during their lives (Davidson 2014; Upadhyay and Silverstein 2014).

5.3 Functions of the Kidneys

The nephron is the functional unit of the kidney, and is composed of a renal corpuscle (a glomerulus and glomerular capsule known as Bowman's capsule) and renal tubules (including the proximal convoluted tubule, the loop of Henle, and the distal convoluted tubule). The glomerulus filters the blood into the renal tubule, and the glomerular filtration rate (GFR) is an indicator of overall kidney function. The GFR is defined as the total amount of fluid filtered through all of the nephrons per unit of time (Levey et al. 2015). Filtration is the process by which cells and large proteins are retained while substances of small molecular weights are filtered from the blood to make an ultrafiltrate fluid. Most of molecules (such as water, glucose, and AAs) in the ultrafiltrate are reabsorbed from the renal tubule into the peritubular capillary and blood circulation. In a healthy adult human, reabsorption by renal tubules recovers about 70 g AAs per day (Young 1991). Moreover, the kidneys excrete a variety of metabolites (such as ammonia, urea, uric acid, methylarginines, homocysteine, and ketone bodies) into urine (McNeal et al. 2018). For example, in humans, about 180 L of ultrafiltrate fluid passes into the renal tubules per day. Most of the fluid is reabsorbed by the tubular cells into the blood circulation, and only about 1.5 L of the fluid is excreted as urine (Kierszenbaum and Tres 2015). Due to their functions in excretion, reabsorption, and filtration, the kidneys play a vital role in maintaining whole-body homeostasis, such as acid-base balance, electrolyte concentrations in plasma, extracellular fluid volume, and blood pressure. The kidneys accomplish these homeostatic functions both independently and in concert with other organs, particularly the endocrine system. Various endocrine hormones coordinate the endocrine functions, including antidiuretic hormone, thyroid hormone, adrenal cortical hormone, renin, angiotensin II, aldosterone, glucocorticoids, mineralocorticoids, prolactin, prostaglandins, and atrial natriuretic peptide, gastrin, among others (McDonald et al. 1976; Afsar et al. 2016; Ahmed and Ramesh 2016). For example, antidiuretic hormone is the hormone of paramount importance in the regulation of water excretion by the mammalian kidneys, but other hormones also influence renal excretion of water. In particular, aldosterone, which is produced by the cortex of the adrenal gland, stimulates the reabsorption of water by renal tubules along with Na^+, thereby increasing blood volume and decreasing urine volume. Furthermore, glucagon-like peptide-1, which is excreted by endocrine cells of the small intestine, can increase the GFR, renal plasma flow, urine output, and the excretion of Na^+, K^+, and HCO_3^- (Afsar et al. 2016). In postnatal mammals and birds, another important function of the kidneys is the regulation of erythropoiesis (the production of erythrocytes) by the bone marrow. Although the kidney is not the site of erythropoiesis in these species, it releases a

hormone called erythropoietin in response to cellular hypoxia. Through erythropoietin receptor-mediated signaling cascades that involve the Janus kinase-2 and its downstream proteins, phosphoinositide-3 kinase (PI3K)/protein kinase A, the extracellular signal-regulated kinase (ERK1/2), p38 mitogen-activated protein kinase (MAPK), and signal transducer and activator of transcription 5 (STAT5), erythropoietin stimulates the bone marrow to produce red blood cells (Watowich 2011).

In amniotes, the kidneys efficiently reabsorb water from the renal tubules into blood, and therefore a relatively small amount of water reaches the bladder (Mahasen 2016). Compared with terrestrial animals, fish have a special need to maintain their internal environment because of the constant exposure to external water and substances. Freshwater fish have a higher concentration of salts in blood than the external environment, contributing to the net osmotic gain of water and diffusional loss of salts across the gills. This conundrum is solved through several strategies, including excretion of relatively dilute urine, active uptake of salts across the gill, and possibly ingestion of more salts in the food (Evans 2002). In freshwater fish species, the kidneys generally remove, as dilute urine, a considerable amount of water that passively enters the body via the gill. This dilute urine is almost completely composed of water. Freshwater fish species have a good size of the renal corpuscle and hence a high water output. On the contrary, in marine fish, the renal corpuscle is small or absent and the renal tubule is short, thereby minimizing the glomerular infiltration of blood and conserving water as well as minimizing the reabsorption of salts by the renal tubules and producing a urine with concentrated salts (Mahasen 2016). In marine fish, excessive salts are removed largely through the kidneys. Of particular note, cartilaginous fishes (sharks, skates, rays, and chimaeras) have an ability to adapt a high-salinity marine environment through a unique urea-based osmoregulation strategy, as their kidneys reabsorb nearly all of the filtered urea in the primary urine (Hyodo et al. 2014).

In fish, each kidney contains two segments: the anterior (head) and the posterior (trunk). The filtration and urine-forming functions are carried out by the posterior kidney, as noted previously. In contrast to mammals and birds, fish lack bone marrow, lymph nodes, and adrenal gland but possess the head kidney to serve as a hematopoietic, lymphoid and endocrine organ for the production of erythrocytes (red blood cells), cytokines, antibodies, and some hormones (Shoemaker et al. 2015). Thus, hematopoiesis occurs in the head kidney in fish, instead of the bone marrow in postnatal mammals and birds. In addition, the head kidney plays an important role in both innate and adaptive immunities in fish (see Sect. 5.7). Furthermore, the head kidney of fish produces glucocorticoids from cholesterol, as well as catecholamines and thyroid hormones from tyrosine. Thus, in fish, the kidney has a high metabolic rate (including AA catabolism and ATP production) to support its integrated *hematopoietic,* immune, and endocrine functions.

5.4 Amino Acid Transporters in the Kidneys

Tissues in the kidney receive AAs that are supplied from the arterial blood. In addition, epithelial cells of the renal tubule reabsorb free AAs from its lumen (the AAs that are filtered through the glomerulus) into the blood. These processes require specific AA transporters and play an important role in maintaining AA homeostasis in plasma (Verrey et al. 2005, 2009). Generally, AAs are transported across the plasma membrane via: (1) simple diffusion (passive and nonsaturable), (2) Na^+-independent systems (facilitated diffusion), and (3) Na^+-dependent systems (active transport). Some Na^+-dependent transport proteins can use Li^+ instead of Na^+, and a few of AA transport proteins are H^+-driven. In the kidney, AA transporters are highly expressed in the luminal brush border membrane of the proximal segments of the renal tubule (Palacín et al. 1998; Verrey et al. 2009). On the basis of sequence similarity, AA transporters are grouped into solute carrier (SLC) families (Table 5.1). For exam-

Table 5.1 Transporters of amino acids in kidneys

Gene	Protein	Cotransport	Predominant substrate	Affinity
SLC6A19	B^0AT1	Na^+	Neutral amino acids	Low
SLC6A15	B^0AT2	Na^+	Pro, Leu, Val, Ile, and Met	High
SLC6A18	XT2	Na^+	Gly and Ala	High
SLC6A20	IMINO or XT3	Na^+ or Cl	Pro, Cys, Ala, Leu, Met, Phe, and Gly	Medium
SLC36A1	PAT1	H^+	Gly, Ala, Pro	Low
SLC36A2	PAT2	H^+	Gly, Ala, Pro	Medium
SLC3A1/SLC7A9	rBAT / $b^{0,+}$ AT		Cationic amino acids or Cys	High
SLC1A2	EAAT2	Na^+, H^+, K^+	Glu, Asp	High
SLC1A1	EAAT3	Na^+, H^+, K^+	Glu, Asp	High
SLC38A4	SNAT4	Na^+	Gly, Ala, Ser, Cys, Gln, Asn, and Met	Medium
SLC1A4	ASCT1	Na^+	Ala, Ser, and Cys	High
SLCA5	ASCT2	Na^+	Ala, Ser, Cys, Thr, and Gln	High
SLC3A2/SLC7A10	4F2 hc/asc1		Gly, Ala, Ser, Cys, and Thr	High
SLC6A6	TauT	Na^+, Cl	Tau, β-Ala	High
SLC16A10	TAT1		Trp, Tyr and Phe	Low
SLC3A2/SLC7A7	4F2hc/y^+LAT1	Na^+ (symport with AA)	Arg, Lys, Gln, His, Met and Leu	High
SLC3A2/SLC7A6	4F2hc/y^+LAT2	Na^+ (symport with AA)	Arg, Lys, Gln, His, Met and Leu	High

Adapted from Boll (2004); Bröer (2006 and 2008), and Wu (2013a)

ple, B^0AT1 (SLC6A19), XT2 (SLC6A18) and IMINO (SLC6A20) systems transport neutral AAs from the lumen of the renal proximal tubule into its epithelial cells (Bröer 2006, 2008; Verrey et al. 2009). Moreover, H^+-dependent transporters, such as PAT1 (SLC36A1) and PAT2 (SLC36A2), have been identified in the kidney (Daniel et al. 2006). Besides, cationic AAs (e.g., arginine, ornithine and lysine) are taken up by cationic AA transporters in a Na^+-independent manner. The cystine transporter consists of a catalytic subunit of the SLC7 family and a disulfide-linked accessory subunit referred to as a heavy chain called rBAT [related to neutral and basic ($b^{0,+}$) AA transporter; SLC3A1; Verrey et al. 2009]. Mutations in the SLC3A1 gene result in cystinuria that is characterized by high concentrations of cystine (an AA with a very low solubility in water) in the urine, leading to the formation of cystine stones in the kidneys, ureter, and bladder. The transport of anionic AAs (e.g., glutamate and aspartate) requires the Na^+-dependent transporters (EAAT1, EAAT2 and EAAT3). Competition of the transport of AAs by the cell occurs when they are structurally similar (e.g., among large neutral AAs, basic AAs, acidic AAs, small neutral AAs, or β-AAs).

5.5 Amino Acid Metabolism in the Kidneys

5.5.1 Gluconeogenesis

Gluconeogenesis is the metabolic pathway for the synthesis of glucose from non-glucose substrates, such as lactate, pyruvate, glycerol, and gluconeogenic AAs. A major function of gluconeogenesis is to provide the body with glucose in response to physiological needs. There are four rate-controlling reactions in gluconeogenesis that are catalyzed by pyruvate carboxylase, phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase (FBPase), and glucose-6-phosphatase (G6Pase). All of these enzymes are present in the kidneys of rats (Anderson and Stowring 1973), chickens (Shen and Mistry 1979; Watford et al. 1981), humans (Møller et al. 2000), and some fish species (Knox et al. 1980; Kirchner et al. 2008; Kumar et al. 2010). PEPCK and G6Pase are mainly expressed in the proximal tubules (Pollock 1989; Sun et al. 2002). Moreover, FBPase and PEPCK are co-localized in the kidney and liver, which contributed almost equally to glucose synthesis in fasting humans (Yánez et al. 2003). Similar to other animals, the gluconeogenic enzymes are

expressed mainly in the liver and kidneys of fish species, and gluconeogenesis does not occur in skeletal muscle (Knox et al. 1980). In the kidneys of some species, whether AAs are the substrates for glucose synthesis depends on the intracellular location of PEPCK (Wu 2013a). As reported for chickens (Watford et al. 1981), the presence of PEPCK in the cytoplasm allows for the generation of NADH from glucogenic AAs and, therefore, the production of glucose from the AAs.

The mammalian kidneys synthesize glucose and release it into the blood circulation under various physiological conditions (Gerich 2010). In the post-absorptive state, renal gluconeogenesis (primarily from glutamine) contributes 20–25% of glucose production in adult humans (Stumvoll et al. 1999), and the kidneys release nearly the same amount of glucose into the circulation as the liver (Mitrakou 2011). In contrast, the contribution of alanine to gluconeogenesis occurs almost exclusively in the liver (Stumvoll et al. 1998). In the body, the production of new glucose molecules occurs mainly through gluconeogenesis in the kidneys and liver, and to a lesser extent via glycogenolysis in the liver and skeletal muscle (Stumvoll et al. 1997). In all animals, the kidney contains very little glycogen and, therefore, produces little glucose through glycogenolysis (Stumvoll et al. 1997; Gerich 2010).

Not all AAs are used for endogenous glucose synthesis (Wu 2013a). Generally, AAs are classified as "glucogenic" or "ketogenic" based on the type of intermediates that are formed from their metabolism. Glucogenic AAs are converted into either pyruvate or one of the intermediates in the Krebs cycle. Leucine and lysine are strictly ketogenic AAs because they are catabolized to acetyl CoA (Fig. 5.1). The kidneys synthesize glucose

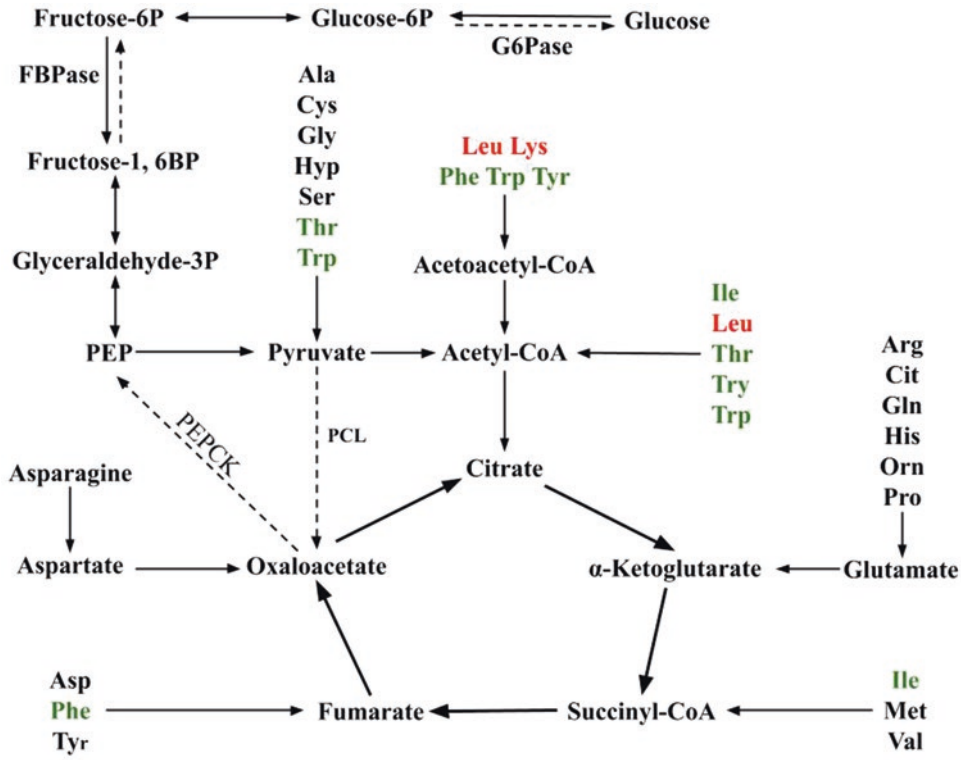

Fig. 5.1 The ketogenic and glucogenic- amino acids metabolism in animals. Amino acids with red color: ketogenic only; Amino acids with green color: both glucogenic and ketogenic; Amino acids with black color: glucogenic only. G6Pase, glucose-6-phosphatase; *PCL* pyruvate carboxylase, PEPCK, phosphoenolpyruvate carboxykinase; *PEP* phosphoenolpyruvate, "----"denotes glucogenic reactions

from many AAs, but glutamine, alanine, glutamate, aspartate, proline, ornithine and arginine are the main AA substrates for the renal gluconeogenesis (Krebs et al. 1963). A mixture of AAs, pyruvate, lactate, and glycerol at normal plasma concentrations can support renal gluconeogenesis at an initial rate of about 0.6 μmol/min per g of kidney (Bowman 1970). As noted previously, the kidney is the major organ for gluconeogenesis from AAs in birds (Watford et al. 1981) and contributes to about 30% of the glucose produced in the starved chickens (Tinker et al. 1986). Renal gluconeogenesis is species-dependent. For example, high expression levels of FBPase and PEPCK are present in the kidneys and liver of some fish species (e.g., cod, salmons and trout), and glucose is produced from AAs (e.g., alanine and glutamine) in their kidneys and liver (Moon and Foster 1995; NRC 2011). However, in many fish species, the kidney lacks PEPCK and is not capable of gluconeogenesis from any potential substrates (Moon and Foster 1995).

Renal gluconeogenesis is regulated by some hormones. Insulin, growth hormone, cortisol, and catecholamines influence renal glucose release in the mammalian kidneys (Wirthensohn and Guder 1986; Schoolwerth et al. 1988). For example, expression of renal gluconeogenic genes in mice is inhibited by both insulin and glucose reabsorption *via* the inactivation of FoxO1 and PGC1α, respectively (Sasaki et al. 2017). The regulation of gluconeogenesis from glutamine by insulin and glucose also occurs in the kidneys of humans (Stumvoll et al. 1999). All of the metabolic pathways for renal glucose synthesis and whole-body homeostasis of glucose are altered in patients with diabetes mellitus (Gerich 2010). Patients with T2DM have an increased release of glucose into the circulation by the kidneys in the fasting and postprandial states, which impairs glucose homeostasis and leads to hyperglycemia (Triscari et al. 1979; Meyer et al. 1998). Epinephrine, norepinephrine, and prostaglandin E-2 (PGE2) also regulate gluconeogenesis in fish (Enes et al. 2009). Some fish species have poor regulation of gluconeogenesis in response to starch intake, which may result from relatively high concentrations of glucogenic AAs in the diets (NRC 2011).

5.5.2 Glutamine and Glutamate

Glutamine is the one of the most important free AAs in the blood of humans and numerous animal species, and plays key roles in diverse physiological processes, including the syntheses of DNA, RNA, protein, aminosugars, NAD, and glucose (Wu 2013a). In the kidney, glutamine catabolism is initiated via four enzymes: (1) phosphate-activated glutaminase, (2) glutamine transaminases K and L, (3) glutamine:fructose-6-phosphate transaminase, and (4) carbamoylphosphate synthase-II. Among these pathways, glutaminase contributes to most of glutamine degradation, which produces glutamate and NH_4^+ primarily in the proximal tubule. (Welbourne 1974). Glutamate is catabolized by glutamate dehydrogenase to NH_4^+ and α-ketoglutarate (α-KG). The latter enters the Krebs cycle for oxidation to CO_2 and water. Overall, the complex oxidation of one mole of glutamine generates two moles of NH_4^+ and 2 mol of HCO_3^-. NH_4^+ is directly excreted into the lumen of the renal tubule through the apical NHE3 (sodium-hydrogen exchanger-3), whereas HCO_3^- is returned into the blood by crossing the basolateral membrane *via* the electrogenic sodium-coupled bicarbonate co-transporter (Weiner et al. 2015). In the kidneys, about 50% of NH_4^+ is reabsorbed into the blood primarily by the thick ascending limb of the loop of Henle via the apical membrane Na^+-K^+-$2Cl^-$ symporter (NKCC2) and NH_4^+ transporter and the basolateral membrane NHE4 (Fig. 5.2). Similar mechanisms are also present in fish species (Claiborne et al. 1982; Evans and Cameron 1986). Because of the renal reabsorption, only about 59% of the ammonia produced by the kidneys appears in the urine. Ammonia accounts for about 10% of total renal nitrogen excretion under basal conditions, but can increase substantially under a variety of clinical conditions such as acidosis, infections, manganese deficiency, urea cycle defects, and excessive intake of dietary protein or AAs (Wu 2013a).

The reabsorption of bicarbonate (HCO_3^-) and excretion of hydrogen ions (H^+) in the kidneys play an important role in maintaining the acid–base balance (Vercoutère et al. 2004). This can be

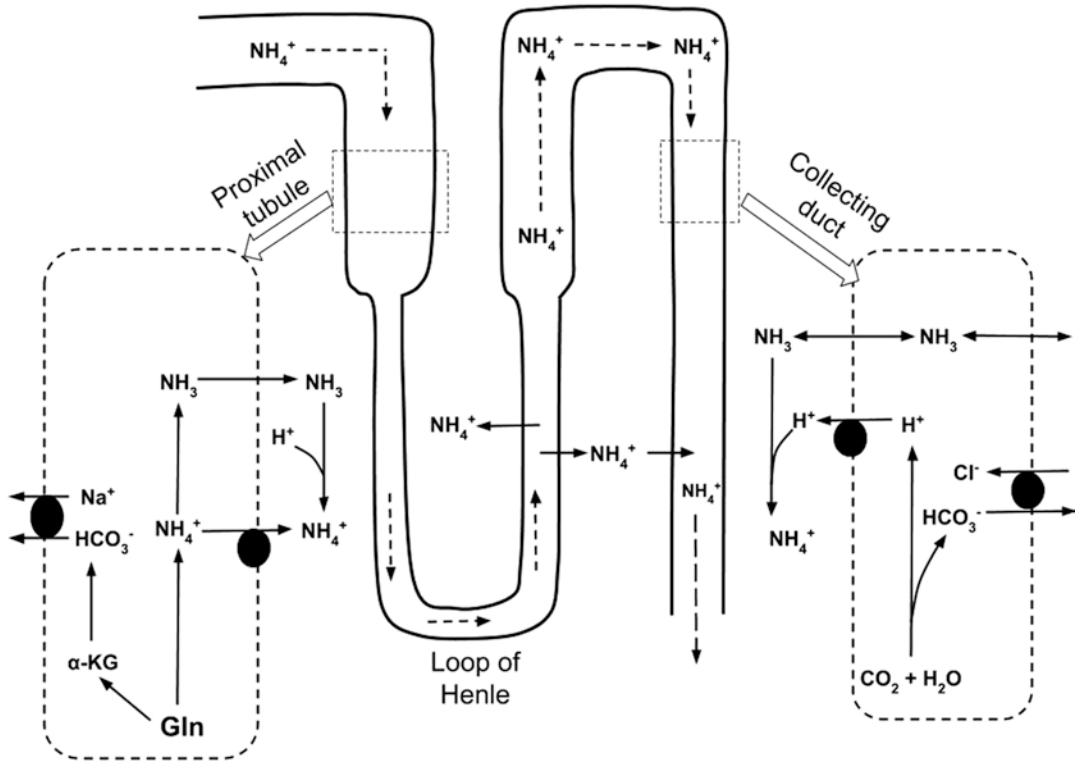

Fig. 5.2 Integrated overview of renal ammonia metabolism. Renal ammoniagenesis occurs primarily in the proximal tubule, involving glutamine uptake, glutamine metabolism forming ammonium and bicarbonate, and apical NH_4^+ (H^+) secretion. Note: ammonia reabsorption in the thick ascending limb, involving apical NKCC2-mediated uptake results in medullary ammonia accumulation. Ammonia is secreted in the collecting duct via parallel H^+ and NH_3 secretion. α-KG, α-ketoglutarate

achieved partly via: (1) the regulation of glutamine uptake through the apical Na^+-dependent neutral AA transporter-1 and the basolateral Na^+-coupled neutral AA transporter-3 (Weiner et al. 2015); and (2) expression of glutaminase. Under acidotic conditions, glutaminase activity, along with the uptake and catabolism of glutamine by the kidney, is greatly enhanced to generate NH_3 for removing excess H^+ as NH_4^+ (Wu 2013a). In rats, acidotic kidneys extract glutamine and produce NH_3 at rates that are 4–5 times greater than those in nonacidotic kidneys (Welbourne 1974). An inhibition of glutamine synthetase contributes to an increase in intracellular ammonia concentration in the kidneys of acidotic rats (Hems 1972). In contrast, in response to alkalosis, the kidneys excrete more HCO_3^- by decreasing H^+ secretion from the tubular epithelial cells as well as the rates of glutamine catabolism and ammonium excretion (Fig. 5.2).

Besides the kidneys, the gut and skeletal muscle also play an important role in inter-organ metabolism of glutamine, which is a major substrate for endogenous synthesis of citrulline and arginine in most mammals (Fig. 5.3). Although the small intestine takes up glutamine from the arterial blood at all times albeit at different rates, the liver either releases or takes up glutamine depending on various physiological conditions (Welbourne 1987). For example, in acidotic animals, the release of glutamine by skeletal muscle is enhanced, the liver becomes a net producer of glutamine, and the uptake of arterial glutamine by the small intestine is reduced, resulting in an increased provision of glutamine for ammoniagenesis in the kidneys. Studies with rats have

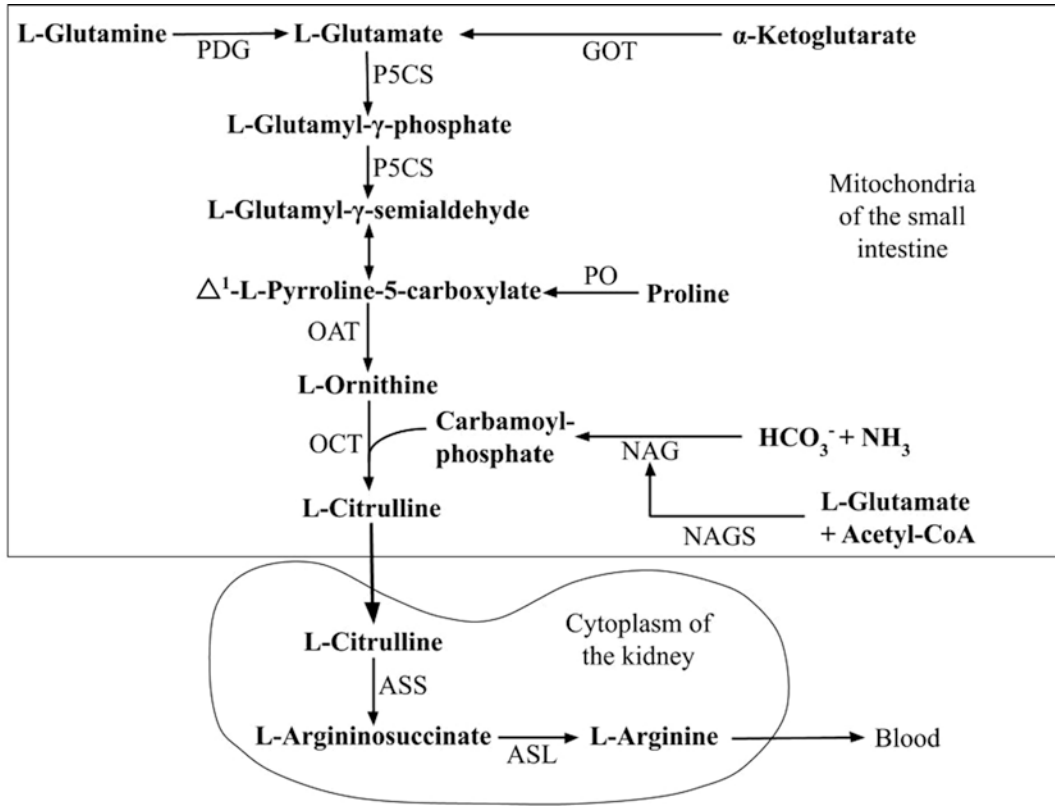

Fig. 5.3 Arginine synthesis in most mammalian animals. The small intestine of most mammals (including humans, pigs, cattle, sheep, and rats) converts glutamine and proline into citrulline, and releases citrulline into the blood. In adult mammals, the kidneys are the major site for the synthesis of arginine from citrulline. Birds lack an ability to form citrulline from glutamine or proline, but their tissues (including the kidneys are capable of converting citrulline into arginine. At present, little is known about endogenous synthesis of arginine in aquatic animals (e.g., fish and shrimp). Note: there is no net synthesis of arginine by the liver under physiological conditions. The conversion of glutamine and glutamate into citrulline occurs exclusively in the mitochondria of enterocytes. Arginine is mainly formed from citrulline in the cytoplasm of almost all cell types. *ASL* argininosuccinate lyase, *ASS* argininosuccinate synthase, *NAG* N-acetylglutamate, *OAT* ornithine aminotransferase, *OCT* ornithine carbamoyltransferase, *PO* proline oxidase, *PDG* phosphate-activated glutaminase, *P5CS* pyrroline-5-carboxylate synthase. This figure is adapted from Wu (2013a, b)

shown that skeletal muscle and liver contribute about 55% and 45%, respectively, of the glutamine extracted by the kidneys during chronic acidosis (Schrock and Goldstein 1981). In contrast to mammals, the skeletal muscle of some fish (e.g., holostean and teleost fish) does not appear to have a net synthesis of glutamine due to a greater activity of glutaminase than glutamine synthetase (Chamberlin et al. 1991). However, this does not necessarily mean a lack of net glutamine synthesis by the skeletal muscle of aquatic animals because of the complex intracellular compartmentation of these two enzymes. In support of this view, Zhou et al. (2018) reported that the skeletal muscle of hybrid striped bass synthesized glutamine from branched-chain AAs as the amino-group donors and release glutamine from the tissue.

As in mammals and birds, glutamine is also crucial for the renal regulation of acid-base balance in fish (Li et al. 2020). The activities of both glutaminase and glutamine synthetase are high in the kidneys of holostean and teleost fish (Chamberlin et al. 1991). There is a metabolic

channeling between glutaminase and glutamine synthetase in the kidneys of the dogfish shark to regulate their ammonia production (King and Goldstein 1983). This pathway involves the increased uptake of glutamine in the arterial blood by the kidneys and the increased degradation of glutamine into ammonia and α-KG during acidosis. However, the majority of ammonia and/or urea is excreted across the gills rather than through the kidney in most fish species (Ip et al. 2001). Both Na^+/H^+ exchange and vacuolar-type H^+-ATPase are present in the epithelium of gills to export H^+ from the fish to the environment (Cameron and Kormanik 1982). The gills generally excrete much more acids from the body than the kidneys during acidosis, but the renal reabsorption of HCO_3^- is required in the systemic regulation of acid-base balance as noted previously (Perry et al. 2003).

5.5.3 Arginine and Nitric Oxide (NO) Production

Arginine is a basic AA in physiological fluids and a precursor for the syntheses of proteins, NO, urea, polyamines, proline, glutamate, creatine and agmatine (Wu and Morris 1998; Wu et al. 2009). The metabolism and functions of arginine has been well summarized (Morris Jr. 2016; Wu et al. 2009, 2016). In adult mammals, the endogenous synthesis of arginine involves the intestinal-renal axis (Reyes et al. 1994; Wu and Morris 1998; Brosnan and Brosnan 2004). Specifically, citrulline is synthesized from glutamine, glutamate and proline in the mitochondria of enterocytes, released from the small intestine, and taken up primarily by kidneys for arginine production. About 85% of the gut-derived citrulline is taken up by the kidneys for quantitative conversion into arginine, which is then released into the renal vein (Brosnan and Brosnan 2004). In neonates, most of the citrulline synthesized in enterocytes is converted locally into arginine (Wu and Knabe 1995; Wu 1997). In cats, the small intestine does not produce citrulline due to the deficiency of pyrroline-5-carboxylate (P5C) synthase, and therefore there is no *de novo* synthesis of arginine (Rogers and Phang 1985). For fish, the endogenous synthesis of arginine is likely limited (NRC 2011), but no information is available regarding P5C synthase activity in any of their tissues. It should be borne in mind that a lack of the hepatic urea cycle is not the reason for possible absence of *de novo* arginine synthesis in some fish. The fact that cats and possibly many species of fish have survived without the ability to synthesize arginine may be attributed to their usual consumption of animal-source foods (containing high-arginine content) as carnivores.

In the kidneys of mammals and birds, citrulline is readily converted into arginine via argininosuccinate synthetase and argininosuccinate lyase, which are localized in their proximal tubule (Silbernagl 1988; Brosnan and Brosnan 2004). An adult human produces about 2 g arginine/day, which is 40%–50% of 4–5 g of dietary arginine intake per day (Brosnan and Brosnan 2004). The kidneys have a high capacity for converting citrulline into arginine but arginine synthesis is limited in vivo by the rate of delivery of citrulline (Dhanakoti et al. 1990). It is unknown whether the kidneys can convert citrulline into arginine in aquatic animals (e.g., fish).

Production of arginine by the kidneys is important for the health of animals. This amino acid has versatile physiological functions directly or indirectly through its metabolites (e.g., NO, agmatine, and ornithine; Wu 2013a,b). As a signaling molecule, NO regulates blood flow, angiogenesis, embryogenesis, immune response, hormone secretion, and protein synthesis (Wu 2013a). Interestingly, the concentration of arginine in plasma declines as the aging kidneys develop progressive injury, which could contribute to endothelial dysfunction and decreased NO production in chronic renal disease (Baylis and Corman 1998). An increase in renal arginine production may serve to sustain systemic NO production in response to endotoxemia (Hallemeesch et al. 2002). As a result, arginine may be regarded as a nutritionally essential amino acid for aging subjects, and dietary supplementation with arginine may be required to maintain sufficient substrate levels for NO production (Weinstein and Anderson 2010). The reduced NO-generating

capacity in aging subjects may also result, in part, from deficiencies of NO synthase and its cofactors such as tetrahydrobiopterin and NADPH (Mistry et al. 2002; Delp et al. 2008).

5.5.4 Methylarginine and Sulfur AA Metabolism

Dimethylarginines, such as asymmetric dimethylarginine (ADMA), N^G-monomethyl-l-arginine (NMMA) and symmetrical dimethylarginine (SDMA), result from the degradation of methylated proteins (Wu 2013a). Production of NO from arginine is reduced by ADMA and NMMA (competitive inhibitors of NOS) as well as SDMA (an inhibitor of arginine transport) (Tsikas et al. 2018). The kidneys play an important role in the metabolism and disposal of these endogenous arginine analogues (Van De Poll et al. 2004). Moreover, the kidneys have an ability to degrade ADMA and NMMA to citrulline via N^G-dimethylarginine dimethylaminohydrolase. Renal excretion also plays a role in the elimination of endogenous dimethylarginines. In humans, approximately 4.5% of the ADMA generated in the body is excreted in the urine, and the remainder is metabolized by the kidneys and liver (Ogawa et al. 1987; Van De Poll et al. 2004). Concentrations of free NMMA, ADMA, and SDMA in the plasma are low in healthy subjects (<1 μM) (Wu 2013a). However, renal dysfunction elevates ADMA or NMMA levels, thereby inhibiting NO synthesis, impairing endothelial function, and promoting atherosclerosis (Sibal et al. 2010; Cooke and Ghebremariam 2011).

Arginine methylation reactions involve the modification of guanidino N atoms and require S-adenosylmethionine (SAM), which is a metabolite of methionine (Wu 2013a). In mammals, the kidneys play an important role in sulfur AA metabolism (Fig. 5.4). In the reaction catalyzed by protein arginine N-methyltransferase, SAM is converted into S-adenosylhomocysteine (SAHC). The latter is also a metabolic precursor of homocysteine in tissues. Garibotto et al. (2010) suggested that the mammal kidneys have a good ability to remove SAHC, but not homocysteine from the bloodstream. Thus, elevated concentrations of SAHC in the whole blood are associated with renal dysfunction, and SAHC may modulate one-carbon flux (Stam et al. 2004). Considering that SAHC is a feedback inhibitor of most methyltransferases, the kidneys may play a major role in the control of the overall transmethylation rates and the circulating levels of homocysteine. Moreover, homocysteine may also inhibit N^G-dimethylarginine dimethylaminohydrolase, causing ADMA to accumulate and suppressing NO synthesis (Stühlinger et al. 2001; Holven et al. 2003). This could impair endothelium-mediated NO-dependent vasodilatation. Of note, the regeneration of methionine from homocysteine is regulated by the one carbon cycle which is mediated by nutrients, such as folic acid, vitamin B_{12}, and vitamin B_6. Thus, dietary supplementation with these vitamins can reduce blood homocysteine levels and provide an effective therapy to improve outcomes in patients undergoing coronary angioplasty (Schnyder et al. 2002). In addition, intake of plant-source proteins, which contain less methionine and cysteine than animal-source proteins (Hou et al. 2019; Li and Wu 2020), may be beneficial for patients with hepatic and renal diseases who have a reduced ability to metabolize homocysteine or excrete this metabolite in urine.

5.5.5 Creatine Synthesis

In mammals and birds, the kidneys participate in the inter-organ synthesis of creatine from arginine, glycine, and methionine (Fig. 5.5). This metabolic pathway requires arginine:glycine amidinotransferase (AGAT) and guanidinoacetate *N*-methyltransferase (GAMT) (Silva et al. 2014). AGAT transfers the guanidino group from arginine to glycine to produce guanidinoacetate and ornithine. This enzyme is expressed primarily in the renal tubule, pancreas, and to much lesser extent, in the liver and other tissues (Wu 2013a). The kidneys are the major site for the production of guanidinoacetate in mammals and birds, as the renal activity of AGAT is the primary

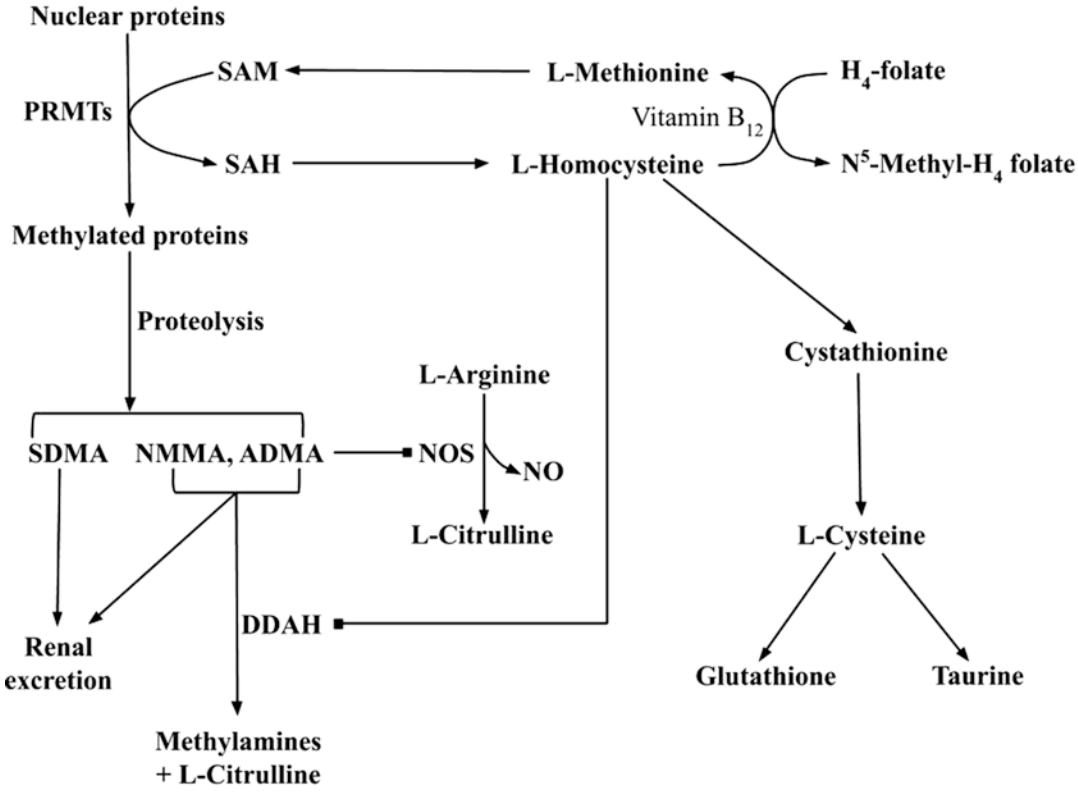

Fig. 5.4 The methylarginines and sulfur amino acid metabolism pathway in animals. Taurine is synthesized from cysteine in the liver of most mammals (except for cats and possibly some of the other carnivores) and birds. Taurine plays important roles as an antioxidant and as well as a regulator of cell signaling and metabolism in the kidneys. *ADMA* asymmetric dimethylarginine, DDAH, N^G-dimethylarginine dimethylaminohydrolase; H_4-folate, tetrahydro-folate; *NMMA* N^G-monomethyl-l-arginine, *NO* nitric oxide, PRMTs, protein arginine methyltransferases; *SAM* *S*-adenosyl-L-methionine, *SAH* *S*-adenosyl-L-homocysteine, *SDMA* symmetrical dimethylarginine

determinant of in vivo creatine synthesis (Wyss and Kaddurah-Daouk 2000). The liver takes up guanidinoacetate from the blood and converts this metabolite into creatine via GAMT, which requires SAM as the cofactor. In skeletal muscle, creatine is spontaneously degraded to creatinine, which is excreted in the urine. The lost creatine must be replaced from diets or de novo synthesis to maintain its total pool in the body (Barcelos et al. 2016). In rats, the kidneys produce a sufficient quantity of guanidinoacetate to replace creatinine lost in the urine (Silva et al. 2014). A 70-kg healthy adult synthesizes 1.7 g creatine per day from 2.3 g arginine, 1.0 g glycine, and 2.0 g methionine (Wu and Morris 1998) to match the daily irreversible loss of creatine (1.7 g/day) as creatinine via the urine (Stead et al. 2006; Edison et al. 2007).

In contrast to mammals and birds, skeletal muscle is the organ for *de novo* creatine synthesis in fish. Borchel et al. (2014) reported that: (a) AGAT is nearly absent from the kidneys of the rainbow trout, (b) GAMT is weakly expressed in their liver, and (c) both AGAT and GAMT are strongly expressed in their skeletal muscle. Similar results were obtained for other four species of fish: maraena whitefsh (Coregonus maraena), pikeperch (Sander lucioperca), European perch (Perca fuviatilis), and the Atlantic herrings (Borchel et al. 2019). Thus, in the skeletal muscle of fish, the guanidinoacetate generated by AGAT is locally used for creatine production by GAMT. In these aquatic animals,

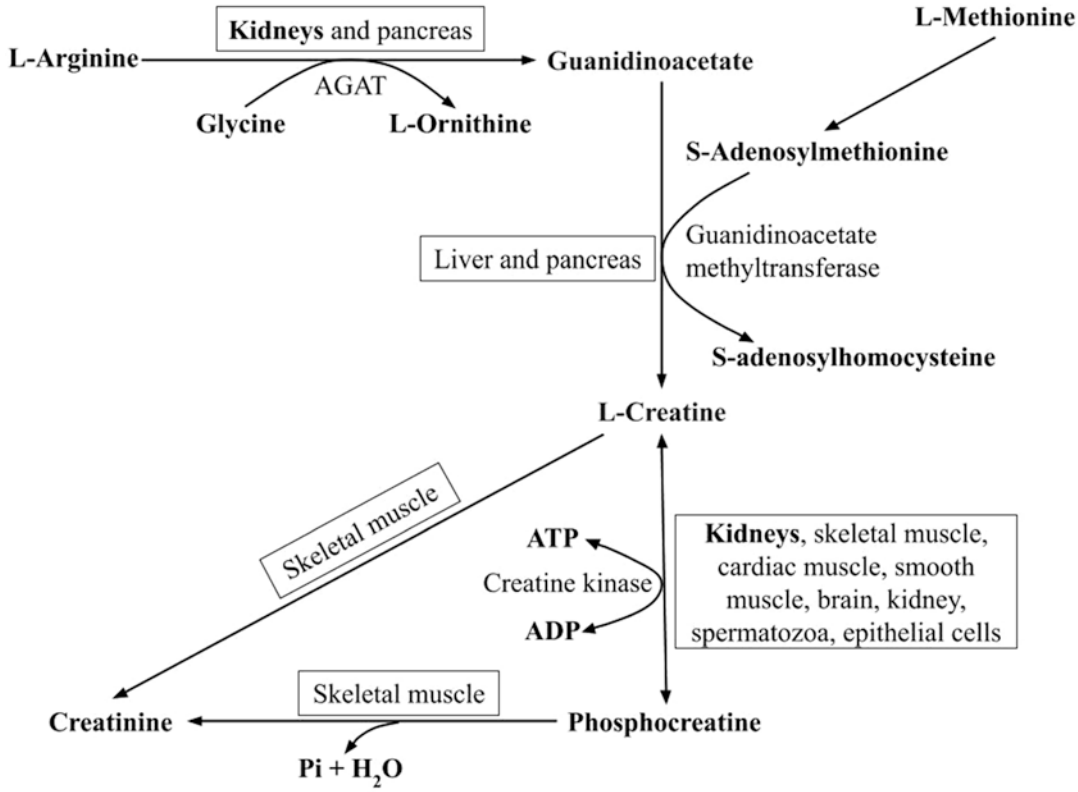

Fig. 5.5 The synthesis of creatine in animals: Creatine is synthesized from arginine, glycine, and methionine in animals via inter-organ cooperation. The creatine can be further converted into creatinine in skeletal muscle. *AGAT* arginine:glycine amidinotransferase

besides the skeletal muscle, the kidneys also strongly express GAMT (Borchel et al. 2014, 2019) and, therefore, are capable of converting diet- and blood-derived guanidinoacetate into creatine.

In mammals, creatine synthesis is regulated by the availability of substrates and renal AGAT activity. Studies with rats have shown that the expression of this enzyme at the pre-translational level is down-regulated by dietary creatine intake but up-regulated by growth hormone (McGuire et al. 1984; Guthmiller et al. 1994; Edison et al. 2007). Thus, dietary supplementation with creatine to rats decreases renal AGAT mRNA levels and activity as well as the production of guanidinoacetate by the kidneys (Edison et al. 2007). In addition, AGAT is inhibited by ornithine (Sipilä 1980) but activated by arginine (Edison et al. 2007). Therefore, arginine supplementation enhances the production of guanidinoacetate and creatine (Edison et al. 2007), which is beneficial for endothelial function (Bodamer et al. 2005). At present, little is known about creatine biosynthesis in fish.

The main function of creatine is to store energy in tissues, primarily skeletal muscle and brain, via creatine kinase, which interconverts creatine and phosphocreatine (Brosnan and Brosnan 2016). This enzyme is expressed at high levels in most of the cells and tissues that have high energy requirements, including the brain, kidneys, retinal photoreceptor cells, spermatozoa, testis, uterus, placenta, sensory hair cells of the inner ear, as well as skeletal, cardiac, and smooth muscles (Wu 2013a). Because of the action of creatine kinase, the concentration of ATP in the brain varies little despite large and rapid changes in turnover rates (Kekelidze et al.

2001). For example, the rate of the creatine kinase-catalyzed reaction rapidly increases with increased ATP demand (e.g., in seizures) but decreases with decreased ATP synthesis (e.g., hypoxia). In a resting state, skeletal muscle transfers excessive ATP to creatine, generating phosphocreatine; in a physically active state, skeletal muscle hydrolyzes phosphocreatine, releasing energy. Approximately 95% of creatine plus phosphocreatine in the body is present in skeletal muscle and brain, indicating the importance of creatine in maintaining ATP homeostasis.

5.5.6 Tyrosine Synthesis

The sources of tyrosine can be diets, protein breakdown, and phenylalanine hydroxylation. The liver and kidneys express phenylalanine hydroxylase, which irreversibly converts phenylalanine into tyrosine (Hufton et al. 1995). In female rats, the kidney (and the liver) is devoid of phenylalanine hydroxylase on Day 20 of gestation, but at birth contains 20% of the adult activity (McGee et al. 1972). In contrast to the liver, the kidneys have a limited ability to degrade tyrosine (Møller et al. 2000; Boirie et al. 2004). In adults, the kidneys extract phenylalanine from the blood and release tyrosine (Kopple 2007). In the post-absorptive state, the human kidneys synthesize an appreciable amount of tyrosine from phenylalanine at the rate of 5.2 μmol/min, compared with the rate of 3.0 μmol/min in the splanchnic bed (the portal-drained viscera plus liver; Møller et al. 2000). Humans and other animals (e.g., dogs and rats) with renal and hepatic disease may be at risk for phenylalanine overloading and tyrosine deficiency (Møller et al. 2000; Kopple 2007), raising the possibility that tyrosine is a nutritionally essential AA under these conditions (e.g., end-stage renal disease; Kopple 2007). The rate of conversion of phenylalanine into tyrosine is approximately 50% lower in patients with end-stage renal disease in comparison with healthy subjects (Boirie et al. 2004). Tyrosine has important physiological functions, including the syntheses of thyroid hormones and neurotransmitters (Wu 2013a,b). In chronic kidney failure, the concentrations of tyrosine and the ratio of tyrosine/phenylalanine are reduced in plasma and many tissues, with either no change or an increase in phenylalanine concentrations (Kopple 2007).

5.5.7 Glycine and Serine Synthesis

Glycine plays an important role in metabolic regulation, anti-oxidative reactions, and neurological function, such as (1) preventing tissue injury; (2) enhancing anti-oxidative capacity; (3) promote protein synthesis and wound healing; (4) improving immunity; and (5) treating metabolic disorders in obesity, diabetes, cardiovascular disease, ischemia-reperfusion injuries, cancers, and various inflammatory diseases (Wang et al. 2013; Wang et al. 2014a,b). Typical intake of dietary glycine meets at most 20% and 14% of daily glycine needs in young pigs (Hou et al. 2015a) and adult humans (Wu 2020b). Thus, these mammals must synthesize a majority of glycine needed daily to ensure their optimal health, growth, and feed efficiency (Li and Wu 2018). Available evidence shows that glycine is a nutritionally essential AA for maximal protein accretion in milk-fed piglets (Wang et al. 2014a).

The pathways for glycine synthesis via different sources are summarized in Fig. 5.6. In rats, the metabolism of hydroxyproline by the kidneys may contribute to a significant production of both glycine and serine. All the enzymes (hydroxyproline oxidase, hydroxyoxoglutarate aldolase, and alanine glyoxalate transaminase) involved in the metabolism of hydroxyproline to glycine are present in the kidneys and cortical tubules (Lowry et al. 1985). Alanine-glyoxylate aminotransferase is responsible for the nearly irreversible transfer of the amino group from alanine to glyoxylate, yielding glycine and pyruvate (Wang et al. 2013). Therefore, the rate of hydroxyproline metabolism is increased by alanine as a nitrogen donor (Lowry et al. 1985). Wu et al. (2019) have estimated that dietary plus endogenously-derived hydroxyproline contributes to most (64%) of the total glycine synthesis in 7-day-old pigs, whereas glucose plus gluta-

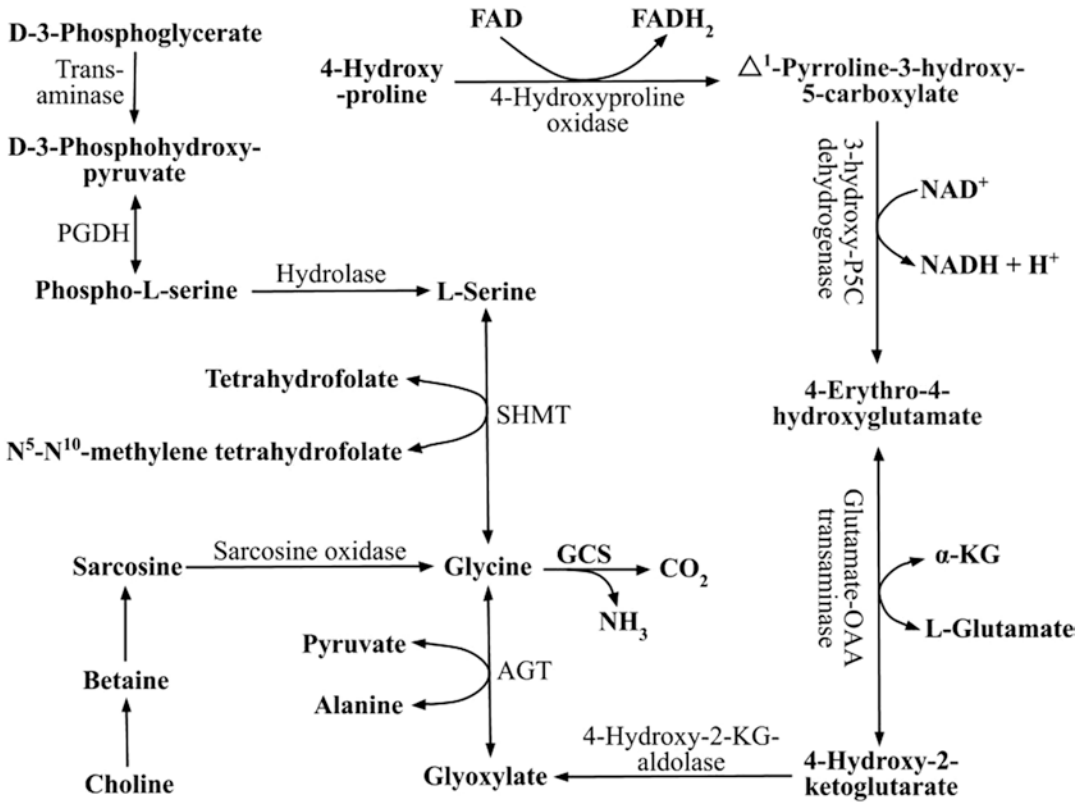

Fig. 5.6 The synthesis of glycine and serine from hydroxyproline, choline or D-3-phosphoglycerate. These synthetic pathways in mammals, fish and birds are cell- and tissue- specific. *SHMT* serine hydroxymethyl transferase

mate contributes to ~25% of total glycine synthesis in the neonates. Consistent with this view, milk and plasma contain large amounts of hydroxyproline-rich small peptides (Hu et al. 2017).

There are multiple pathways to degrade glycine in mammals and birds (Coon et al. 1974; Wu 2013a). In the liver and kidneys of these animals, glycine can be converted into serine by serine hydroxymethyl transferase at a low rate or degraded to NH_3 and CO_2 by glycine cleavage system (GCS) at a higher rate, with the regeneration of tetrahydrofolate. The low activities of renal GCS and serine hydroxymethyl transferase are necessary for the net production of glycine by the kidneys (Lowry et al. 1985; Wu et al. 2019). Thus, although the kidneys of mammals and birds may release serine into the blood circulation, the concentration of serine in plasma is enhanced only moderately by dietary glycine supplementation (Wang et al. 2013). Interestingly, the kidneys of fish have a higher activity of serine dehydratase (converting serine into pyruvate and NH_4^+) as compared with mammals, and are a major site for serine catabolism in the body (Jürss and Bastrop 1995).

5.5.8 Branched-Chain Amino Acid (BCAA) Metabolism

BCAAs play an important role in whole-body nitrogen metabolism under both physiological and pathological conditions (Cano et al. 2006). In dogs, the kidneys take up BCAAs (valine, leucine, and isoleucine) after consuming AAs in a meal (Kuhlmann and Kopple 1990). In the post-absorptive state, whole-blood-renal AA exchanges are characterized by the release of leucine from the kidneys that accounts for one-third

of whole-body leucine production, with no net renal exchange for valine or isoleucine (Tizianello et al. 1983). This indicates a different metabolic fate of leucine than the other two BCAAs in the kidneys. The plasma pool of BCAAs in the post-absorptive state is regulated by their release from tissues (e.g., skeletal muscle and kidneys) due to proteolysis, their uptakes by the tissues, and their oxidation in a tissue-specific manner (Felig 1975; Abumrad and Miller 1983; Price et al. 1998). Catabolic factors, such as acidosis and inflammation, are responsible for increases in intramuscular protein breakdown and BCAA degradation (Kopple et al. 2005), in association with an enhanced activity of branched-chain α-ketoacid (BCKA) dehydrogenase (May et al. 1987; Lim et al. 1998). In mammals and birds, BCKAs are extensively oxidized to CO_2 and water in the kidneys due to a high activity of BCKA dehydrogenase. Likewise, in fish species, the kidneys seem to be important for the catabolism of BCAAs because a high activity of BCAA transaminase is present in the posterior kidney (Hughes et al. 1984). Dietary supplementation with leucine or valine increases the activity of BCAA transaminase in the posterior kidney of Lake trout, *Salvelinus namaycush* (Hughes et al. 1984). Thus, in all animal species studied, the kidneys play an important role in regulating whole-body BCAA homeostasis.

5.5.9 Homoarginine Synthesis

L-Homoarginine (hArg) has an additional −CH2 group on its main carbon chain than L-arginine. Thus, hArg is a structural homologue of arginine. Synthesis of hArg by rats and humans was discovered by Ryan and Wells (1964), with the major sites of synthesis including the kidneys and liver (Ryan et al. 1968, 1969; Hou et al. 2015b). Less than 0.025% and < 0.045% of ingested arginine is metabolized to hArg in pigs and rats, respectively (Hou et al. 2016). Concentrations of hArg in plasma are relatively low (approximately 2 μM) in healthy humans (Marescau et al. 1985) and rats (Hou et al. 2015b), increase up to 20 μM in hyperargininemic patients (Marescau et al. 1985), and decrease in diabetic mice (Wetzel et al. 2019). The enzyme responsible for hArg synthesis in animals is unknown, but there are suggestions that mitochondrial arginine:glycine amidinotransferase (AGAT) catalyzes the transfer of the amidino group from L-arginine to L-lysine to form hArg, with L-ornithine being a product (Tsikas and Wu 2015). The concentrations of hArg in the brain, kidney and liver of rats are about 1.5, 100 and 115 μM, respectively (Hou et al. 2015b). Although AGAT activity in the liver is 20 times greater than that in the kidneys, concentrations of hArg in the liver are only 15% greater than those in the kidneys, possibly due a relatively low concentration of arginine in the liver (~0.05 mM) as compared with a much higher concentration (~1.5 mM) in the kidneys, and (b) the possibility of a higher rate of hArg catabolism in the liver than in the kidney.

hArg can regulate the metabolism of arginine and other nutrients by inhibiting arginine transport across the cell membranes, arginase, as well as liver and bone alkaline phosphohydrolases, while serving as a substrate for NO synthase (Tsikas et al. 2018). Whether hArg has a beneficial or an adverse effect on NO production likely depends on cell type, extracellular and intracellular concentrations of arginine, and activities of competing pathways for hArg and arginine metabolism. There is evidence that low concentrations of hArg in plasma are associated with a high risk of cardiovascular (Atzler et al. 2015) and renal (Wieczorek-Surdacka et al. 2019) diseases in humans and animals. Accordingly, dietary supplementation with hArg [via either drinking water (50 mg/L) or a mini-osmotic pump (0.72 mg/kg body weight per day)] for 12 weeks prevents kidney damage in diabetic mice (Wetzel et al. 2019).

5.6 Benefits of AAs on Renal Function

The current recommended protein intake for healthy adult humans with minimal physical activity is 0.8 to 1.0 g/kg body weight/day (Wu 2016). Excessive protein intake may promote

renal damage by chronically increasing glomerular pressure and hyperfiltration in mammals (Brenner et al. 1982; Martin et al. 2005; Beasley et al. 2014). In dogs, transition from a carbohydrate meal to a meat meal resulted in a 50–100% increase in glomerular filtration rate (GFR; King and Levey 1993). High protein intake may accelerate renal disease in adults with mild renal insufficiency or in peritoneal dialysis patients, leading to a progressive loss of renal capacity and function (Johnson et al. 2003; Knight et al. 2003). In another study, increased GFR and renal hypertrophy as well as hormonal changes occur at moderate rates after the consumption of a high-protein diet (e.g., 2.6 g protein/kg body weight/day vs 0.1 to 0.4 and 1.0 to 1.4 g protein/kg body weight/day for 2 weeks; King and Levey 1993; Martin et al. 2005). However, an early study indicated that glucagon, insulin and growth hormone were not involved in an increase of GFR induced by protein intake (Bergstrom et al. 1985), indicating a complex interplay among nutritional and physiological factors. Reddy et al. (2002) reported that consumption of low-carbohydrate and high-protein diets (19 g carbohydrate and 164 g protein for the first 2 weeks and 33 g carbohydrate and 170 g protein for the subsequent 4 weeks) increased risks for stone formation and bone loss, compared with a regular (control) diet (285 g carbohydrate and 91 g protein). However, interpretation of this result is confounded because the dietary intakes of energy and minerals (including calcium) were lower in the high-protein group, compared with the control group. Nonetheless, dietary protein restriction is a common treatment for patients with renal disease by alleviating uremic symptoms due to the better control of hyperparathyroidism, hyperphosphatemia and hyperkalemia as well as improvements in the epithelial integrity of the renal tubule (Pedrini et al. 1996; Chauveau et al. 2007). Changes from a low to a high intake of dietary protein can allow for adaptive alterations in renal size and function without adverse effects (Skov et al. 1999). Results of clinical studies indicate that consumption of a weight-loss diet containing 90–120 g protein per day does not affect renal function in overweight subjects or in obese adults with type-II diabetes, compared with the counterparts consuming 55–70 g protein per day (Wu 2016). Aquatic animals naturally require 100–200% greater dietary protein than land mammals (Wu 2018), it is unclear whether such a high intake of protein over a prolonged period of time may adversely affect the health of fish and shrimp. Thus, it is important to study the roles of AAs not only as major metabolic fuels for the kidneys of aquatic animals but also as protectors of their renal health.

Restricted intakes of dietary protein below physiological requirements for AAs may be harmful to health by accelerating the development of protein-energy wasting, leading to adverse consequences such as malnutrition and increased risk for death (Garibotto 1999). Protein-energy wasting is a strong predictor of the adverse outcomes that are characterized by a decline in body protein mass and energy reserves. Unfortunately, this metabolic condition is often under-appreciated in early to moderate stages of chronic kidney disease (Kovesdy et al. 2013). A protein-restricted diet supplemented with some AAs or the α-ketoacids or hydroxyacid analogues of AAs (e.g., phenylalanine and methionine) may have potential beneficial effects on improving renal function and survival (Abel, et al. 1973; King and Levey 1993). For example, oral administration of tyrosine, tyrosine-containing dipeptides, or *N*-acetyl-tyrosine can replete the plasma and intracellular pools of tyrosine and improve nitrogen balance in chronic renal failure patients on a low protein diet (Druml et al. 1989). The AA supplements for those patients include valine, leucine, isoleucine, phenylalanine, threonine, tryptophan, lysine, methionine, tyrosine, and histidine (Alvestrand et al. 1983; Cano et al. 2006). Patients with chronic kidney disease on dialysis have been recommended to reduce protein intake from 1.2 to < 0.8 or even < 0.6 g/kg body weight per day (Kalantar-Zadeh et al. 2011). BCAA and BCKA supplements may be integrated into a therapeutic strategy that includes protein restriction for these subjects (Cano et al. 2006). However, high levels of dietary BCAAs can exert a deleterious effect on renal disease and should be avoided at all times (Pillai and Verrey 2019).

Functional AAs hold great promise in prevention and treatment of metabolic diseases, such as renal dysfunction (Wu 2013b). A deficiency of AAs or glutathione depletion may contribute to disturbances in renal structure and function (Epstein et al. 1982). An increase in renal blood flow in response to a short-term AA infusion can protect the kidneys from acute ischemic insults in animal models (King and Levey 1993). Glycine has a positive effect on reducing medullary injury in perfused kidneys (Silva et al. 1991) and mild ischemia-reperfusion injury in the kidneys in vivo, in part by decreasing initial damage and preventing chronic hypoxia (Yin et al. 2002). Thus, glycine can be used to prevent or treat ischemic and/or toxin-induced injury to the kidneys. Similarity, Baines et al. (1990) reported that small neutral AAs, such as glycine and alanine, prevented tubular disruption through their physicochemical effects on stabilizing the tertiary structure of membrane proteins. Some AAs play a role in the renal cell cycle and apoptosis, function as osmolytes during the stress response, scavenge reactive oxygen species (ROS), and modulate blood flow (Chesney et al. 2010). For example, taurine reduces oxidant levels in diabetic nephropathy (Trachtman et al. 1995), and protects against Cd-induced renal oxidative damages (Manna et al. 2009) or cyclosporine A-induced hypertension and nephrotoxicity (Hagar et al. 2006). In addition, arginine corrects renal failure-associated endothelial dysfunction (Hand et al. 1998) and hypertension (Wu et al. 2000). L-Citrulline may also be beneficial for ameliorating renal disorders, while facilitating the removal of ammonia through arginine synthesis by proximal renal tubules and other cell types.

5.7 Functions of AAs in the Immune System of Fish Kidneys

As noted previously, the anterior (head) kidney is an important immune organ in fish, in addition to their thymus and spleen. The head kidney contains cytokine-producing T-lymphocytes (T-cells), antibody-producing B-lymphocytes (B-cells), and ROS-producing macrophages (Shoemaker et al. 2015). Protein synthesis in immunocytes require AAs. Our recent studies have shown that AAs, such as glutamate and glutamine, are more important energy substrates than glucose and fatty acids in the kidneys of fish (Jia et al. 2017). These findings indicate an essential role of the head kidney and AAs in innate and adaptive immunities in fish. There are many lines of evidence that functional AAs have beneficial effects on the immune system of fish via improving the kidney functions. For example, arginine stimulates the proliferation of T-lymphocytes in response to mitogens (Ochoa et al. 2001) and increases the number of cell surface receptors in fish (Cheng et al. 2011). In Jian carp, the transcript levels of inflammatory response genes in the head kidney are up-regulated by increasing dietary arginine intake (Chen et al. 2015). In addition, glutamine supplementation improves the development of B-cells in the head kidney of fish in the head kidney (Hu et al. 2015). Similarly, glutamine supplementation promotes the development of the head kidney in channel catfish (Pohlenz et al. 2012). Finally, leucine supplementation augments antioxidant activities, immune-gene expression, and ant-inflammatory responses in the head kidney of Labeo rohita fingerlings (Giri et al. 2015). Thus, AAs and their metabolism in the kidneys play an important role in mounting immune responses to challenges by bacterial, viral, parasitic, and fungal pathogens.

5.8 Conclusion and Perspectives

Results from studies with humans and different animal species indicate that the kidneys play a vital role in AA metabolism and hemostasis through the following mechanisms: the reabsorption of free AAs and other nutrients by the proximal tubules into the blood; the production of glucose from AAs via gluconeogenesis; regulation of the acid-base balance via the inter-organ metabolism of glutamine; the synthesis of arginine from citrulline and the conversion of arginine into NO; the generation of polyamines from arginine and ornithine; the generation of tyrosine

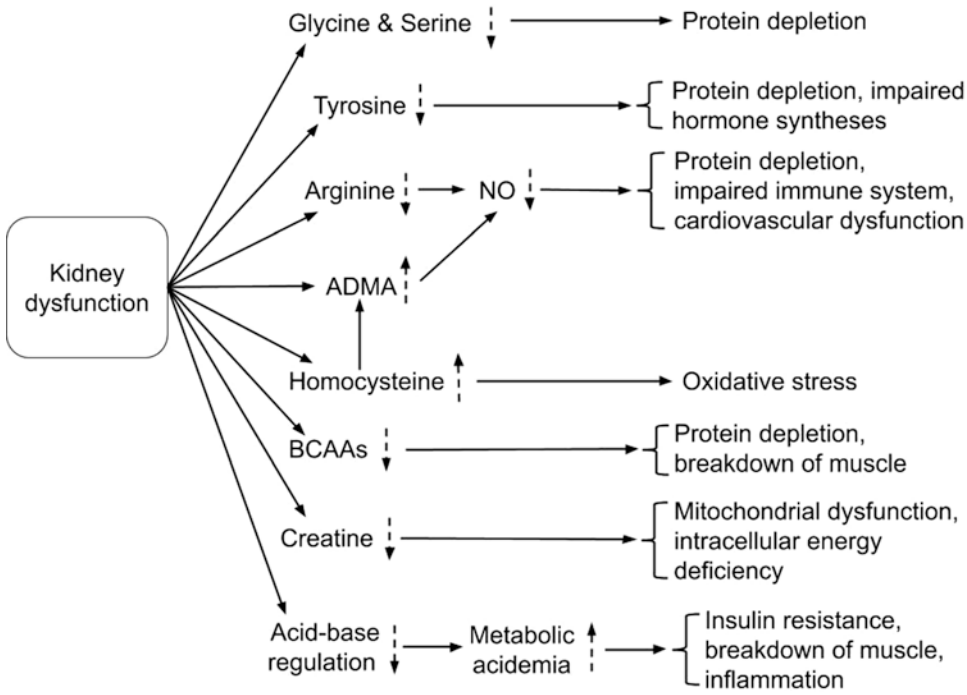

Fig. 5.7 The diseases development from the impaired amino acids metabolism in kidney dysfunction (like chronic kidney failure). *ADMA* asymmetric dimethylarginine, BCAAs, branched-chain amino acids

from phenylalanine; glycine and serine syntheses; the catabolism of BCAAs; the metabolism of methylarginines and sulfur AAs; ATP production; and immune response. Renal dysfunction alters the pathways of AA metabolism and, therefore, whole-body AA homeostasis, leading to health problems, including excessive muscle protein breakdown, inflammation, mitochondrial dysfunction, impaired immune system, and cardiovascular diseases (Fig. 5.7). Although a reduced intake of protein is usually recommended to subjects with chronic renal failure in humans and other mammals, moderate supplementation with AAs (including valine, leucine, isoleucine, phenylalanine, threonine, tryptophan, lysine, methionine, tyrosine, histidine, glycine, glutamine, and taurine) may provide a beneficial effect to meet physiological requirements.

Most of the knowledge on renal AA metabolism was gained from studies with land mammals and birds. At present, little is known about AA synthesis or catabolism in the kidneys of fish. In aquatic animals, the nutritional values of AAs are often evaluated on the basis of growth, protein accretion, survival, immunity, anti-oxidative ability, or meat quality. We propose that protective effects of AAs on renal structure and function should also be an important criterion with which to assess dietary requirements of fish for these nutrients. It is likely that renal AA metabolism differs between fish and terrestrial animals. Although much research has been done with humans, mice and rats regarding the roles of the gut, intestinal microbiome, liver, skeletal muscle and adipose tissue in metabolic disorders (including diseases), there is only a limited number of published studies regarding the improvement of renal functions in livestock and poultry production or aquaculture (Wu 2020a). Further studies are warranted to address this critical issue in order to further enhance the quantity and sustainability of animal production.

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6 Amino Acids in Health and Endocrine Function

Nick E. Flynn, Max H. Shaw, and Jace T. Becker

Abstract

Dietary amino acids play an important role in maintaining health. Branched chain amino acids can adversely increase blood pressure whereas arginine and citrulline can reduce it. D-amino acids play important roles in several cell types including testis, the nervous system and adrenal glands. Several amino acids also can have dramatic effects on diabetes; branched chain amino acids, phenylalanine and tyrosine have been implicated while others, namely arginine and citrulline can improve outcomes. Leucine has been shown to play important roles in muscle primarily through the mTOR pathway though this effect does not translate across every population. Glutamine, arginine and D-aspartate also exert their muscle effects through mTOR. Relationships between amino acids and endocrine function include that of glucocorticoids, thyroid function, glucagon-like peptide 1 (GLP-1), ghrelin, insulin-like growth factor-1 (IGF-1) and leptin. Leucine, for example, can alleviate the effect of dexamethasone on muscle protein accretion. Interestingly, amino acid transporters play an important role in thyroid function. Several amino acids have been shown to increase GLP-1 levels in non-diabetics when administered orally. Similarly, several amino acids increase ghrelin levels in different species while cysteine can decrease it in mice. There is evidence to suggest that the arginine/NO pathway may be involved in modulating some of the effects of ghrelin on cells. In regard to IGF-1, branched chain amino acids can increase levels in adults while tryptophan and phenylalanine have been shown to increase levels in infants. Finally, leptin levels can be elevated by branched chain amino acids while restricting leucine in high fat diets can increase leptin sensitivity.

Keywords

Dietary · Hypertension · D-amino acid · Diabetes · mTOR · Glucocorticoids · Thyroid · Glucagon like protein-1 · Ghrelin and leptin

Abbreviations

NO	nitric oxide
IGF-1	insulin-like growth factor 1
GLP1	Glucagon-like peptide 1
BCAA	branched chain amino acids

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6.1 Introduction

The primary purpose of this review is to provide a broad update on the impact of amino acids on both health and endocrine function. This article intentionally does not focus on detailed molecular mechanisms related to these aspects of amino acids in relation to health as there are other reviews that effectively accomplish this (Wu 2009, El Hiani et al. 2019; Agostinelli 2020). With regard to amino acids in health, we will cover dietary amino acids, hypertension, D-amino acids, diabetes and muscle amino acid sensing. In regard to endocrine function we will cover glucocorticoids, thyroid function, glucagon-like peptide 1 (GLP-1), ghrelin, insulin-like growth factor-1 (IGF-1) and leptin.

6.2 Dietary Amino Acids and Health: An Update

Both the source and corresponding intake of amino acids are known to affect health (Wu 2016, 2020). A summary of the effects discussed here can be found in Table 6.1. Recent studies suggest a link between dietary amino acid intake and a variety of health parameters including: bone mineral density, signalling molecule levels, cellular pathways, hypertension and reproduction. It is important to emphasize that the source of dietary amino acids (e.g. plant vs animal) has been shown to have a significant impact on the incidence of diabetes in epidemiology studies (Ke et al. 2018). Analysis of amino acid intake using discordant twins suggest that intake of certain amino acids, namely alanine, arginine, glycine, leucine and lysine, are associated with higher bone mineral density (Jennings et al. 2016).

Several studies suggest that amino acid intake can affect hormone and cytokine levels. Administering purified amino acids to broiler chickens elevated IGF-1 levels while reducing IFN-γ and TNF-α levels compared to control. This supplementation also led to increased carcass and breast weight (Wandita et al. 2018). Administration of a mixed amino acid solution to dairy cattle followed by ghrelin injection led to marked increases in insulin and glucagon accompanied by a greater decline in plasma glucose (Fukumori et al. 2011). When L-theanine, an amino acid in tea leaves (Wu 2013), is administered to performance athletes, a decrease in post-exercise IL-10 levels which suggests that it exerts an effect on cellular TH1/TH2 balance (Juszkiewicz et al. 2019). Conversely, administration of β-alanine during military training led to an increase in IL-10 in soldiers (Hoffman et al. 2018). There are, however, conflicting results as to whether arginine supplementation in healthy individuals can affect growth hormone, IGF-1 and insulin production (da Silva et al. 2014). The effects of arginine on individuals likely depend on their nutritional and physiological status. For example, oral arginine has been shown to actually reduce growth hormone levels after resistance training in strength trained males (Forbes et al. 2014). In obese subjects, dietary supplementation with 30 g arginine/day for 90 days did not affect the concentrations of insulin, growth hormone, or thyroid stimulating hormone in serum, but reduced systolic blood pressure and serum glucose concentration in females, as well as serum concentrations of free fatty acids in both males and females (McNeal et al. 2018).

Administration of dietary amino acids based on prior studies, however, does not always provide the same clinical result in select patient populations. Arginine and lysine, both known for their ability to stimulate growth hormone secretion in other studies, could not mediate an increase in growth hormone secretion in elderly heart failure patients (Smeets et al. 2017).

Some of the effects of dietary amino acids actually start at the sensory level of the tongue. T1R1 and T1R3 receptors on the tongue detect amino acids in food while various organs (intestine, pancreas and heart) are capable of detecting extracellular amino acids. Furthermore, there are mechanisms in the body including GCN2, LYNUS and mTOR which can adjust cellular pathways based upon amino acid sensing and Lushchak provides a good review of these mechanisms in relation to amino acid sensing (Lushchak et al. 2019).

Elevated dietary intake of aromatic amino acids, specifically phenylalanine obtained from

Table 6.1 Dietary amino acids in health, endocrine function and physiology

Dietary Amino Acid(s)	Species/Subjects/Cell types	Positive effect (improvement)	Negative effect (adverse response)
Ala, Arg, Gly, Leu and Lys	Human	Bone density	N/A
Purified amino acids	Broiler Chickens	IGF-1 Carcass and breast weight	IFN-γ and TNF-α
Mixed amino acid solution + ghrelin injection	Dairy cattle	Insulin Glucagon	Plasma glucose
L-Theanine	Performance athletes	Brain function	IL-10
β-Alanine	Military training	IL-10	N/A
Arginine	Resistance trained males	Growth hormone	N/A
Arginine and Lysine	Elderly heart failure patients	No effect on growth hormone at the dose used	N/A
Aromatic amino acids	Human	Blood pressure	N/A
Cysteine	Human	Reproductive system	N/A
Arginine + B vitamins	Humans	Reduced blood pressure	N/A
Citrulline	Prehypertensive and hypertensive patients	Reduced blood pressure	N/A
Ile, Leu, Val, Tyr and Phe	Hypertensive patients	N/A	Impaired fasting blood glucose at high dose
Branched chain amino acids + high fat diet	Human (obese)	N/A	Obesity-induced insulin resistance
Leucine	Mice	Improved glucose tolerance and insulin signaling	N/A
Citrulline and Citrulline + Arginine	Type 2 diabetic rats	Reduced endothelial senescence	N/A
Arginine	Humans	Improved insulin sensitivity/ secretion	N/A
Glutamine	Healthy humans	Reduced blood glucose	N/A
Leucine	Human skeletal muscle	Insulin signaling in muscle	N/A
Leucine + Vitamin D	Older male humans	Muscle protein synthesis	N/A
Leucine	Cultured cells	Muscle protein accretion	N/A
Glutamine	Type 2 diabetic patients	Glucagon like protein-1	N/A
Tryptophan	Humans	Glucagon like protein-1	N/A
Glu, Gln, Lys, Thr or Val	Sheep	Ghrelin release	N/A
Tryptophan or Leucine	Mice	N/A	Ghrelin levels
Cysteine	Mice	N/A	Ghrelin levels
BCAA + carbohydrates	Humans	IGF-1	N/A
Leucine	Resistance trained men	IGF-1	N/A
Tryptophan + Phenylalanine in formula	Infants	IGF-1	N/A
Branched chain amino acids	Nondiabetics	Leptin	N/A
Lysine restricted diets	Piglets	N/A	Reduced leptin levels
Leucine	Rats	Leptin sensitivity	N/A

N/A not applicable or reported

animal sources, may be associated with hypertension (Teymoori et al. 2018). The same group also demonstrated that diets high in animal protein and dairy resulted in an amino acid pattern high in branched chain, aromatic, alcoholic amino acids and proline which may be associated with an increased risk of hypertension (Teymoori et al. 2017) that we will explore later. Finally, the positive effects of coconut water on the reproductive system may, indeed, be due to cysteine (Kunle-Alabi et al. 2017).

6.3 Amino Acids and Hypertension

Links between amino acid intake and hypertension have only recently been recognized. Branched chain amino acids (BCAA) and the aromatic amino acids tyrosine and phenylalanine have been associated with both hypertension and impaired fasting glucose (Weng et al. 2015). It is worth noting that these amino acids are also elevated in patients with β blocker induced impaired fasting glucose (Cooper-Dehoff et al. 2014). Given the well known association between diets high in saturated fats and hypertension, it would be worth comparing the amino acid content of animal vs plant sources of amino acids in relation to hypertension.

While some amino acids have detrimental effects on hypertension, others can apparently be beneficial. A recent clinical trial demonstrated that combining administration of arginine and B vitamins can restore endothelial function and lower blood pressure in mild to moderate hypertensive patients (Menzel et al. 2018). Citrulline has been demonstrated to relieve hypoxia induced hypertension in newborn pigs (Fike et al. 2015) and can reduce blood pressure in overweight men in response to exercise (Figueroa et al. 2016). Given the high concentration of citrulline in watermelon, one study was able to demonstrate that administration of watermelon extract could effectively reduce blood pressure (Massa et al. 2016) in prehypertensive and hypertensive patients. It is believed that much of the effects of arginine and citrulline on blood pressure are exerted through nitric oxide (NO) pathways, but it would be worth examining their associated effects on other areas explored in this particular article.

6.4 D-Amino Acids and Health

Because they are not incorporated into nascent proteins as a result of protein synthesis mechanisms in cells, D-amino acids were quite often ignored in regard to their effect on cellular metabolism. We now know that this is certainly not the case. Table 6.2 provides the major effects of D-amino acids on health discussed below.

D-aspartate serves signalling roles in several parts of the body including the reproductive and nervous systems. Immature Leydig cells were shown to accumulate D-asparate and increase androstenedione and testosterone synthesis in parallel to D-aspartate levels (Raucci et al. 2014). Similarly, D-aspratate regulates the release and synthesis of leutenizing hormone and testosterone in both humans and rats (Topo et al. 2009). Additionally, D-aspartate administration has been shown to increase androgen receptor expression while reducing estrogen receptor expression in rat testis (Santillo et al. 2014). D-asparate is degraded by D-aspartate oxidase thus regulating its levels in the body. This enzyme also degrades D-glutamate. Studies in animals where this enzyme is knocked out demonstrate an increase in D-asparate levels while D-glutamate levels remain unchanged (Han et al. 2015). Synthesis pathways for most D-amino acids are poorly understood in mammals and remain a fertile area for future research. D-aspartate could arguably be produced by a serine dehydratase enzyme, but apparently is not produced by glutamic-oxaloacetic transaminase-1 enzyme (Tanaka-Hayashi et al. 2015). D-asparate promotes L-glutamate release in selected areas of the brain and has been suggested as a potential treatment for schizophrenia (Errico et al. 2018).

Three notable D-amino acids, D-aspartate, D-serine and D-alanine are known to interact with NMDA receptors in the nervous system (Kiriyama and Nochi 2016). These receptors are involved in learning and memory. D-serine is not uniformly distributed in the nervous system. The cerebrum contains the highest levels but even in this location there is a divergent distribution. This distribution pattern suggests that D-serine may exhibit localized effects of N-methyl-D-aspartate glutamate receptors which the authors suggest may play a role in treating neurological diseases. (Suzuki et al. 2017). Release of D-serine is mediated through the alanine–serine– cysteine transporter-1 (Rosenberg et al. 2013). D-serine may originate from the glia in the cerebral cortex and serve as the coagnosist for NMDA receptors. Similar to

Table 6.2 D-amino acids in health

D- amino acid	Organ/Cells/Species	Effect
D-aspartate	Leydig cells (human)	↑ androstenedione and testosterone synthesis
D-aspartate	Reproductive system (human)	↑ Leutinizing hormone
D-aspartate	Reproductive system (rat)	↑ Leutinizing hormone, androgen receptor
D-aspartate	Brain	↑ Glutamate release
D-aspartate, D-serine and D-alanine	Nervous system (human)	↑ NMDA receptor function
D-alanine	Anterior pituitary gland (rat)	↑ ACTH synthesis
D-alanine	Adrenal gland (rat)	↑ Glucocorticoid secretion (via ACTH)

D-aspratate, modifying levels of D-serine may play a role in treating neurological disorders (Fossat et al. 2012). Furthermore, D-aspartate supplementation has been proposed as a new way to promote myelin recovery (de Rosa et al. 2019).

D-alanine has been shown to affect hormonal secretion in the pineal gland, anterior pituitary and adrenal glands (Errico et al. 2000, Etoh et al. 2009). It is also interesting to note that D-alanine levels exhibit circadian rhythm changes in concentration (Karakawa et al. 2013). Intestinal flora may be responsible for D-alanine production while intestinal absorption could explain diurnal variations in D-alanine levels (Karakawa et al. 2013).

6.5 Amino Acids and Diabetes

Amino acid profiles are significantly altered in individuals with metabolic disorders, specifically obesity, type 2 diabetes and metabolic syndrome. For example, valine, isoleucine, glutamic acid and proline levels increased in these metabolic disorders, while glycine decreased in one study (Okekunle et al. 2017). Another study noted that the development of diabetes in individuals may be associated with isoleucine, leucine, valine, tyrosine and phenylalanine levels (Wang et al. 2011). As mentioned above, these same five amino acids are also associated with impaired fasting blood glucose in patients with hypertension (Weng et al. 2015). As mentioned above, these amino acids are also elevated in patients with β blocker induced impaired fasting glucose (Cooper-Dehoff et al. 2014).

Branched-chain amino acids provide an interesting association with diabetes. Overconsumption of food has notably been associated with elevated levels of BCAA (Elshorbagy et al. 2018) thus making the understanding of the relationship between diabetes and these amino acids, both as a whole and on an individual amino acid basis vital. To further emphasize this point, consumption of diets high in fat accompanied by branched chain amino acid consumption is associated with obesity induced insulin resistance (Newgard et al. 2009).

On an individual basis, leucine provides an interesting window into the relationship between BCAA and diabetes. Leucine administration to mice fed on a high fat diet significantly improves glucose tolerance and insulin signalling while reducing hepatic steatosis and adipose tissue inflammation (Macotela et al. 2011). When combined with resistance training, leucine can synergistically reduce muscle loss in diabetic rats by increasing protein synthesis (Martins et al. 2017). In contrast, reduced leucine intake has been shown to improve hepatic sensitivity to insulin which may help improve insulin resistance (Fei Xiao et al. 2011). Similarly, leucine supplementation caused a delay in muscle IR/PI3K signaling, leading to impaired glucose tolerance (Balage et al. 2011). These conflicting findings regarding BCAA (individual vs combinatorial) suggests that these amino acids exert different effects depending on what other amino acids they are consumed with and in what ratios. To that end, the co-administration of glucose with leucine and phenylalanine to healthy individuals

results in an additive effect on insulin production. Findings from this study suggest that the effect of leucine on insulin production is apparently different than that of phenylalanine (Iverson et al. 2013). Other amino acids worth discussing in relation to diabetes are arginine/citrulline and glutamate/glutamine.

Supplementation with citrulline and arginine or citrulline alone can reduce endothelial senescence in dyslipidemic type 2 diabetic rats fed a high glucose diet (Tsuboi et al. 2018). It has also been noted that L-arginine supplementation may reduce the onset of type 2 diabetes via a reduction in oxidative stress. Subjects treated with L-arginine demonstrated an improvement in both insulin sensitivity and secretion (Monti et al. 2018).

Glutamine and glutamate, as well as their ratio, may serve as markers for the incidence of diabetic retinopathy in type 2 diabetics (Rhee et al. 2018). The mechanism is not entirely understood, but it is interesting to point out that the glutamine/glutamate ratio does correlate to insulin resistance in some study groups (Cheng et al. 2012). This is contrasted by findings which indicate that in healthy individuals glutamine can slow gastric emptying and reduce blood glucose spike associated with consuming a high energy glucose drink (Du et al. 2018). It is thought that glutamine accomplishes this by increasing pyloric motility (Chang et al. 2013). Interestingly, both L-glutamine supplementation and whole protein diet low in glutamine can help restore the first phase insulin response in type 2 diabetics (Samocha-Bonet et al. 2015). Given that glutamine can be readily synthesized in cells and plasma glutamine levels were not monitored, it is possible that the additional dietary protein led to an undetected increase in glutamine production. Glutamate plays a vital role in islet cell function (Maechler and Wollheim 1999) and it has been suggested that NMDA receptors may be important targets for treating type 2 diabetes (Otter and Lammert 2016).

Pharmaceutical treatment of diabetes apparently does have an effect on certain amino acid levels. It is interesting to note that metformin, used to control high blood glucose, does elevate plasma levels of histidine while lowering levels of phenylalanine and tyrosine (Preiss et al. 2016). L-amino acid transporters have been demonstrated to play a key role in regulating beta cell function and signalling and they could, indeed, be a good target for drug design in the treatment of type 2 diabetes (Cheng et al. 2016).

6.6 Amino Acid Signaling in Muscle

A significant level of research has emerged regarding amino acid signalling in muscle and particularly leucine. Many of these effects are mediated through the mTOR signaling pathway which has been reviewed elsewhere (Wu 2009, El Hiani et al. 2019). Leucine has a synergistic effect on insulin signaling in muscle by supporting select phosphorylation events (Di Camillo et al. 2014). It activates mTOR signaling in human myotubes via phosphorylation and was shown to be independent of insulin. This did not affect amino acid transport expression, but did increase expression of human vacuolar sorting protein. (Gran and Cameron-Smith 2011). Leucine also has been shown to upregulate muscle slow fiber and glucose oxidative metabolism through the mTOR signaling pathway (Sato et al. 2018). Furthermore, there is evidence that combining leucine supplementation with vitamin D supplementation can improve muscle protein synthesis in older men (Chanet et al. 2017).

The effect of leucine on muscle in certain populations is certainly worth mentioning. Leucine serves a more potent anabolic role than total protein in older women (McGlory et al. 2018). Additionally, regulation of mTOR signaling in older rats may be reduced in skeletal muscle and increased in adipose tissue (Zeanandin et al. 2012). Sarcopenia is a muscle disease which involves the loss of both muscle mass and strength over time. Leucine may serve a beneficial role in treating sarcopenia (Tessier and

Chevalier 2018). Whey supplement enriched with leucine negated lower protein synthetic rates in sarcopenic males (Kramer et al. 2017). Expanding this to other BCAA, BCAA supplementation prevents liver cirrhosis-induced sarcopenia (Kitajima et al. 2018).

It is worth mentioning that other amino acids have been shown to affect the mTOR pathway in muscle. Glutamine, for example, also affects mTOR by decreasing phosphorylation (Deldicque et al. 2008). Arginine has been shown to reverse altered mTOR signalling in a model of Pompe disease (Lim et al. 2017). Modifying BCAA ratios can affect mTOR expression (Duan et al. 2017). These researchers were also able to demonstrate that modifying ratios of BCAA had beneficial effects on myocytes with regard to differentiation and amino acid expression.

6.7 Amino Acid Metabolism and Glucocorticoids

Glucocorticoids play an important role in modulating amino acid metabolism and subsequent signalling. For example, in the nervous system they can protect cells from toxic concentrations of glutamate. This effect is mediated by decreasing cytosolic calcium concentrations in cortical neurons (Suwanjang et al. 2013). Asymmetric dimethylarginine, an endogenous inhibitor of NO production, can be antagonized by homoarginine (Tsikas and Wu 2015). It is interesting to note that homoarginine levels are lower while asymetric dimethylarginine levels are higher in Duchenne muscular dystrophy. This leads to an impairment of NO production via arginine which can be alleviated through glucocorticoid treatment (Horster et al. 2015). Amino acids, on the other hand, can ameliorate effects of glucocorticoids. For example, leucine has been shown to alleviate the effect of dexamethasone on muscle protein accretion by acting synergistically on mTOR and AMPK systems in cultured cells (Wang et al. 2016).

6.8 Amino Acids and Thyroid Function

Amino acid transporters may work cooperatively to facilitate transport of various iodocompounds to regulate cellular thyroid status (Zevenbergen et al. 2015). L-type amino acid transporters, in particular, may play a role in mediating this (Hinz et al. 2015, Krause and Hinz 2017). Zevenbergen was able to demonstrate that there was differential transport between the five L-type amino acid transporters that were studied. Metabolism of alternative amino acids may provide an additional mechanism whereby thyroid hormones can be produced in the thyroid. To this end, metabolism and production of D-aspartate via actions of D-aspartate oxidase and D-aspartate racemase, may provide another mechanism whereby thyroid hormones can be produced due to production of H_2O_2 during metabolism (Enza Topo et al. 2010).

6.9 Amino Acids and Glucagon-like Peptide 1

Glucagon-like peptide 1 (GLP1) is a 30 amino acid peptide hormone produced in epithelial endocrine cells and the brain in response to meal intake (Sekar et al. 2016). It is produced via differential processing of proglucagon in these cells. Proglucagon is expressed in the intestine, β cells and the brain, but pancreatic processing yields the precursor molecule responsible for the production of GLP1. Effects of this molecule include inhibition of gastric acid production and stimulation of glucagon secretion. It plays a needed role in stimulating insulin release in response to other signalling events (Drucker and Nauck 2006). As a result, the control of GLP1 via amino acids and their associated metabolism merits discussion.

Administration of oral amino acid solutions were shown to increase levels in non-diabetic patients (Lindgren et al. 2015). This was not mirrored in intravenous administered amino acid solutions. Glutamine has been shown to increase GLP-1 levels in Type 2 diabetics (Samocha-Bonet et al. 2011, 2015). It has been suggested that this

response is due to an activated glutamate dehydrogenase (Andersson et al. 2018). Interstingly, human milk elicits a significant increase in concentration in healthy adult subjects (Gunnerud et al. 2012). This ties directly into the observation that mature human milk is extremely high in glutamine (Zhang et al. 2013). With regard to other amino acids, administration of L-tryptophan but not L-leucine to lean and non-diabetic subjects can modulate levels of GLP1 in blood (Meyer-Gerspach et al. 2016).

6.10 Amino Acids and Ghrelin

Ghrelin, discovered 20 years ago, can be produced by several cells including the small intestine, pancreas, brain and stomach which is the primary producer. It is known to stimulate production of growth hormone and ACTH (Gray et al. 2019). Other effects include increase in appetite, elevated gut motility and increased gastric acid secretion. It increases lipolysis and has recently been demonstrated to affect insulin release by β cells. Recently, Wu et al. (2020) have shown that ghrelin protects aging mice from fasting-induced muscle atrophy. Several amino acids are known to affect ghrelin levels in organisms.

Lysine-restricted diets have been shown to reduce ghrelin expression in piglets (Yin et al. 2017). Note that this amino acid is abundant in animal-source fooods but is relatively deficient in most plant-source foods (Hou et al. 2019; Li and Wu 2020). Duodenal supply of glutamate, glutamine, lysine, threonine and valine have been shown to enhance ghrelin release in sheep (Elsabagh et al. 2018). In humans, both tryptophan and leucine exhibit a stronger reduction than glutamine on plasma levels of ghrelin (Steinert et al. 2017), while L-cysteine has been shown to reduce ghrelin plasma levels in mice (McGavigan et al. 2015).

There is evidence to suggest that the arginine/NO pathway may be involved in modulating some of the effects of ghrelin on cells. Ghrelin has been shown to enhance uptake of food and oxidation of carbohydrates in a NO dependent manner using L-NAME, a NO donor (Abtahi et al. 2017). Similarly, NO has been implicated in mediating effects of ghrelin on potassium currents in the hippocampus (Lu et al. 2018). Another pathway which may involve amino acids and ghrelin could be the extracellular signal regulated kinase pathway since glutamate has been shown to inhibit ghrelin expression in cell lines through this pathway (Chacrabati et al. 2017).

6.11 Amino Acids and IGF-1

Insulin-like growth factor 1 (IGF-1) is important in mediating fetal and postnatal growth and development. The interplay between IGF-1 and growth hormone has sometimes been debated. Many believe that there are codependent and independent effects in regard to these two signaling molecules.

Amino acids can certainly exhibit an effect on IGF-1 levels in cells and production. Consumption of carbohydrates with branched chain amino acids resulted in an increase in serum IGF-1 levels in humans (Li et al. 2015). Administration of L-leucine to resistance trained men results in increased IGF-1 levels in muscle (Church et al. 2016). It was also determined that infants fed formula containing tryptophan and phenylalanine exhibited elevated IGF-1 compared to controls (Fleddermann et al. 2017). There is evidence that administration of several amino acids can increase IGF-1 levels under specialized metabolic conditions. In this respect, administration of an amino acid supplement containing eight essential amino acids resulted in an increase in IGF-1 levels in healthy men placed on bed rest (Brooks et al. 2014). This could lead to specialized treatment paradigms where reducing muscle atrophy due to illness or restricted activity is desired. All of these findings need to be tempered with the understanding that other amino acids, such as citrulline, can certainly provide similar beneficial physiological effects without directly affecting insulin action (Jourdan et al. 2015).

6.12 Amino Acids and Leptin

Leptin is produced by adipose tissue cells and affects the hippocampus to modulate eating behaviors and energy expenditure. It plays a major

role in regulating feed intake. Amino acids can have significant effects on leptin levels. Branched chain amino acids are positively correlated with leptin levels in nondiabetics (Katagiri et al. 2018). Lysine restricted diets have been shown to reduce leptin concentrations in the blood of piglets (Yin et al. 2017) while leucine can improve leptin sensitivity in rats fed a high fat diet (Yuan et al. 2015).

6.13 Future Work

In summary, there are several identified areas of future study that emerged from this review. These include studying the fertile area of D-amino acid metabolism and actions as well as the multiple effects of branched chain amino acids on health and endocrine function. The interplay between amino acid transporters, the endocrine system and other physiological functions (e.g. D-amino acid metabolism, thyroid and β-cells) certainly deserves more attention. Finally, the recently discovered effects of arginine and nitric oxide on ghrelin function suggests that the nitric oxide field is by no means played out.

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Amino Acids in Reproductive Nutrition and Health

7

Haijun Gao

Abstract

Amino acids are not only the building blocks of proteins, an indispensable component of cells, but also play versatile roles in regulating cell metabolism, proliferation, differentiation and growth by themselves or through their derivatives. At the whole body level, the bioavailability and metabolism of amino acids, interacting with other macronutrients, is critical for the physiological processes of reproduction including gametogenesis, fertilization, implantation, placentation, fetal growth and development. In fertilization and early pregnancy, histotroph in oviductal and uterine secretions provides nutrients and microenvironment for conceptus (embryo and extraembryonic membranes) development. These nutrients include select amino acids in histotroph (arginine, leucine and glutamine of particular interest) that stimulate conceptus growth and development, as well as interactions between maternal uterus and the conceptus, thus impacting maintenance of pregnancy, placental growth, development and functions, fetal growth and development, and consequential pregnancy outcomes. Gestational protein undernutrition causes fetal growth restriction and predisposes cardiovascular, metabolic diseases and others in offspring via multiple mechanisms, whereas the supplementation of glycine, leucine and taurine during pregnancy partially rescues growth restriction and beneficially modulates fetal programming. Thus, amino acids are essential for the fertility of humans and all animals.

Keywords

Amino acid · Nutrition · Reproduction · Metabolism · Uterus · Pregnancy · Mechanistic target of rapamycin

7.1 Introduction

Nutrition is critical for any organism's growth and reproduction because nutrients not only provide building blocks for the growth of any cell, organ and whole body, but also regulate the development of reproductive organs, onset of reproduction and fetal growth and development (Wallace et al. 2006; Wang et al. 2012; Bloomfield et al. 2013). Among nutrients, amino acids not only function as the building block of proteins, one of the major components of cells, tissues, organs or organisms, but also plays versatile roles in reproduction, development and production

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(Wu et al. 2004, 2014; Wu 2009). Here we review the essential functions of amino acids, particularly derived from diet, in reproductive processes including gametogenesis, conceptus development and the long term impacts of their nutritional status during pregnancy on the health and diseases of adult offspring. Because published studies that address the role of amino acid in reproduction focus on amino acid transport and metabolism, the related main findings will be the focus of this review. Mammals (e.g., humans, pigs, sheep and cattle) will be included, but knowledge from other vertebrates such as rodents is also discussed if appropriate.

7.2 Amino Acids in Gametogenesis

7.2.1 Oogenesis

In mammals, oogenesis is a long and complicated process, which requires the hormonal and nutritional interactions between oocyte and surrounding granulosa and/or cumulus cells, dependent on the stage of oogenesis. This event is also affected by the environment (Wu et al. 2019). To date, our understanding of the metabolic control of oocyte development primarily comes from the oocyte's in vitro maturation, while little is known about its metabolism in the early stage of oogenesis (Gu et al. 2015). Dietary deficiency of essential amino acids rapidly induces cessation of the rat estrous cycle (Narita et al. 2011), and preovulatory exposure to a protein-restricted diet disrupts amino acid kinetics and alters mitochondrial structure and function in the rat oocyte (Schutt et al. 2019). Therefore, amino acid metabolism is indispensable for oocyte development. Decreased amino acid transport proteins levels and increased glucose/lipid content in oocytes have been implicated in meiotic defects, organelle dysfunction and epigenetic alteration (Gu et al. 2015). To date, amino acid transport and metabolism in mammalian oocyte growth and development remains largely unknown.

The expression of amino acid transporter(s) in oocytes and follicular cells demonstrates a developmental stage-dependent manner. In mice, amino acid transport systems $b^{0,+}$, L, and asc/ASC are active throughout oocyte growth and maturation; amino acid transport systems X_{AG}^-, $B^{0,+}$, A, and CAT/y^+ are not active in growing or meiotically maturing oocytes; amino acid transport systems GLY, β, and x_c^- are activated in oocytes during meiotic maturation (Pelland et al. 2009). In particular, glycine transport was mainly via system GLY and cysteine/glutamate transport was via system x_c^- in immature oocytes, while system β, L, GLY, x_c^-, and $b^{0,+}$ were detected in matured oocytes (Haghighat and Van Winkle 1990; Van Winkle et al. 1990, 1992). Similarly, follicular cells (granulosa and cumulus cells) also demonstrated development stage-dependent expression of amino acid transporters and ability to promote amino acid transport into oocyte (Eppig et al. 2005). The extent to which follicular cells enhance uptake of a particular amino acid into oocytes depends on at least three physiologically important variables, the stage of follicular development, the presence of other amino acids in the environment, and gap junctional communication (Haghighat and Van Winkle 1990). It is noteworthy that cumulus and granulosa cells have distinct pattern of expression of amino acid transporters, for instance, *Slc38a3* (a transcript encoding a sodium-coupled neutral amino acid transporter that has high substrate preference for alanine) was abundantly expressed in cumulus cells (Eppig et al. 2005), while granulosa cells promote transport of Gly, Ala, taurine and Lys into oocytes (Colonna et al. 1983; Haghighat and Van Winkle 1990; Pelland et al. 2009).

The prolife of amino acids in follicular fluids in antral follicles changes with the progression of follicular development. In pigs, regardless of follicle size, Gly, Ala, Gln, and Pro were the most abundant amino acids in follicular fluid (Hong and Lee 2007). As follicle size increased in antral follicles, the concentration of Asn significantly increased, but the concentrations of other amino

acids, except Arg and Trp, significantly decreased (Hong and Lee 2007).

Oocytes and follicular cells have different metabolic requirements. While growing oocytes preferentially metabolize pyruvate over glucose, the somatic compartment of ovarian follicles is more glycolytic. Accumulating evidence supports other nutrients, amino acids including Gln, Arg and Leu, and fatty acids, play an important role in the maturation of oocytes (Collado-Fernandez et al. 2012). Thus, the unusually high abundance of amino acids such as Gln may complement the metabolism of glucose and fatty acids via transamination and participation of the TCA cycle (Cetica et al. 2003), and serve as the energy substrate to support oocyte development (Collado-Fernandez et al. 2012). During oocyte maturation, the metabolism rate is increased and the oxidative metabolism is supported by an increase in glucose oxidation via the TCA cycle. This cycle is facilitated by pyruvate, Gln and Gly metabolism (Rieger and Loskutoff 1994). The utilization of amino acids also represents the developmental competence of oocyte in early embryonic development (Thompson et al. 2007).

7.2.2 Spermatogenesis

Similar to oogenesis, spermatogenesis is an intricate and complex process, which occurs in the two specialized compartments in seminiferous tubules, basal and Sertoli cell formed compartments. To date, little has been known about the metabolism of germ cells in the basal compartment where spermatogonia use glucose as a fuel for ATP production. More developed germ cells, such as spermatids, are unable to use glucose, despite of expressing all enzymes for glycolysis, and utilize lactate for ATP production (Boussouar and Benahmed 2004). The metabolism of sperm cells closely interacts with that of Sertoli cells when sperm germ cells enter meiosis. In general, Sertoli cells are involved in the regulation of spermatogenesis, providing nutritional support for germ cells. The characteristic of Sertoli cells in metabolism is its potent capacity of glycolysis, and low capacity of oxidative metabolism (Robinson and Fritz 1981; Grootegoed et al. 1986). Lactate is primarily derived from glycolysis in Sertoli cells (Robinson and Fritz 1981). The export of lactate from Sertoli cells by specific monocarboxylate transporters is responsible for improved lactate supply to germ cells and Sertoli cells preferentially use lipids as an energy source primarily via beta oxidation of fatty acids (Xiong et al. 2009). Recent studies suggest that despite being an energy substrate, glucose is not the main metabolite used for ATP synthesis in Sertoli cells (Riera et al. 2009), because Sertoli cells possesses a strong capacity of metabolizing fatty acids via the mitochondrial β-oxidation pathway (Xiong et al. 2009) and of the oxidation of amino acids, primarily Gln and Leu and also other amino acids, such as Ala and Val (Kaiser et al. 2005). Macronutrients have regulatory roles in Sertoli cells. First, glucose metabolism could modulate the oxidation of Ala and Val by competing with acetyl-CoA. Second, glucose metabolism stimulates the conversion of Val into lipids. Third, Gln inhibits the oxidation of Leu, Val, and Ala, but does not alter the conversion of these amino acids into lipids. Fourth, Gln also inhibits the incorporation of Ala into proteins (Kaiser et al. 2005). Fifth, Ala is the main glucogenic amino acid, since it can be converted into pyruvate that can be used as a substrate by Sertoli cells for several biochemical pathways, including the TCA cycle and possibly gluconeogenesis (Kaiser et al. 2005; Rato et al. 2012).

7.3 Amino Acids in Conceptus Development

In most mammalian species, fertilization and early mitotic divisions of the zygotes occur in the oviduct, followed by hatching from zona pellucid, implantation, placentation and fetal development in the uterus. Thus, oocytes containing nutrients, the oviductal fluid, uterine fluid and maternal circulation serve as the nutrient sources for conceptus development.

7.3.1 Fertilization Stage

Fertilization occurs in the oviduct for most mammals. The oviduct provides the minienvironment for fertilization and the subsequent development of zygotes and early embryo. In bovine oviduct fluid, concentrations of amino acids were not affected by day of cycle (Days 0, 2, 3, 4 and 6). Asp, Glu, Ser, Gly, Ala, Tyr, Phe and Lys in oviductal fluid were present in higher concentrations than in plasma. Gly was the most abundant amino acid, and the concentration of many amino acids in oviduct fluid were higher than their plasma levels, indicating their transport into the oviduct (Hugentobler et al. 2007). To date, how these amino acids are transported from maternal circulation to the oviductal fluid remains unknown; however, recent studies support the view that the presence of multiple embryos in the oviduct could induce many differentiated gene expressions in the oviductal epithelial cells (Maillo et al. 2015).

Amino acids in the oviductal fluid may affect the process of fertilization including sperm penetration and pronuclear formation. In pigs, sperm penetration was not altered by amino acid treatment during oocyte in vitro maturation (IVM), but monospermic fertilization was increased by Gln, Asp, and Val. All amino acids except Asp and Asn stimulated male pronuclear formation after IVF. Arg and Ala treatment during IVM improved blastocyst formation (Hong and Lee 2007). Interestingly, gonadotrophins (FSH and LH) plus 11 amino acids interacted with cysteamine (a mercaptoethylamine compound that is endogenously derived from the CoA degradation) to improve oocyte maturation, while enhancing the decondensation of spermatozoa and maternal pronuclear formation. However, the addition of 10% serum or gonadotrophins with or without amino acids did not support male pronuclear formation without cysteamine. This suggests the importance of cysteamine in the formation of male pronuclear formation. In contrast, female pronuclear formation was apparently similar between controls and IVM oocytes (Kito and Bavister 1997).

7.3.2 Peri-Implantation Stage

Embryonic loss represents a major constraint in both human reproduction and the livestock industry. There is a high rate of loss of early pregnancies in humans and 75% of this loss represents a failure of implantation (Wilcox et al. 1988). Much pregnancy wastage is caused by abnormal embryos (Roberts and Lowe 1975); however, it has been estimated that 27% of normal embryos are lost at the time of or soon after implantation (Clark 2003). Similarly, the incidence of embryonic loss in sheep is 20–30%, of which two-thirds occurs during the peri-implantation period between Days 12 and 18 of gestation (Nancarrow 1994), when rapid trophectoderm growth and conceptus elongation occur (Guillomot and Leroy 1993). In addition, up to 40% of cattle embryos die within 3 weeks of fertilization (Hugentobler et al. 2007). Therefore, peri-implantation stage is critical for improving pregnancy outcomes.

In most mammals, an embryo at the morula stage enters uterine lumen and further develops in the minienvironment provided by uterine endometrium and uterine secretions termed histotroph. The latter includes secretions of uterine epithelia and molecules transported into the uterine lumen, a complex mixture of enzymes, growth factors, cytokines, lymphokines, hormones, transport proteins and nutrients. Histotroph is critical for growth and development of ovine conceptuses as they undergo morphological transitions from spherical to tubular to filamentous forms, as well as differentiation between Days 13 and 16 of pregnancy and immediately prior to implantation (Bazer 1975; Spencer and Bazer 2004). In ewes, survival and elongation of the conceptus as well as growth of trophectoderm are dependent on uterine secretions (Heyman et al. 1984; Flechon et al. 1986), as conceptuses fail to elongate beyond the tubular stage of development in ewes lacking uterine glands (Gray et al. 2001). Histotroph supports conceptus survival and development during the critical peri-implantation period of pregnancy in mammals (Bazer 1975; Spencer and Bazer 2004).

7.3.2.1 Amino Acid Profile in Uterine Secretions

Among the components in histotroph, amino acids play critical roles in embryonic development, especially the trophectoderm development during peri-implantation stage. In reality, amino acid turnover has been identified as an indicator of embryonic viability in humans and cattle (Houghton et al. 2002; Sturmey et al. 2010). To date, the amino acid profile in the uterine secretions has been measured in sheep (Gao et al. 2009d), pigs (Bazer et al. 2015a), cattle (Forde et al. 2014), and humans (Kermack et al. 2015).

In sheep, total recoverable glucose, Arg, Gln, Leu, Asp, Glu, Asn, His, beta-Ala, Tyr, Trp, Met, Val, Phe, Ile, Lys, Cys, Pro, glutathione, calcium, and sodium were greater in the uterine fluid of pregnant compared with cyclic ewes between Days 10 and 16. In cyclic ewes, only modest changes in the total amounts of glucose, Asn, Cit, Tyr, Trp, Met, Val, Cys, glutathione, calcium, and potassium were detected between Days 3 and 16. However, in pregnant ewes, amounts of glucose, Arg, Gln, Glu, Gly, Cys, Leu, Pro, glutathione, calcium, and potassium in uterine fluids increased 3- to 23-fold between Days 10 and 14 and remained high to Day 16. Of particular interest were increases in glucose, Arg, Leu, and Gln in uterine flushings of pregnant ewes between Days 10 and 16 of pregnancy (Fig. 7.1). Total amounts of His, ornithine (Orn), Lys, Ser, Thr, Ile, Phe, Trp, Met, and Cit in uterine fluids also increased, but to a lesser extent during early pregnancy. These novel results indicate activation of pregnancy-associated mechanisms for transport of nutrients into the uterine lumen, and they provide a framework for future studies of nutrients, including glucose, amino acids, and glutathione, required to activate nutrient-sensing cell signaling pathways for growth, development, and survival of conceptuses, as well as for optimization of culture media for in vitro studies of conceptus development (Gao et al. 2009d). The detailed amino acid profile in the ovine uterine flushings from cyclic and early pregnant ewes were described in our original publication (Gao et al. 2009d). Similarly, there were significant changes in amino acids in uterine flushing of pigs during

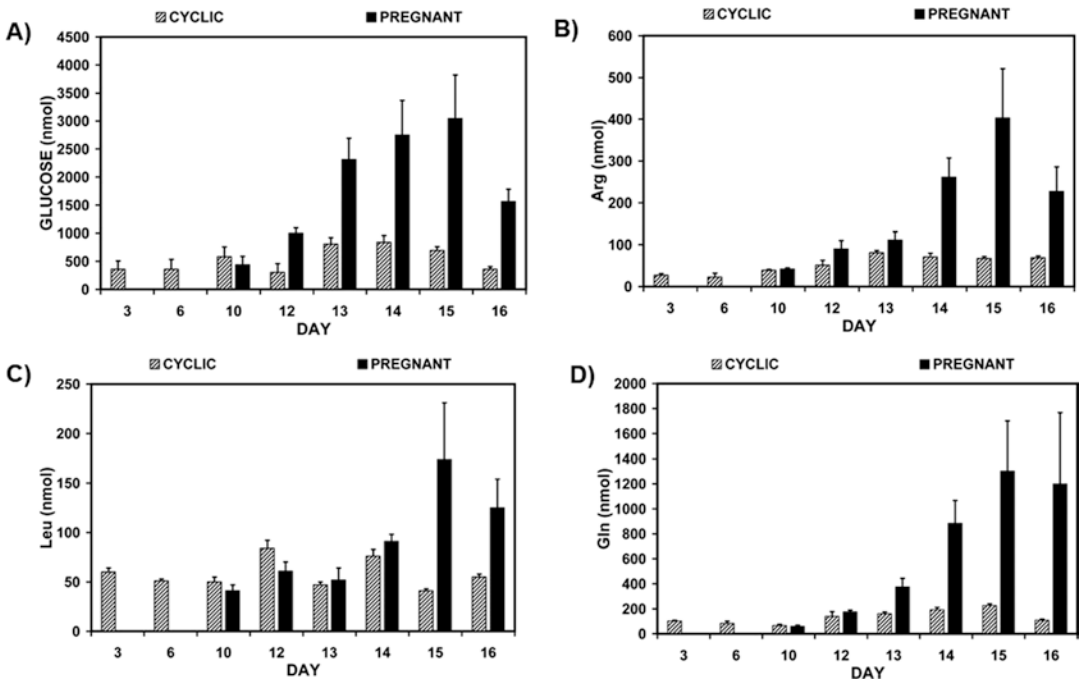

Fig. 7.1 Total recoverable Glucose (**a**), arginine (**b**), leucine (**c**) and glutamine (**d**) (nmol) in uterine flushings from cyclic and pregnant ewes. Effects of day, pregnancy status and day by pregnancy status were significant ($P < 0.05$)

the estrous cycle (Days 5–15) and pregnancy (Days 9–15). Among all amino acids investigated, concentrations of Arg, Gln, Leu, His, Orn, Lys, Asp, Ser and Cys were affected by the status of pregnancy or cyclicity; concentrations of Arg, His, Asn, Gly, Ala, Cys, Glutathione, Tyr, Trp and Pro were changed with the days investigated; concentrations of Gln, Glu, Ser, Cys and Trp were affected by the interaction of status of pregnancy and Days (Bazer et al. 2015a). Amino acid profiles in bovine uterine secretions were also changed with the status of the estrus cycle and/or pregnancy. The concentrations of multiple amino acids (Asp, Arg, Gln, His, Lys, Ile, Leu, Phe, tTyr, Glu, Asn and Val) in bovine uterine luminal fluid were increased during peri-implantation (Days 7–19 of pregnancy investigated) (Forde et al. 2014).

In literature, there is only one study of amino acid profile in human uterine secretions. In non-pregnant subjects, concentrations of 18 amino acids were not significantly altered by age, BMI, cycle phase or the presence of specific benign gynecological pathologies, although concentrations of several amino acids in the uterine fluid were increased by Western diet, including Asn, His, Ser, Gln, Val, Phe, Ile and Leu. In addition, there were no significant correlations between serum amino acid concentrations and those in the uterine fluid (Kermack et al. 2015).

7.3.2.2 Nutrient Sensing: Mechanistic Target of Rapamycin (MTOR) Signaling

MTOR, a highly conserved serine-threonine protein kinase, senses and responds to changes in amino acid levels and energy sufficiency, as well as select agents, such as hormones and mitogens (Dennis et al. 1996; Gingras et al. 1999, 2001), thus integrating both extracellular and intracellular factors to control growth and development of cells and tissues. MTOR and associated proteins comprise two structurally and functionally distinct complexes, mTORC1 and mTORC2 (Guertin et al. 2006; Wullschleger et al. 2006; Liao et al. 2008). The MTOR-associated proteins appear to determine specificity of the two different cell signaling pathways (Schmelzle and Hall 2000; Gao et al. 2002; Zhang et al. 2003; Sarbassov et al. 2005) mediated by mTOR complexes, associated regulators and effectors.

MTORC1 and MTORC2 mediated cell signaling pathways may be critical for growth and development of the conceptus, as well as implantation. First, in mice, disruption of the *MTOR* gene leads to post-implantation lethality due to impaired cell proliferation and hypertrophy in both the embryonic disc and trophoblast (Gangloff et al. 2004; Murakami et al. 2004) and dysfunction of MTOR complex 1 and 2 leads to fetal lethality occurring at different stages of development (Guertin et al. 2006; Jacinto et al. 2006; Shiota et al. 2006). Second, MTOR signaling regulates translation of proteins in the uterus, including insulin-like growth factor-2 (IGF2), ornithine decarboxylase (ODC1) and nitric oxide synthases (NOS) (Nielsen et al. 1995; Kimball et al. 1999; Martin and Sutherland 2001), which play important roles in trophoblast cell proliferation, differentiation and migration in varied species including sheep and pigs (Kim et al. 2008, 2011, 2013; Kong et al. 2012, 2014; Wang et al. 2014a, b, 2015, 2016; Lenis et al. 2018). Third, MTOR promotes translation of the "polypyrimidine tract" mRNA family (Jefferies et al. 1994) that is critical to fetal and placental development (Ohlsson et al. 1989; Zhou and Bondy 1992; Wathes et al. 1998), as well as trophoblast development, differentiation, and motility (Martin and Sutherland 2001; Martin et al. 2003; Wu et al. 2004). Fourth, emerging evidence indicates that the MTOR cell signaling pathways regulate expression of the glucose transporters SLC2A1 (Buller et al. 2008; Zhou et al. 2008) and SLC2A12 (Schmid et al. 2008), and amino acid transporters SLC1A5 (neutral amino acid transporter) (Fuchs and Bode 2005; Fuchs et al. 2007) and SLC7A5 (L-type amino acid transporter 1) (Liu et al. 2004; Fuchs and Bode 2005), which together with other amino acid transporters mediate the transport of glucose or amino acids from blood circulation into histotroph including oviductal and uterine secretions, critical microenvironments for fertilization and early conceptus development.

Our previous study demonstrated, for the first time, that MTOR and its complexes are present in both ovine uterine endometrium and conceptus during peri-implantation stage (Gao et al. 2009c). The mRNAs for MTOR, LST8, MAPKAP1, RAPTOR, RICTOR, TSC1, TSC2, RHEB, and EIF4EBP1 were localized to luminal, superficial glandular, and glandular epithelia and stromal cells of uteri from cyclic and pregnant ewes, as well as trophectoderm and endoderm of conceptuses between Days 13 and 18 of pregnancy. In endometria of pregnant ewes, increases in abundance of mRNAs for RICTOR, RHEB, and EIF4EBP1, as well as RHEB protein, correlated with rapid conceptus growth and development during the peri-implantation period. In addition, P4 and IFNT stimulated expression of RHEB and EIF4EBP1 in uterine endometria. These results suggest that the MTOR cell signaling pathway mediates interactions between the maternal uterus and peri-implantation conceptuses and that P4 and IFNT affect this pathway by regulating expression of RHEB and EIF4EBP1. To support this notion, further in vivo and ex vivo studies have demonstrated the critical role of MTOR signaling in early conceptus development in response to IGF2, SPP1 (osteopontin), select nutrients (glucose, Arg, Leu and Gln) and manipulation of production of NO and polyamine (Mehrotra et al. 1998; Wu and Morris 1998; Martin and Sutherland 2001; Martin et al. 2003; Kwon et al. 2004; Wu et al. 2005).

7.3.2.3 Amino Acid Transport

Amino acid transporters are required for amino acid transport from blood circulation into tissue and cells where amino acids are utilized. In human placentae, amino acid transport requires active amino acid transporters which belong to different families and systems (Regnault and Hay 2006; Grillo et al. 2008), but little is known about their expression in the uterine endometrium and conceptuses in livestock. Our studies, taking the advantage of prolonged period of peri-implantation in ovine conceptus development, first revealed the expression of multiple amino acid transporters in ovine uterine endometrium and conceptus (Gao et al. 2009a, b).

Cationic amino acids including Arg are primarily transported by SLC7A1-3. SLC7A1 mRNA was most abundant in endometrial luminal (LE) and superficial glandular (sGE) epithelia in both cyclic and early pregnant ewes, SLC7A2 mRNA was most abundant in LE and mid to deep glandular (GE) epithelia on Days 14–20 of gestation and SLC7A3 mRNA was expressed ubiquitously in uterine endometrial cells. SLC7A1, SLC7A2, and SLC7A3 mRNAs were expressed in trophectoderm and endoderm of conceptuses. More importantly, the expression of SLC7A2 in uterine endometrium was induced by P4 and further stimulated by IFNT (Gao et al. 2009a), which may be responsible for the marked increase of Arg in uterine secretions during early pregnancy (Gao et al. 2009d). The detailed mRNA expressions of neutral and acidic amino acids transporters in ovine uterine tissue and conceptus are summarized in Table 7.1. Briefly, SLC1A2, SLC1A3, SLC3A1, SLC6A14, SLC6A19, SLC7A6, SLC38A3, and SLC38A6 mRNAs were weakly expressed in the ovine endometrium. However, SLC1A4, SLC1A5, SLC7A8, and SLC43A2 mRNAs were detectable in uterine luminal epithelia (LE), superficial glandular epithelia (sGE), and/or glandular epithelia (GE). SLC1A1 and SLC7A5 mRNAs were most abundant in LE/sGE and GE. SLC1A3 and SLC38A4 mRNAs were most abundant in uterine stroma. SLC38A6 mRNA was detected only in cells with a stromal distribution suggesting immune lineage. SLC1A5 mRNA was expressed primarily in LE/sGE and stromal cells, and it was more abundant in uteri of pregnant ewes. Furthermore, P4 induced and IFNT further stimulated SLC1A5 expression in LE/sGE. Endometrial SLC1A1, SLC7A5, and SLC43A2 mRNAs demonstrated both temporal and cell SLC specific changes. Several mRNAs were detectable in trophectoderm (SLC6A19, SLC7A5, SLC7A6, and SLC43A2), while others were more abundant in endoderm (SLC1A4, SLC1A5, SLC6A19, SLC7A5, SLC7A6, SLC7A8, and SLC43A2) of conceptuses. These results document coordinate changes in expression of transporters that are likely responsible for increases in amounts of neutral and acidic amino acids in the uterine

Table 7.1 Gene name and function of members of solute carrier families 1, 3, 7 and 43

Gene abbreviation	Full name of gene in HGNC and function
SLC1A1 [a]	Solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system X_{AG}), member 1
SLC1A2 *	Solute carrier family 1 (glial high affinity glutamate transporter), member 2
SLC1A3 * [b]	Solute carrier family 1 (glial high affinity glutamate transporter), member 3
SLC1A4 [a,d]	Solute carrier family 1 (glutamate/neutral amino acid transporter), member 4
SLC1A5 [a,b,d]	Solute carrier family 1 (neutral amino acid transporter), member 5
SLC3A1 *	Solute carrier family 3 (cystine, dibasic and neutral amino acid transporters, activator of cystine, dibasic and neutral amino acid transport), member 1
SLC6A14 *	Solute carrier family 6 (amino acid transporter), member 14
SLC6A19 * [d]	Solute carrier family 6 (neutral amino acid transporter), member 19
SLC7A5 [b,d]	Solute carrier family 7 (cationic amino acid transporter, y + system), member 5; transport large neutral amino acid
SLC7A6 * [d]	Solute carrier family 7 (cationic amino acid transporter, y + system), member 6; transport cationic and neutral amino acids
SLC7A8 [a,d]	Solute carrier family 7 (cationic amino acid transporter, y + system), member 8; transport large and small neutral amino acids
SLC38A3 *	Solute carrier family 38, member 3; transport glutamine, histidine, and asparagine
SLC38A4 * [b]	Solute carrier family 38, member 4; transport alanine, histidine, glutamine etc.
SLC38A6 [c]	Solute carrier family 38, member 6; transport neutral amino acids
SLC43A2 [a,d]	Solute carrier family 43, member 4; transport large neutral amino acids

*Weak to undetectable in uterine endometrial epithelia
[a]Detectable in endometrial luminal, superficial glandular and/or glandular epithelia
[b]Expression most abundant in endometrial stroma
[c]Detected in unidentified cells in endometrial stroma that appeared to be macrophages
[d]Detected in conceptus trophectoderm and endoderm

lumen to support conceptus growth, development, and survival (Gao et al. 2009b).

Similar to sheep, temporal changes in the expression of amino acid transporters in the endometrium and conceptus occurred during early pregnancy in cattle (Days 7–19 of pregnancy) and SLC1A1, −1A4, −1A5, −38A2, −38A4, −38A7, −43A2, −6A14, −7A1, −7A5 and −7A7 in the endometrium, some of which were modified by P4 (Bazer et al. 2015a). Temporal changes in expression of the cationic AA transporters SLC7A1, SLC7A4 and SLC7A6 occurred in the endometrium during the estrous cycle/early pregnancy coordinate with changes in conceptus expression of SLC7A4, SLC7A2 and SLC7A1. Only one acidic AA transporter (SLC1A5) increased in the endometrium while conceptus expression of SLC1A4 increased. The neutral AA transporters SLC38A2 and SLC7A5 increased in the endometrium in a temporal manner while conceptus expression of SLC38A7, SLC43A2, SLC38A11 and SLC7A8 also increased.

It is noteworthy that due to the lack of specific inhibitor for certain amino acid transporters and the substrate sharing among different amino acid transporters, it is difficult to analyze the activity of single amino acid transporter in vivo or ex vivo. More advanced techniques are highly demanded in the study of amino acid transport in both uterine endometrium and conceptus during peri-implantation and thereafter. Overexpression of genes or specific knockout of genes/proteins can provide a powerful tool to study a role of amino acid transporters in conceptus survival, growth and development.

7.3.2.4 Select Amino Acid Metabolism in Conceptus During Peri-Implantation Stage

Arginine

Arg is a conditionally essential amino acid for conceptus survival, growth and development (Wu et al. 2013, 2017, 2018). In general, Arg exerts its functions primarily through its metabolites and is a precursor for important biological molecules

including urea, creatinine, Orn, Pro, nitric oxide, agmatine (Agm) and polyamines (Wu and Morris 1998). Arginine metabolism via nitric oxide and polyamines plays critical roles in conceptus development (Mehrotra et al. 1998; Wu and Morris 1998; Martin and Sutherland 2001; Martin et al. 2003; Kwon et al. 2004; Wu et al. 2005). In addition, Arg activates the mTOR cell signaling pathway to induce proliferation, migration and adhesion of ovine and pig trophectoderm cells required for implantation, survival and growth of blastocysts, as well as survival, growth, and health of mammalian conceptuses (Kong et al. 2012, 2014; Wang et al. 2014a, 2016; Bazer et al. 2015a).

In the ovine conceptus, mRNA expressions of SLC7A1-3 are present during peri-implantation stage. In vivo morpholino antisense oligonucleotide (MAO)-mediated knockdown of SLC7A1 mRNA in ovine trophectoderm report that SLC7A1 accounts for 73% of arginine uptake, thus being the key transporter of arginine by conceptus trophectoderm (Wang et al. 2014a). Interestingly, MAO knockdown of SLC7A1 also reduced the abundance of ornithine decarboxylase, and nitric oxide synthase (NOS3) proteins, arginine-related amino acids [Cit (76%) and Orn (40%)] and polyamines, which likely accounts for the retarded development (Wang et al. 2014a).

In ovine trophectoderm cells, Arg is used for biosynthesis of NO and polyamines (putrescine, spermidine and spermine) (Wang et al. 2014b, 2015; Lenis et al. 2018), which are critical to the morphological transition of conceptuses from the spherical to filamentous forms and signaling for pregnancy recognition by IFNT from conceptus trophectoderm. The NOS3 is rate-limiting enzymes in the production of NO in ovine conceptus, while ODC1, is the rate limiting enzyme in the production of polyamines from ornithine which is derivatives of arginine catalyzed by arginase I/II. In addition, Arg is also converted to agmatine by ADC and agmatine is converted to putrescine by AGMAT in the uterus and conceptus trophectoderm to generate polyamines or agmatine may have direct effects on the uterus or conceptus (Wang et al. 2014b). Both putrescine and NO stimulate cell proliferation via activation of the TSC2-MTOR signaling cascade, whereas only putrescine increased IFNT production (Wang et al. 2015). MAO-mediated knockdown ODC1 and ADC mRNAs was most detrimental to conceptus development and their production of IFNT. Agm, polyamines, amino acids, and adequate secretion of IFNT are critical for establishment and maintenance of pregnancy during the peri-implantation period of gestation in sheep (Lenis et al. 2018) (Fig. 7.2).

Arg also stimulates cell migration of ovine trophoblast cells and demonstrates synergistic effects on adhesion medicated by SPP1 in ovine trophectoderm cells. Those cooperative effects of arginine and SPP1 were mediated by focal adhesion assembly-MTORC2-cytoskeletal reorganization and MAPK pathways, which may play an important role in rapid elongation of ovine conceptuses during the peri-implantation period of pregnancy (Wang et al. 2016).

The acute stimulation of L-arginine on MTOR signaling has been reported in ovine and porcine trophoblast cells. In ovine trophoblast cells, Arg activates mTOR cell signaling and phosphorylation of RPS6, V-AKT murine thymoma viral oncogene homolog 1 (AKT1), glycogen synthase kinase 3-beta (GSK3B), mTOR and RPS6 kinase (RPS6K) proteins (Kim et al. 2010). Similarly, Arg activates MTOR signaling dose-dependently in porcine trophectoderm cell line (Kong et al. 2012) via the production of putrescine (Kong et al. 2014).

Leucine

Among all amino acids investigated before, the role of Leu in regulating activities of EIF4EBP1 and RPS6KB1 via MTOR signaling is well established (Proud 2002; Ban et al. 2004). Leu is the most effective single amino acid as other amino acids have little or no effect on phosphorylation or dephosphorylation of EIF4EBP1. Leu has a potent capability of regulating kinase activity of RPS6KB1 through phosphorylation (Ban et al. 2004). It has been proposed that Leu modulates MTOR function, in part, by regulating mitochondrial function and AMPK because it may serve both as a mitochondrial fuel through oxidative carboxylation and as an allosteric activator of

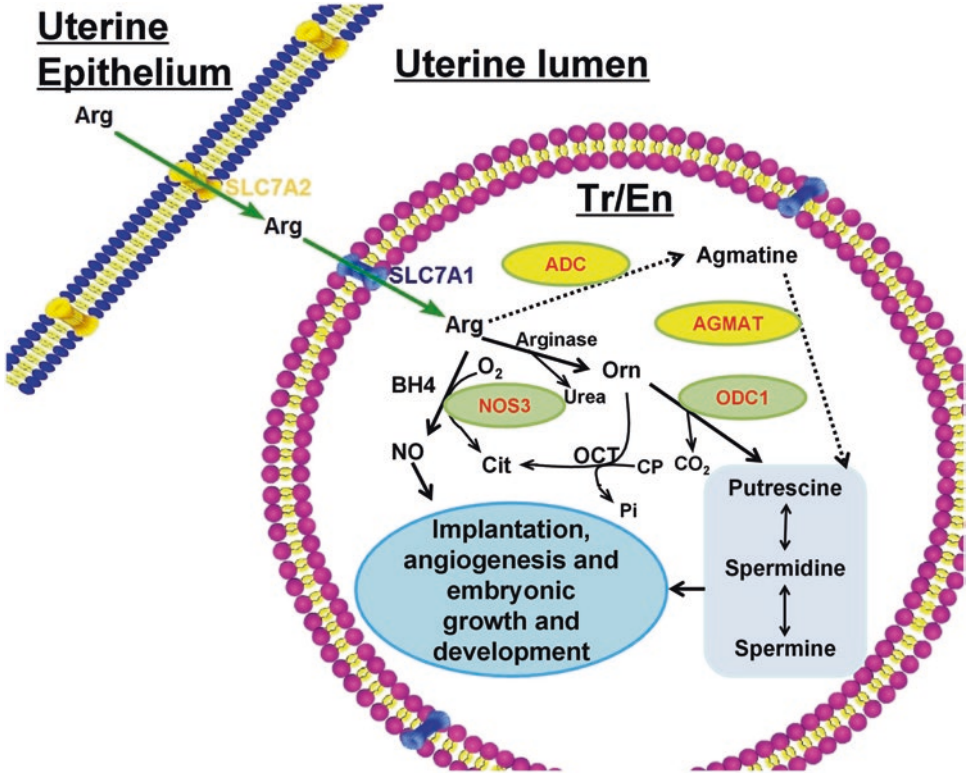

Fig. 7.2 Biosynthesis of nitric oxide and polyamines from L-arginine in ovine trophectoderm cells. Arg in uterine lumen, primarily transported by solute carrier family 7 member 2 (SLC7A2) in uterine endometrial epithelia, is transported into trophectoderm (Tr) and/or endoderm (En) cells by the solute carrier family 7 member 1 (SLC7A1) where Arg can be converted to nitric oxide (NO) by nitric oxide synthase 3 (NOS3) or can be converted to ornithine by arginase and then ornithine is converted to putrescine by ornithine decarboxylase (ODC1). However, the sheep conceptus can also convert arginine to agmatine via arginine decarboxylase and agmatine can be converted to putrescine by agmatinase. *ADC* arginine decarboxylase, *BH4* tetrahydrobiopterin, *OCT* optimal cutting temperature, *AGMAT* agmatinase. (Gao et al. 2009a; Bazer et al. 2015b)

glutamate dehydrogenase (Tokunaga et al. 2004). In ovine trophectoderm cells, leucine stimulates MTOR-RPS6K-RPS6 cell signaling pathways to stimulate hypertrophy, hyperplasia, and migration (Kim et al. 2011). Similarly, in porcine trophectoderm cells, physiological levels of Leu stimulate activities of mTOR and RPS6K, and proliferation of trophectoderm cells (Kim et al. 2013). More importantly, dietary supplementation of Leu during pregnancy may promote fetal growth and pregnancy outcome (Teodoro et al. 2012; Liu et al. 2018); therefore, Leu has the potential to be applied in the livestock industry and human clinic to overcome fetal growth restriction.

Glutamine

To date, little is known about the Gln metabolism during early conceptus development in livestock. In ovine fetuses, however, Gln may be synthesized from branched-chain amino acids (BCAA) including Ile, Leu, and Val by BCAA transaminase in the placenta (Goodwin et al. 1987). Gln metabolism provides reducing equivalents for energy production in ovine (Wales and Du 1994) and bovine (Rieger et al. 1992) conceptuses, possibly to compensate for glucose metabolism (Gardner et al. 1993). In addition, Gln is essential for the synthesis of nucleotides, $NAD(P)^+$, and aminosugars (glucosamine-6-phosphate, UDP-*N*-acetylgalactosamine, and UDP-*N*-

acetylglucosamine), and a precursor for synthesis of all macromolecules containing amino sugars (Wu 2013; Flynn et al. 2002). Gln can be converted into citrulline, the precursor of Arg, in ovine placentae (Kwon et al. 2003a) and inhibit NO production from Arg (Wu et al. 2001); therefore, Gln and Arg are closely linked in conceptus metabolism. There is evidence that dietary supplementation with glutamine to swine during late gestation enhances pregnancy outcomes (Wu et al. 2011).

Proline

Little is known about proline metabolism and function in the conceptuses in early pregnancy; however, ovine placentae have a high capacity for Pro catabolism and polyamine production as Pro is synthesized from pyrroline-5-carboxylate (P5C) by cytosolic NAD(P)H-dependent P5C reductase, and P5C is formed mainly from Orn, Gln and Glu (Wu and Morris 1998; Wu et al. 2008b). Arg can also be converted into Pro in ovine placentae via the arginase pathway (Kwon et al. 2003b). Pro is a major substrate for polyamine synthesis via Pro oxidase, Orn aminotransferase and Orn decarboxylase in both ovine and porcine placentae. Pro can be converted into ornithine, which is subsequently converted into putrescine, spermidine and spermine via ornithine decarboxylase (ODC), spermidine synthase and spermine synthase, respectively in the cytosol (Wu et al. 2005). In addition, allantoic and amniotic fluids contain enzymes to convert Pro into Orn for delivery into the circulation of the conceptus (Kwon et al. 2003b). The important role of Pro in fetal development is supported by a positive association between fetal growth and Pro availability during pregnancy. Reduced placental and reduced fetal growth are associated with reductions in placental Pro transport, Pro oxidase activity, and concentrations of polyamines in gestating dams with either naturally occurring or malnutrition-induced growth retardation, while increasing Pro availability in maternal plasma through nutritional or pharmacological modulation in pigs and sheep enhances concentrations of Pro and polyamines in placentae and fetal fluids, as well as fetal growth (Wu et al. 2008a). In support of an important role of Pro in mammalian pregnancy, Liu et al. (2019a) recently reported that dietary supplementation with Pro to gestating mice enhanced the number of live-born pups. Interestingly, the F1 generation female offspring from Pro-supplemented dams had higher concentrations of Glu and Tau in plasma; of putrescine and spermidine in placental tissues; and of Gly, Tau, and spermidine in amniotic fluid at E12.5, as compared with F1 generation female offspring from dams without Pro supplementation (Liu et al. 2019b).

Other Amino Acids

Other amino acids play important roles in conceptus development. For example, Ala, and Ser, together with Gln, are major glucogenic precursors in humans (Wu 2013) and ewes (Clark et al. 1976). Ser also plays an important role in one-carbon unit metabolism essential for 2′-deoxythymidylate synthesis and methylation (Snell and Fell 1990). Ser participates in the synthesis of phosphatidylserine and ceramide (signaling molecules). These events are critical for DNA synthesis and consequently, cell proliferation. Gly and Ser are interconvertible via serine hydroxymethyltransferase, which also contributes to one-carbon unit metabolism essential for synthesis of purine and pyrimidine nucleotides in DNA synthesis and cell proliferation. Interestingly, Gly is the most abundant amino acid in ovine uterine arterial plasma (Kwon et al. 2003a), and uterine fluids from cyclic cows (Hugentobler et al. 2007).

7.3.3 Placentation Stage and Thereafter

In contrast to the extensive studies during peri-implantation stage, little is known about the amino acid transport and metabolism in placentation stage in most species. Placentation refers to the formation and arrangement of placenta, leading to a fully functional placenta, which maintains pregnancy, nurtures the fetus and modulates the bidirectional interactions between the mother

and fetus. Like the formation of any organs, the development of placenta undergoes cell proliferation, death and differentiation in two major types of cells, trophoblast and endothelial cells, which execute two primary functions of the placenta, nutrient transport from the maternal circulation to fetal capillaries and hormone synthesis and release.

Studies of amino acids in the placenta can be primarily divided into two fields of research, metabolic pathways and transport systems (Vonnahme et al. 2015). Previous research has shown net uteroplacental consumption of Ile, Leu, and Val, while Met was the only essential amino acid showing a net uteroplacental release (Chung et al. 1998). The ovine placenta has the capability of Gln synthesis due to the activity of Gln synthetase, which catalyzes the transamination of Glu, derived from α-ketoglutarate (Wu et al. 2015), a metabolite of branch-chained amino acids in the placenta (Chung et al. 1998). BCAA, especially Leu can activate mTOR signaling, a nutritional sensor, which stimulates cell growth and protein synthesis via increased rates of mRNA translation through the phosphorylation of eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) and the ribosomal protein S6 kinase 1, and also cell proliferation, differentiation and migration. The regulatory mechanisms of amino acids on mTOR signaling have been described above. Supplementation of Leu to dams on a protein deficient diet can restore fetal growth and minimize the decreases in fetal organ mass and carcass fat, which is associated with increased mTOR signaling in the fetus (Teodoro et al. 2012). On the other hand, mTOR signaling regulates several type of amino acid transporters in trophoblast cells or cell lines at transcriptional and posttranslational levels (Edinger and Thompson 2002). In human primary trophoblast cell cultures, rapamycin, the inhibitor of mTOR complex 1, reduced the activity of system A, system L, and taurine amino acid transporters, but not protein expression, thus, amino acid transporter activation may be independent of protein synthesis (Edinger and Thompson 2002).

7.3.4 Beneficial Effects of Dietary Arginine Supplementation on Embryonic/Fetal Survival and Growth in Mammals

Dietary supplementation of Arg has been studied extensively in pregnant pigs in the past decade, with the dose and timing of supplementation being determined to maximize the benefits of Arg in reproductive performance of pregnant dams. To date, dietary Arg at the doses of 0.4, 0.5, 0.83, 1 and 1.7% has been supplemented to pregnant sows at the early, mid and late gestation. Most of these studies demonstrated the beneficial effect of Arg on pregnancy outcomes including increased live litter size and birthweight (Wu et al. 2017, 2018). An increase in the number of live-born pigs markedly increases the profit margin associated with reproduction and lactation performance in dams and reduced low-birthweight piglets greatly improves the management of neonatal pigs and maximizes pre-weaning survival and growth (Wu et al. 2010). However, dietary Arg supplementation immediately post-mating at the dose of 0.83% may have deleterious effects on reproductive performance of pigs (ovulation, luteinization and fetal development) (Li et al. 2010), and in stark contrast, dietary Arg supplementation starting 14 post-mating increased reproductive performance (Berard and Bee 2010; Li et al. 2014). In addition, Arg supplementation from early to late gestation increased pregnancy outcomes (increased live litter size and birth weight), but whether Arg supplementation at late gestation is beneficial is controversial (Bass et al. 2017; Nuntapaitoon et al. 2018). Thus, maternal dietary Arg supplementation holds great promise in improving reproductive efficiency in livestock industry.

Besides pregnant pigs, other species also benefit from the supplementation of Arg during pregnancy, including sheep, rats and humans. Intravenous administration of Arg prevents IUGR in underfed ewes (Lassala et al. 2010) and diet-induced obese ewes (Carey Satterfield et al. 2012). Dietary supplementation of pregnant rats with Arg increased the numbers of implantation sites and litter size by approximately three (Zeng

et al. 2008, 2013). More interestingly, intravenous Arg supplementation also improved fetal-placental growth and prevented IUGR in pregnant women by reducing placental apoptosis and improving fetal growth and development (Shen and Hua 2011), increasing birth weight at term (Xiao and Li 2005; Singh et al. 2015), reducing diastolic blood pressure and prolonging pregnancy in patients with gestational hypertension with or without proteinuria (Gui et al. 2014). Thus, Arg supplementation could be potentially used to prevent or treat IUGR and pregnancy related disorders.

7.4 Amino Acids in Developmental Origins of Health and Diseases

7.4.1 Protein Restriction During Maternal Gestation and Associated Fetal Growth Restriction and Fetal Programming of Adult Diseases

Evidence from numerous human studies and animal experiments supports the Barker Hypothesis, which propose that the in utero environment especially nutrition affects fetal growth and development and has long term effects on offspring health and diseases in adulthood (Fleming et al. 2015; Daniels 2016; Sferruzzi-Perri and Camm 2016). Pregnant rats with dietary protein insufficiency have been widely used as an animal model in the study of fetal programming of adult diseases, or developmental origin of adult health and diseases and other experimental models include mice (Gheorghe et al. 2009; Mortensen et al. 2010; Gonzalez et al. 2016) and non-human primate (Roberts et al. 2018). One of important findings in the study of gestational protein insufficiency is the association between fetal growth restriction and predisposition of adult diseases. Gestational protein insufficiency programmed diseases include cardiovascular diseases (hypertension, cardiac and arterial disorders), metabolic diseases (obesity and diabetes) and endocrine disorders (Sferruzzi-Perri and Camm 2016). To date, although many mechanisms have been proposed, the placenta is emerging as a critical player in fetal programming (Burton et al. 2016; Sferruzzi-Perri and Camm 2016; Myatt and Thornburg 2018). Placentas of dams with gestational protein insufficiency demonstrate impaired growth and placental efficiency (the ratio of fetal to placental weight) (Gao et al. 2012a), which is associated with reduced expression and/or activities of amino acid transporters and MTOR signaling (Jansson et al. 2006; Rosario et al. 2011; Sferruzzi-Perri and Camm 2016).

7.4.2 Amino Acids in Maternal and Fetal Plasma in Response to a Low Protein Diet During Pregnancy

One may assume that the abundance of amino acids in maternal plasma will be decreased if pregnancy dams are fed a low protein diet. In striking contrast to this assumption, in pregnant rats fed the low protein diet during mid and late pregnancy, the total concentration of amino acids in maternal plasma is not reduced by protein insufficiency, resulting from increased levels of so-called "non-essential amino acids" and reduced levels of essential amino acids (Gao et al. 2012a) and altered maternal metabolism related to diet intake (Gao et al. 2015a, b) and insulin secretion (Gao et al. 2017), which may represent a successful adaptation to maternal nutritional stresses. Similarly, protein-deficient gilts maintain maternal plasma concentrations of amino acids by mobilizing maternal protein stores and decreasing oxidation of amino acids during the first half of gestation (Wu et al. 1998). The "non-essential amino acids" likely play an important role in the adaptation of the conceptus to a nutritional insult for survival.

To date, few studies measured fetal plasma amino acids in response to maternal dietary protein insufficiency due to the technical limits in fetal plasma sampling in most experimental animals. In rats with gestational protein insuffi-

ciency, among all the measured amino acids, Thr is the only amino acid whose fetal plasma levels are remarkably reduced in late pregnancy (Rees et al. 1999). However, the dietary supplementation of Thr failed to rescue the fetal growth restriction and programmed adult diseases in offspring (Rees et al. 2006). In contrast, in pigs, reduced concentration of multiple amino acids were seen in both fetal plasma and allantoic fluid during mid-pregnancy (fetal plasma: Ala, Arg, BCAAs, Gln, Gly, Lys, Orn, Pro, Tau, Thr and urea; allantoic fluid: Ala, Arg, BCAA, Cit, Cys, Gly, His, Met, Pro, Ser, Tau, Thr and Tyr) (Wu et al. 1998). This discrepancy in fetal amino acid profile in response to gestational protein insufficiency may result from species differences in the metabolism, stage of pregnancy as well as different diet components.

7.4.3 Mechanisms of Fetal Growth Restriction

To date, emerging evidence suggests the following mechanisms to be responsible for fetal growth restriction. Reduced mTOR signaling in the placenta. Gestational protein insufficiency in rats leads to reduced mTOR signaling, and decreased expressions and activities of several sodium-dependent neutral amino acid transporters such as system A in the placenta (Jansson et al. 2006), possibly due to reduced plasma BCAAs (Gao et al. 2012a) and insulin (Gao et al. 2017) which are known stimulators of MTOR signaling.

Activation of the amino acid response (AAR) pathway in the placenta and IGFBP-1 activity. The mammalian AAR pathway (activating transcription factor-3 and 4) in the placenta are upregulated by a maternal low-protein diet (Strakovsky et al. 2010) and the activation of AAR stimulates both IGFBP-1 secretion and hyperphosphorylation (pSer101/pSer119/pSer169), decreasing IGF-1 bioavailability and its activity as potent regulator of fetal growth (Karl 1995).

Altered renin-angiotensin system (RAS) in maternal, uterine and placental compartments. RAS plays a critical role in regulating blood flow, including the blood flow in maternal-utero-fetal units. The low protein diet alters the expression of RAS in maternal and utero-placental units, including enhanced angiotensin II production in maternal lung (Gao et al. 2012d, 2016), increased expression of angiotensin receptor type I in uterine artery (Gao et al. 2012d), reduced expression of angiotensin converting enzyme II (ACE2) in placental labyrinth zone (Gao et al. 2012c). All these alterations contribute to the reduced utero-fetal blood flow, a determinant of fetal growth (Lang et al. 2003), and local accumulation of angiotensin II in placental labyrinth zone inhibits amino acid transport in trophoblast cells (Shibata et al. 2006).

Increased testosterone. The LP diet enhances the plasma levels of testosterone in pregnant rats (Zambrano et al. 2005; Gao et al. 2012b). Increased testosterone inhibits the expression and activity of neutral amino acid transporters (Sathishkumar et al. 2011), thus resulting in IUGR as well as associated hypertension and other disorders in adult offspring (Chinnathambi et al. 2012, 2013).

Impaired mitochondrial function. Gestational protein insufficiency causes the mitochondrial abnormality with increased oxygen uptake and impaired oxidative phosphorylation (Rebelato et al. 2013), negatively affecting placental functions including amino acid transport which requires continuous energy supply. Improving anti-oxidative responses and reducing obesity likely play an important role in mitigating mitochondrial dysfunction (Ji et al. 2017).

7.4.4 Prevention of Fetal Programming in Response to Maternal Gestational Protein Restriction

Supplementations of Gly (Jackson et al. 2002), taurine (Mortensen et al. 2010) and Leu (Teodoro et al. 2012) during pregnancy have been reported to benefit fetal growth and long-term health in response to maternal protein

insufficiency, although the underlying mechanisms remain unclear. Dietary supplementation of Gly throughout pregnancy normalized the predisposed hypertension in offspring from pregnant rats fed a low protein diet (Jackson et al. 2002), possibly by reversing vascular dysfunction in mesenteric artery and improving NO release in maternal circulation. The supplementation of taurine in maternal gestational protein restriction partly rescues fetal growth retardation, restores fatty acid metabolism in the liver and oxidative phosphorylation and TCA cycle in skeletal muscle (Mortensen et al. 2010), normalizes proliferation and vascularization and cytokine sensitivity in pancreatic islets in offspring (Boujendar et al. 2002; Merezak et al. 2004). The supplementation with Leu also reversed this growth deficit, minimizing the difference or restoring the mass of organs and carcass fat, the liver and muscle protein, and the RNA concentrations in offspring of rats with gestational protein restriction, possibly by the activation of the mTOR signaling pathway (Teodoro et al. 2012). Dietary supplementation of Leu to premating SD rats improved the within-litter birth weight uniformity, antioxidative capability, and immune function (Liu et al. 2018). However, a recent study with a large cohort of human subjects suggested that the dietary amino acid pattern, rich in branched-chain, aromatic, and aliphatic amino acids, and proline could increase the risk of hypertension (Teymoori et al. 2017). The controversy on the benefits of dietary amino acids in pregnancy and non-pregnancy may result from the differences in the metabolic patterns of amino acids (including ammonia production) and metabolic adaptations during pregnancy (Herring et al. 2018).

7.5 Summary

Amino acids are critical for animal production and human reproduction because they modulate major processes of reproduction, including gametogenesis, fertilization, implantation, placentation, and fetal growth and development. Peri-implantation stage of pregnancy is associated with significant embryonic loss in both humans and livestock, and the remarkable increases of amino acids in the uterine secretions, hormonally regulated expression of amino acid transporters in the uterine endometrium, and the activation of MTOR signaling pathway provide an intricate regulatory system in conceptus growth and development, implantation and maintenance of pregnancy. Select amino acids in uterine secretions, particularly Arg, Leu and Gln, together with glucose, stimulate trophectoderm or trophoblast cell proliferation, differentiation and growth through the MTOR cell signaling. Arg exerts its stimulatory effects on placental and fetal growth primarily through its derivatives, NO and polyamines, whose production in the trophectoderm/trophoblast cells are regulated by key enzymes NOS3, ODC1, ADC, AGMAT, and proline oxidase. Dietary or intravenous Arg supplementation improves reproductive performances of domestic animals and humans and could serve as a potential means to prevent or treat fetal growth restriction. Dietary supplementation with Gln during late gestation can also improve fetal growth. Extreme nutritional conditions, including gestational protein undernutrition, cause fetal growth restriction and long-term effects on the health and disease in offspring via impaired MTOR and amino acid response signaling in the placenta, altered RAS in maternal, placental and fetal compartments, and elevated testosterone. Dietary supplementation of Gly, Leu and taurine to dams with a severe protein deficiency can partially rescue fetal growth restriction and fetal programming of adult diseases.

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8 Impacts of Amino Acids on the Intestinal Defensive System

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Abstract

The intestine interacts with a diverse community of antigens and bacteria. To keep its homeostasis, the gut has evolved with a complex defense system, including intestinal microbiota, epithelial layer and lamina propria. Various factors (e.g., nutrients) affect the intestinal defensive system and progression of intestinal diseases. This review highlights the current understanding about the role of amino acids (AAs) in protecting the intestine from harm. Amino acids (e.g., arginine, glutamine and tryptophan) are essential for the function of intestinal microbiota, epithelial cells, tight junction, goblet cells, Paneth cells and immune cells (e.g., macrophages, B cells and T cells). Through the modulation of the intestinal defensive system, AAs maintain the integrity and function of the intestinal mucosa and inhibit the progression of various intestinal diseases (e.g., intestinal infection and intestinal colitis). Thus, adequate intake of functional AAs is crucial for intestinal and whole-body health in humans and other animals.

Keywords

Amino acids · Intestinal microbiota · Tight junction · Macrophages · T cells

Abbreviations

AAs	amino acids
AJ	adheren junction
Akt	protein kinase B
AMPK	AMP-activated protein kinase
Ang4	RNase angiogenin 4
APRIL	a proliferation-inducing ligand
BCAAs	branched-chain amino acids
CaMKK2	calcium/calmodulin-dependent kinase 2

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Crs1c cryptdin-related sequence 1c
DAO D-amino acid oxidase
DSS dextran sulfate sodium
ERK extracellular regulated protein kinases
ETEC enterotoxigenic *Escherichia coli*
FcRn neonatal Fc receptor
GABA gamma-aminobutyric acid
IELs intraepithelial lymphocytes
IFN interferon
IL interleukin
IR ischemia/reperfusion
MAPK mitogen-activated protein kinase
mTORC1 mechanistic target of rapamycin complex 1
NK natural killer
PI3K phosphatidylinositol 3′ -kinase
ROS reactive oxygen species
S6K1 ribosomal protein S6 kinase 1
Sirt1 sirtuin-1
TEER transepithelial electrical resistance
TJ tight junction
TNF tumor necrosis factor
ZO zonula occludens

8.1 Introduction

Interactions with pathogens and toxins are a fact of life for almost all animals, and this is more pronounced in the intestine than any other organs. The small intestine is responsible for nutrient digestion and absorption (Wu 2018). In addition, the gut is the home to a diverse community of indigenous microorganisms. Thus, both the small intestine and the large intestine are constantly exposed to various antigens from food and water, as well as a large number of bacteria that coexist in the intestinal lumen. The gastrointestinal tract has evolved with a sophisticated barrier defense system to protect against this exposure and to distinguish "self" from "foreign". This defense system includes indigenous commensal microorganisms, epithelial layer, and the lamina propria (Fig. 8.1). Intestinal microbiota is associated with the intestinal defensive system through its regulation on intestinal or systemic innate and adaptive immunities (Honda and Littman 2016; Thaiss et al. 2016), as well as direct effects on pathogens via colonization resistance or competition for nutrients (Endt et al. 2010; Seekatz and Young 2014). The epithelial layer includes absorptive enterocytes, hormone-secreting enteroendocrine cells, mucus-secreting goblet cells, antimicrobial-secreting Paneth cells, intraepithelial lymphocytes (IELs), microfold cells, and dendritic cells. The lamina propria harbors various immune cells, such as dendritic cells, neutrophils, macrophages, B lymphocytes (B cells), T lymphocytes (T cells), and fibroblasts. Based on published studies (Johansson and Hansson 2016; Mukherjee and Hooper 2015; Pabst et al. 2016), the intestinal epithelium produces and releases secretory IgA, antimicrobial proteins and mucins in a cell-specific manner.

Recent years have witnessed growing interest in the biochemistry and physiology of amino acids (AAs) in mammals, such as arginine, glutamine, glycine, and tryptophan (Fan et al. 2019; Le Floc'h et al. 2018; Hou and Wu 2017; Wu 2013). Notably, dietary contents of AAs are crucial for intestinal physiology, especially the intestinal defensive immune (Li et al. 2007; Ren et al. 2016a, b). The review highlights our current understanding of the influences of dietary AAs on intestinal defensive system in humans and animal models, including intestinal microbiota, cells in the epithelial layer and immune cells in the lamina propria.

8.2 Amino Acids and Intestinal Microbiota

Intestinal microbiota is present in virtually any metazoans, ranging from invertebrates to vertebrates. It affects numerous physiological functions of the gut (Lee and Hase 2014; Ren et al. 2016b; Subramanian et al. 2014; Thaiss et al. 2016) and is linked to the pathogenesis of various diseases (Anhe et al. 2014; Lee and Hase 2014; Louis et al. 2014; Qin et al. 2014; Thaiss et al. 2016) through the microbiome and its metabolic products (Lee and Hase 2014; Ren et al. 2016d). Intestinal microbiota has critical roles in intesti-

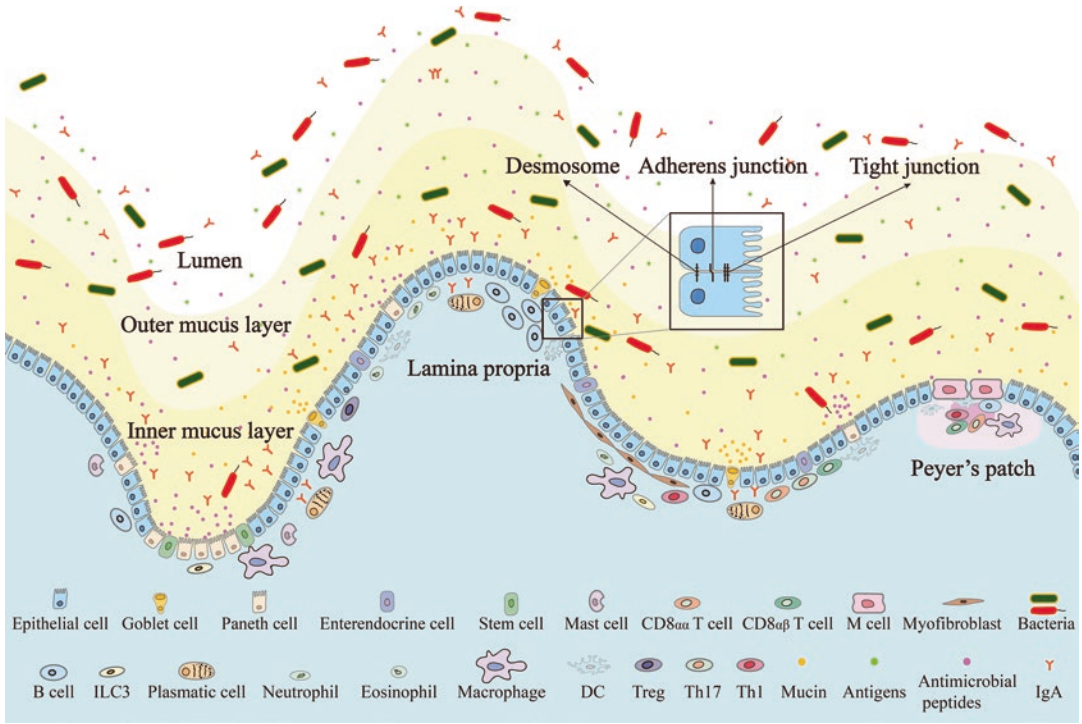

Fig. 8.1 The mucosal barrier defense system in the intestine. This defense system includes indigenous commensal microorganisms, epithelial layer, and the lamina propria. The epithelial layer consists of absorptive enterocytes, hormone-secreting enteroendocrine cells, mucin-secreting goblet cells, antimicrobial-secreting Paneth cells, intraepithelial lymphocytes (IELs), microfold cells, and dendritic cells. The lamina propria harbors various immune cells [e.g., dendritic cells (DC), neutrophils, macrophages, B cells, and T cells], fibroblasts, and blood vessels. ILC3 = group 3 innate lymphoid cell

nal immune response through its regulation of intestinal or systemic innate and adaptive immunities (Honda and Littman 2016; Thaiss et al. 2016), as well as by direct effects on pathogens via colonization resistance (Endt et al. 2010; Seekatz and Young 2014). For example, infection by *Clostridium difficile*, which is the leading health care-associated illness, usually follows the disruption of the indigenous gut microbiota after antibiotic treatment, leading to the loss of colonization resistance against the pathogen (Britton and Young 2014; Seekatz and Young 2014; Theriot et al. 2014). A successful treatment strategy for *C. difficile* infection is fecal microbiota transplantation from healthy individuals, which can recover the gut microbiome after transplantation (Fuentes et al. 2014; Seekatz et al. 2014).

Dietary AAs regulate the diversity, composition and metabolism of intestinal microbiota (Dai et al. 2011, 2015). For example, arginine decreases the net utilization of lysine, threonine, isoleucine, leucine, glycine and alanine by jejunal or ileal mixed bacteria (Dai et al. 2012). Arginine supplementation shifts the population of microbes in the jejunum and ileum of mice to favor the growth of *Bacteroidetes* by decreasing the number of *Firmicutes*, but increasing the abundance of *Bacteroidetes* (Ren et al. 2014a). Arginine also enhances the abundance of *Lactobacillus* in the jejunum and the abundance of *Streptococcus* in the ileum (Ren et al. 2014a). Thus, feeding *Lactobacillus reuteri* DSM 17938 to newborn mice increased the concentration of beneficial AAs and their metabolites in the large intestine, while regulating gut microbiota and immune responses (Liu et al. 2019b). In addition, dietary supplementation with glutamine to mice decreases the abundance of *Firmicutes* in their jejunum and

ileum, while increasing the abundance of *Streptococcus* and *Bifidobacterium* in their jejunum (Ren et al. 2014b). Furthermore, adding proline to the diet of Huanjiang mini-pigs decreases the amounts of *Klebsiella pneumoniae, Peptostreptococcus productus, Pseudomonas,* and *Veillonella* spp. in distal colonic contents (Ji et al. 2018). Likewise, dietary supplementation with gamma-aminobutyric acid (GABA) regulates the community richness and diversity of the ileal microbiota, as well as the abundances of the dominant microbial populations in weaned piglets (Chen et al. 2019b). Interestingly, dietary lysine restriction decreases the bacterial diversity and increases the abundance of *Actinobacteria*, *Saccharibacteria*, and *Synergistetes* in the intestine at the phylum level, as well as the abundances of *Moraxellaceae*, *Halomonadaceae*, *Shewanellaceae*, *Corynebacteriaceae*, *Bacillaceae*, *Comamonadaceae*, *Microbacteriaceae*, *Caulobacteraceae*, and *Synergistaceae* in the intestine at the family level (Yin et al. 2017).

The exact mechanisms whereby AAs modulate intestinal microbiota need further investigation. It is possible that AA supplementation or restriction alters the intestinal microenvironment, and then influences the composition and function of the intestinal microbiota. Notably, beneficial effects of AAs on gut health are associated with similar changes in the intestinal microbiota, but some AAs exert specific effects. Also, the influences of AAs on the intestinal microbiota depend on their supplemental dosages. For example, dietary supplementation with 0.5 and 1% aspartate to mice reduces the ratio of *Firmicutes* to *Bacteroidetes* in the ileum and feces, but dietary supplementation with 2% aspartate increases this ratio in the feces (Bin et al. 2017).

Results of our recent studies indicate that arginine or glutamine supplementation promotes the activation of intestinal innate immunity, including expression of factors (e.g., toll-like receptors) and activation of signaling pathways [e.g., mitogen-activated protein kinase (MAPK)] associated with intestinal innate immunity (Ren et al. 2014a, b). Thus, dietary supplementation with arginine or glutamine enhances the ability of the host to clear infections by pathogens (e.g., porcine circovirus type 2 and *Pasteurella multocida*) (Chen et al. 2014; Ren et al. 2012, 2013a, b, c, d), especially intestinal pathogens (e.g. enterotoxigenic *Escherichia coli*) (Liu et al. 2017a). However, whether arginine or glutamine promotes the clearance of these pathogens in the host through the intestinal microbiota remains to be explored.

Intestinal microbiota also affects the host AA metabolism and, therefore, the defensive responses. For example, the intestinal microbiota (*Clostridium sporogenes*) uses aromatic AAs (tryptophan, phenylalanine and tyrosine) as substrates to produce metabolites (e.g., indolepropionic acid), which in turn affect intestinal permeability and systemic immunity (Dodd et al. 2017). The enrichment of the intestinal microbiota that synthesizes the branched-chain amino acids (BCAA), such as *Prevotella copri* and *Bacteroides vulgatus*, and that have a low capacity to take up BCAAs, are associated with high concentrations of BCAA in serum (Pedersen et al. 2016). Indeed, the levels of AAs in the ileum differ markedly between conventionally reared and germ-free mice, indicating that the gut microbiota greatly affects the metabolism of AAs in the ileum (Mardinoglu et al. 2015). Those AAs include arginine, asparagine, histidine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine and glutamine (Mardinoglu et al. 2015). It is unknown whether this alteration in AA metabolism is associated with the abnormities of intestinal immunity in germ-free mice, such as Paneth cell dysfunction (Zhang et al. 2015). During enterotoxigenic *Escherichia coli* infection, hosts (i.e., piglets and mice) experience remarkable alterations in the intestinal microbiota, especially increases in the abundance of *Lactococcus lactis* subsp. (Ren et al. 2016d). *Lactococcus lactis* subsp. regulates the host immune responses against enterotoxigenic *Escherichia coli* infection through producing GABA, which promotes intestinal expression of IL-17 to activate the mechanistic target of rapamycin complex 1 (mTORC1)-ribosomal protein S6 kinase 1 (S6K1) signaling (Fig. 8.2) (Ren et al. 2016d). Besides glycine and the L-isoform of AAs, the mouse intestine contains high levels of free D-AAs derived from the microbiota (Kepert et al. 2017; Sasabe et al. 2016). Interestingly, the intesti-

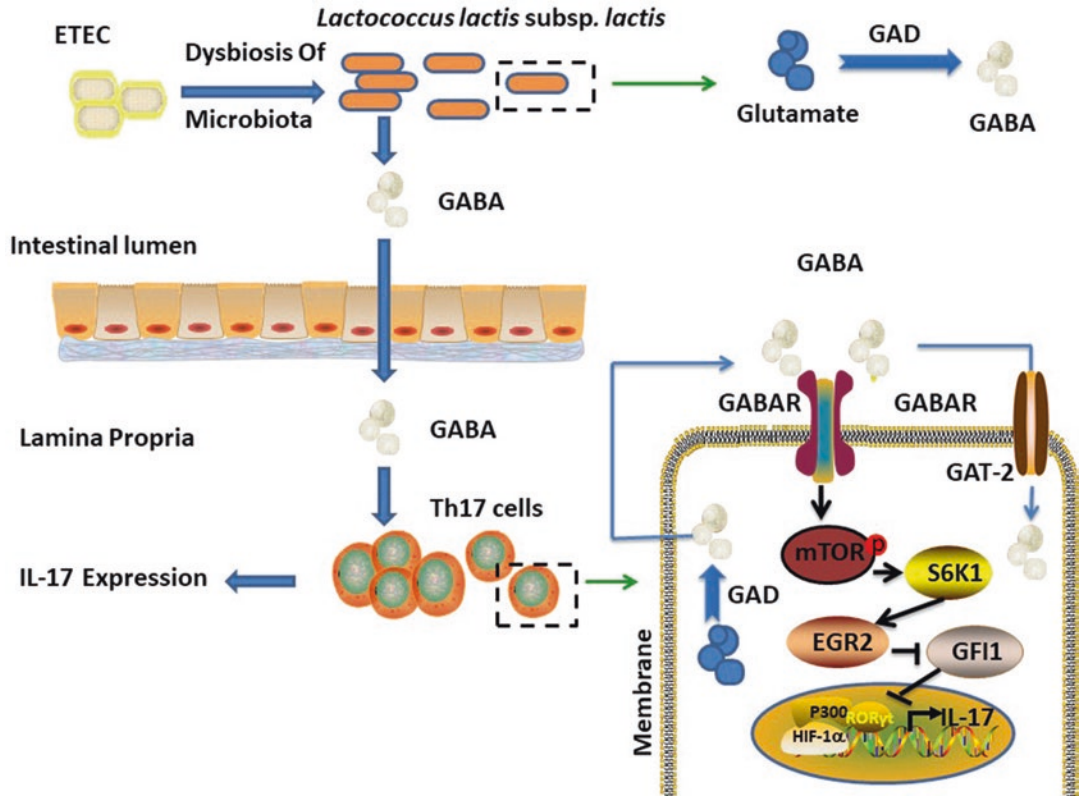

Fig. 8.2 γ-Aminobutyrate (GABA) mediates intestinal interleukin-17 expression during infection by enterotoxigenic *Escherichia coli* (ETEC). During ETEC infection, the pathogen induces dysbiosis in the gut microbiota, increasing *Lactococcus lactis subsp*. The *Lactococcus lactis subsp.* produces GABA from glutamate through the action of glutamate decarboxylase (GAD). GABA is sensed by Th17 cells through GABA receptors (GABAR), leading to the activation of the mTOR pathway. The mTOR signaling promotes IL-17 expression during infection through the mTOR-S6K1-EGR2-GFI1 pathway. GABA transporter 2 is negatively associated with Th17 response during intestinal infection by terminating the GABA signaling through the translocation of GABA from the extracellular to the intracellular space

nal microbiota stimulates the release of D-amino acid oxidase (DAO) from intestinal epithelial cells (including goblet cells) into the intestinal lumen, resulting in the oxidative deamination of intestinal D-AAs to yield a potent antimicrobial product, H_2O_2, thereby protecting the mucosal surface in the small intestine from the cholera pathogen (Sasabe et al. 2016). DAO has also been shown to modify the composition of the microbiota and production of intestinal sIgA (Sasabe et al. 2016). This illustrates the importance of D-AAs in nutrition and metabolism. Collectively, there is significant crosstalk between host AAs and the intestinal microbiota, and this interplay regulates the intestinal defensive responses and the progression of intestinal infection.

8.3 Amino Acids and Intestinal Epithelial Cells

Besides the absorption of nutrients (including AAs, glucose, fatty acids, and electrolytes), intestinal epithelial cells (generated from intestinal epithelial stem cells) represent an effective barrier ling the gastrointestinal mucosal surface, and regulate the functions of intestinal immune cells as well as intestinal homeostatic and inflam-

matory responses (Nowarski et al. 2017). For example, the villous epithelial cells that express the neonatal Fc receptor (FcRn) play a role in binding intestinal antigens (McDole et al. 2012; Schulz and Pabst 2013). FcRn on villus epithelial cells aids in the secretion of IgG across the intestinal epithelium into the lumen, and also contributes to the uptake of intestinal antigens from the lumen through the IgG-dependent process (Yoshida et al. 2004, 2006). Also, the expression of *Ifnlr1* [the receptor for interferon (IFN)-λ] on intestinal epithelial cells in the small intestine and colon is critical for enteric IFN-λ antiviral activity in mice (Baldridge et al. 2017). Importantly, *Ifnlr1* expression in intestinal epithelial cells affects the efficacy of IFN-λ in resolving persistent murine norovirus infection, and is necessary for the sterilizing innate immune effects of IFN-λ (Baldridge et al. 2017). Although p40 (a *Lactobacillus rhamnosus* GG-derived protein) treatment directly on B cells shows little effect on IgA production, p40 promotes the expression of a proliferation-inducing ligand (APRIL) in intestinal epithelial cells, resulting in an increase in fecal IgA levels, as well as IgA^+B220^+, IgA^+CD19^+, and IgA^+ plasma cells in the lamina propria of mice (Wang et al. 2017). Collectively, intestinal epithelial cells are closely associated with intestinal immunity responses.

It is well known that AAs, such as glutamate, cysteine, glutamine and glycine, promote protein synthesis in intestinal epithelial cells and their growth via various cellular signaling, such as the mTOR signaling (He et al. 2016; Honda and Littman 2016; Wang et al. 2014a, 2016; Ye et al. 2016), as well as nutrient metabolism, glutathione synthesis, and ATP production (Li et al. 2020). For example, arginine enhances DNA synthesis, cell-cycle progression, and mitochondrial bioenergetics in intestinal epithelial cells through mechanisms involving activation of the phosphatidylinositol 3′-kinase (PI3K)-protein kinase B (Akt pathway) (Tan et al. 2015). Given the importance of AAs in these physiological processes, we surmise that AAs may affect intestinal defensive responses by regulating the expression and secretion of immune regulators in intestinal epithelial cells. For example, BCAA stimulate the expression of β-defensin from porcine intestinal epithelial cells possibly through activation of the sirtuin-1(Sirt1)/extracellular regulated protein kinases (ERK) signaling pathway (Ren et al. 2016a). In addition, tryptophan inhibits the secretion of interleukin (IL)-8 in intestinal epithelial cells after tumor necrosis factor (TNF)-α challenge through the calcium-sensing receptor (Mine and Zhang 2015). Glycine attenuates the production of reactive oxygen species (ROS) in intestinal epithelial cells via promoting the synthesis of glutathione and expression of glycine transporter 1, while reducing the activation of the MAPK signaling pathway (Wang et al. 2014a). Amino acids also regulate the function of intestinal epithelial cells and the intestinal immunity. For example, AA starvation in intestinal epithelial cells induces autophagy responses in intestinal epithelial cells, resulting in lower levels of ROS and IL-1beta as well as a reduction in the abundance of IL-17A-producing $CD4^+$ T cells (Ravindran et al. 2016). Collectively, epithelial cells are involved in intestinal immune responses, such as antigen recognition, IgA production, and the killing of pathogens. Some AAs (e.g., arginine, BCAA and glycine) regulate protein synthesis in intestinal epithelial cells, their proliferation and migration, as well as the generation and secretion of immune regulators by the cells.

8.4 Amino Acids and Intercellular Junction

Between intestinal epithelial cells, there are intercellular junctions that include an apical tight junction (TJ), subjacent adheren junction (AJ), and desmosomes, controlling the movement of fluids and solutes in the paracellular space and the establishment of cell polarity (Luissint et al. 2016; Tsukita et al. 2001). Tight junctions reside include claudins, TJ-associated MARVEL domain-containing proteins (TAMPs, including occludin, MARVELD2, and MARVELD3), and members of the cortical thymocyte marker in the Xenopus family, such as junctional adhesion molecules (Luissint et al. 2016; Raleigh et al. 2010). The AJ is an ancient junctional complex that initiates and maintains epithelial cell-cell

contacts, while the desmosomes provides mechanical strength to the epithelium. The key transmembrane protein in the epithelial AJ is E-cadherin, while the desmosomes include desmoglein and desmocollin proteins (Green and Simpson 2007; Ivanov and Naydenov 2013). The maintenance of the intestinal epithelial barrier is dependent on the crosstalk among TJs, AJs, and desmosomes (Luissint et al. 2016). A functional intestinal epithelium allows for selective absorption of nutrients, while restricting the passage of pathogens and food-borne antigens. However, various intestinal pathogens have been reported to target the intestinal epithelial barrier to induce disassembly and barrier defects. For example, the enterotoxin produced by *Clostridium perfringens* has been reported to bind claudins 3, 4, 6, 7, 8, 9, and 14, resulting in their internalization from the TJ and therefore compromising mucosal barrier function (Fernandez Miyakawa et al. 2005; Saitoh et al. 2015; Veshnyakova et al. 2010).

Dietary AAs are important regulators of intercellular function, especially the expression and abundance of TJs. This notion is supported results from both *in vitro* and *in vivo* experiments. For example, tryptophan enhances the abundances of occludin, claudin-4, zonula occludens (ZO)-1 and 2 in intestinal porcine epithelial cells (Wang et al. 2015a). Similarly, glutamine decreases the TJ permeability, but increases the monolayer transepithelial electrical resistance (TEER), the abundances of transmembrane proteins (including occludin, claudin-4, ZO-1, ZO-2, and ZO-3) through activation of the calcium/calmodulin-dependent kinase 2 (CaMKK2)-AMP-activated protein kinase (AMPK) signaling (Jiao et al. 2015; Wang et al. 2016). Subsequent investigations with piglets also demonstrate the positive influence of physiological levels of AAs on the expression of TJ proteins. Specifically, dietary supplementation with glutamine to weanling piglets augments the abundances of occludin, claudin-1, ZO-2, and ZO-3 proteins in the jejunum (Wang et al. 2015b). Besides glutamine, dietary supplementation with putrescine or proline to neonatal piglets between day 1 of age and weaning at 14 day of age increases the abundances of ZO-1, occludin, and claudin-3 proteins in the jejunum (Wang et al. 2015c). Similarly, studies with post-weaning pigs have shown that dietary supplementation with 1% glutamine (Wu et al. 1996), 1% proline (Wu et al. 2011), or 1-2% glycine (Wang et al. 2014b) ameliorated intestinal atrophy and improved their growth performance, whereas dietary supplementation with 0.2% putrescine dihydrochloride improved intestinal integrity and decreased the incidence of diarrhea (Liu et al. 2019a). Note that glutamine, glycine and proline are highly abundant in animal-source proteins such as meat & bone meal, poultry by-products, and chicken visceral digest (Wu and Li 2020), whereas the content of glycine and proline is relatively low in all plant-source proteins (Hou et al. 2019).

Animals are frequently exposed to stressful conditions in their life times. Importantly, AAs are beneficial for maintaining the adequate expression of intestinal TJ proteins in subjects with various intestinal diseases, such as intestinal inflammation that is associated with the defect of TJ functions. In the dextran sulfate sodium (DSS)-induced colitic mouse model, which is similar to human ulcerative colitis, dietary supplementation with arginine or glutamine increases the abundance of the claudin-1 protein in the colon (Ren et al. 2014c). Likewise, glutamine administration increases the abundance of the ZO-1 protein in the small-intestinal mucosa of DSS-treated mice (Pai et al. 2014). Similarly, in rats with methotrexate-induced mucositis, glutamine or arginine supplementation enhances the jejunal abundances of claudin-1, occludin and ZO-1 proteins through ERK and NF-κB pathways (Beutheu et al. 2014). In addition to intestinal inflammation, AAs are also essential for the homeostasis of TJ proteins in other situations. For example, although a western-style high-fat diet lowers the levels of occludin and ZO-1 proteins in the upper part of the mouse small intestine, oral administration of arginine restores the abundances of occludin and ZO-1 proteins (Sellmann et al. 2017a). Liang et al. (2018) reported that dietary supplementation with 0.2% tryptophan to weanling pigs increased the abundances of ZO-1 and occludin proteins in the colon. Furthermore, dietary supplementation

with 0.2% and 0.4% tryptophan to weanling pigs augmented the abundances of the jejunal ZO-1, ZO-3 and claudin proteins in a dose-dependent manner, whereas dietary supplementation with 0.4% tryptophan also enhanced the abundance of the jejunal occludin protein (Liang et al. 2019). Thus, much evidence shows that intercellular junctions, including TJ, AJ and desmosomes, play a critical role in the hemostasis of the intestinal epithelium. Dietary AAs are essential for the expression of the TJ proteins, especially in various intestinal diseases that are characterized by defects in the intestinal mucosal barrier (Table 8.1). However, it remains unknown how AAs affect the location of intestinal TJ proteins or the homeostasis of the intestinal AJ and desmosomes. This remains to be an active area of research in AA physiology and nutrition.

8.5 Amino Acids and Goblet Cells

In addition to enterocytes, the second subtype of cells in the intestinal epithelium is the mucus-producing goblet cells. Goblet cells are specialized secretory cells lining intestinal mucosal epithelia. The differentiation of goblet cells from intestinal epithelial stem cells is tightly regulated by the sterile α motif pointed domain epithelial specific transcription factor (*Spdef*), which responds to the downstream of both Notch and Wnt signaling. *Spdef*-null mice show a reduction in mature, differentiated goblet cells in the intestine, whereas overexpression of *Spdef* in the intestine displays an expansion of Muc2-expressing goblet cells at the expense of other intestinal cell types (Gregorieff et al. 2009; Noah et al. 2010). Goblet cells have critical roles in maintaining intestinal homeostasis through secreting a variety of factors, such as mucins and trefoil factors (Johansson and Hansson 2016; McCauley and Guasch 2015). The secretion of these factors from goblet cells depends on various stimuli, such as microbial factors, growth factors and inflammatory cytokines (Deplancke and Gaskins 2001; McCauley and Guasch 2015; Wlodarska et al. 2014), as well as the availability of threonine (a major AA in mucins; Wu 2018). These factors entrap external insults such as pathogens, toxins, and allergens, and prevent their translocation into the blood and other extra-intestinal tissues (Johansson and Hansson 2016). Besides the secretory function, goblet cells have recently been implicated as antigen-presenting cells because goblet cells in the small intestine present intestinal luminal antigens to the underlying dendritic cells so that dendritic cells can sense intestinal insults without a break in intestinal barrier integrity (Knoop et al. 2015; McDole et al. 2012).

Increasing evidence has shown that dietary AAs actively maintain the number of intestinal goblet cells and the expression of mucins in the intestine. For example, in healthy mice, dietary supplementation with 1.0% glutamine for 2 weeks promotes the expression of mucin-4 in the jejunum (Ren et al. 2014b). Similar observation has also been reported in various models of intestinal diseases. For example, in rats with DSS-induced colitis, dietary supplementation with a mixture of AAs (containing L-threonine, L-serine, L-proline, and L-cysteine) attenuates reductions in the number of Muc2-containing goblet cells in the intestinal epithelium of the ulcerated area and mucin production in the colon, while restoring the mucin AA composition and mucosal content (Faure et al. 2006). Likewise, in rats with experimental diversion colitis, glutamine supplementation increases the number of goblet cells in the colonic lamina propria (Pacheco et al. 2012). Also, in enterotoxigenic *Escherichia coli* (ETEC) infected mice, glutamine promotes the expression of mucin-2 in the jejunum (Xu et al. 2017), providing another line of evidence for a crucial role of the functional AA in gut integrity and function (Rhoads and Wu 2009).

Under certain experimental conditions, some AAs have little effect on or even reduce the number of intestinal goblet cells. For example, glutamine supplementation to weaning mice did not affect the number of goblet cells, or the expression of markers for goblet cells (Chen et al. 2018a). In male 50-day-old Wistar rats, dietary supplementation with 2.0% glutamine for 10 days

Table 8.1 Effects of amino acids on intestinal tight junction proteins

Amino acids	Models	Effect on TJs	References
Tryptophan	IPEC	Tryptophan increases the protein abundances of occludin, claudin-4, ZO-1 and ZO-2.	Wang et al. (2015a)
	Pigs	Tryptophan supplementation enhances the mRNA levels of claudin-3 and ZO-1.	Liu et al. (2017b)
Methionine	Rats	Methionine restriction increases the mRNA levels of claudin-3 and changes the posttranslational modification of occludin.	Ramalingam et al. (2010)
	Renal epithelial cells	Methionine restriction decreases the protein abundances of claudin-3 and 7, but dramatically increases the abundances of claudin-4 and 5.	Mullin et al. (2009)
Glycine	IPEC	Glycine supplementation enhances the protein abundances of claudin-3, claudin-7 and ZO-3.	Li et al. (2016)
Valine	Grass carp	Valine deficiency decreases mRNA levels of claudin-b, claudin-3, occludin and ZO-1, but increases the mRNA level of claudin-15.	Feng et al. (2015b)
Phenylalanine	Grass carp	Phenylalanine supplementation increases the mRNA levels of ZO-1, occludin and claudin-c.	Feng et al. (2015a)
Leucine	Grass carp	Leucine supplementation increases the mRNA levels of occludin, ZO-1, claudin-b, claudin-3 and claudin-12.	Jiang et al. (2017b)
Isoleucine	Hen	Excess digestible isoleucine level does not change mRNA levels of claudin-1 and occludin.	Dong et al. (2016)
	Grass carp	Isoleucine deficiency down-regulates the mRNA levels of claudin-3, claudin-b, claudin-c, occludin and ZO-1, but up-regulates the mRNA level of claudin-12.	Feng et al. (2017)
Proline	Piglet	Proline increases the protein abundances of ZO-1, occludin and claudin-3.	Wang et al. (2015c)
Glutamine	IPEC	Glutamine increases the protein abundances of occludin, claudin-4, junction adhesion molecule (JAM)-A, ZO-1, ZO-2 and ZO-3.	Wang et al. (2016)
	Weanling piglet	Glutamine increases the protein abundances of occludin, claudin-1, ZO-2, and ZO-3.	Wang et al. (2015b)
	Caco-2 cells	Deprivation of glutamine decreases protein abundances of claudin-1, occludin and ZO-1.	Li et al. (2004)
Glutamate	IPEC	Glutamate enhances the mRNA and protein abundances of occludin, claudin-3, ZO-2 and ZO-3.	Jiao et al. (2015)
	Caco-2 cells	Glutamate supplementation increases the mRNA levels of ZO-1 and occludin during MTX treatment.	Beutheu et al. (2013)
	Carp	Glutamate supplementation increases mRNA levels of ZO-1, occludin, claudin-2, 3 and 7 during LPS challenge.	Jiang et al. (2017a)
Arginine	Caco-2 cells	Arginine supplementation increases the mRNA levels of ZO-1 and occludin during MTX treatment.	Beutheu et al. (2013)
	Grass carp	Arginine supplementation enhances the mRNA levels of occludin, claudin-3 and claudin-c.	Chen et al. (2019a)
Threonine	Broiler chickens	Threonine administration increases the mRNA levels of claudin-3 and ZO-1 during LPS challenge.	Chen et al. (2018b)
Histidine	Grass carp	Histidine deficiency down-regulates the mRNA levels of claudin-b, claudin-c, claudin-3, claudin-12, claudin-15, occludin and ZO-1.	Jiang et al. (2016)
Citrulline	Mice	Citrulline increases the protein abundances of occludin and ZO-1.	Sellmann et al. (2017b)

was reported to decrease the numbers of goblet cells in the villi and crypt of the jejunum or ileum (Martins et al. 2016). Similarly, glutamine supplementation reduced the number of goblet cells in the villi and crypt of jejunum or ileum in rats with Walker-256 tumor (Martins et al. 2016). However, the provision of glutamine from the basal diet was not known in all these studies. In weaned piglets, tryptophan supplementation had little effect on the numbers of goblet cells in the duodenum, jejunum and ileum (Tossou et al. 2016). Similarly, threonine supplementation did not influence the numbers of goblet cells in the jejunum and colon or the amounts of mucins in the scrapings of the jejunum and colon in weaning piglets (Trevisi et al. 2015). The possible reasons for these discrepancies include animal models, intakes of the AAs from the basal diets, supplemental dosages of the AAs, and the methods used for the analysis of goblet cells. Thus, the influences of AA in intestinal goblet cells need further investigation. It is interesting to determine whether specific AAs (e.g., glutamine, arginine and glycine) regulates the differentiation of intestinal goblet cells from intestinal epithelial stem cells.

8.6 Amino Acids and Paneth Cells

With the lineage of secretory cells from intestinal epithelial stem cells, Paneth cells produce antimicrobial peptides, which are rich in proline (Hou et al. 2017). Various cellular signaling pathways affect the differentiation of Paneth cells, such as Notch, PKC λ/ι and mTORC1 (Heuberger et al. 2014; Nakanishi et al. 2016; Zhou et al. 2015). Unlike the enterocytes, Paneth cells reside at the base of the small intestinal crypts of Lieberkühn, where epithelial stem cells are also present.

Paneth cells secrete a wide variety of peptides and proteins, such as lysozyme, α-defensin peptides and secretory phospholipase A2 isotype II (Clevers and Bevins 2013; Porter et al. 2002; Salzman and Bevins 2013). Most of these peptides and proteins have antimicrobial effects, which target microorganisms, including the resident microbiota of the small intestine and the intruding pathogens that can potentially penetrate the mucus layer to invade the crypt or other parts of the intestinal epithelium (Ayabe et al. 2000; Bevins and Salzman 2011). Thus, Paneth cells help protect the gut from pathogenic microbes and shape the composition of the intestinal resident microbiota (Brandl et al. 2007; Salzman et al. 2010; Veshnyakova et al. 2010).

Paneth cells also secrete ligands that provide trophic support for the adjacent epithelial stem cells (Sato et al. 2011). These peptides and proteins are stored in the large secretory granules of Paneth cells and secreted into the crypt lumen via mechanisms mediated by KCa3.1 calcium-activated potassium channels in response to a variety of stimuli, including bacterial products (Ayabe et al. 2000, 2002).

Dietary AAs have been reported to regulate the production of antimicrobial peptides by Paneth cells. For example, arginine supplementation upregulates the expression of *cryptdins 1*, *4*, and *5*, cryptdin-related sequence 1c (*Crs1c*), and RNase angiogenin 4 (*Ang4*) in the jejunum and ileum (Ren et al. 2014a). Similarly, glutamine supplementation increases the mRNA levels for *cryptdins 1*, *4*, and *5* in the jejunum, *cryptdins 4* in the ileum, and *Reg3g* in the colon (Ren et al. 2014b). In ETEC-infected mice, arginine or glutamine supplementation also promotes the expression of the *Crs1c* and *Reg3g* genes (Liu et al. 2017a). Although these results indicate the beneficial function of arginine or glutamine in Paneth cells, the underlying mechanisms are largely unknown. It remains to be determined whether other functional AAs regulate the differentiation of Paneth cells or the expression of antimicrobial peptides in the cells. Collectively, arginine or glutamine can modulate the synthesis of antimicrobial peptides in Paneth cells. However, the roles of other AAs in the secretion

of intestinal antimicrobial peptides and the differentiation of Paneth cells remain to be explored.

8.7 Amino Acids and Intestinal Immune Cells

There are various types of immune cells in the intestine, including IELs, microfold cells, dendritic cells, macrophages, B cells, and T cells (Fig. 8.1). The intestine has now been characterized as the largest lymphoid organ in humans and other mammals. The intricate intestinal immune system consists of the outer epithelial layer and the inner lamina propria. The components of the outer section include IELs, the dendritic cell extensions and microfold cells. Intraepithelial lymphocytes are an important line of the first defense that maintains the integrity of intestinal epithelial cells, and dendritic cells help to determine the type of immune response as needed by presenting luminal antigens. In pigs, IELs respond well to T-cell mitogens after weaning but not during the preweaning period (Wu 1996). Microfold cells also mediate the transcytosis of antigens across the epithelium. The inner section of the intestinal defense locates below the IELs, and includes dendritic cells, neutrophils, macrophages, immunoglobulin (Ig) A-producing B cells, natural killer (NK) cells, NK T-cells, conventional T-cells, and T-regulatory cells.

The numbers of macrophages, T cells, and B cells in the intestinal mucosa are greater in weanling mammals (e.g., pigs) than in preweaning ones (Wu 1995). These immune cells initiate inflammation and injury in the gut. Available evidence shows that AAs are important regulators of the activation and function of intestinal immune cells. For example, glutamine promotes the secretion of IgA and increases the abundance of IgA-producing B cells in the intestine (Ren et al. 2016b; Wu et al. 2016). Glutamine also highly shapes the polarization of macrophages through mechanisms, including glutamine-UDP-*N*-acetylglucosamine pathway, glutamine-derivedα-ketoglutarate via glutaminolysis, and glutamine-dependent anerplerosis or the GABA shunt (Ren et al. 2019a; Xia et al. 2019). Dietary deficiency of AAs significantly reduces the number of $F4/80^+CD11b^+$ macrophages and the number of $IL\text{-}10^+F4/80^+CD11b^+$ macrophages in the mouse small intestine (Ochi et al. 2016). The influence of dietary AAs on small-intestinal macrophages may depend on mTOR signaling because an inhibition of this signaling by rapamycin also reduces the number of $IL\text{-}10^+F4/80^+CD11b^+$ macrophages in the mouse small intestine (Ochi et al. 2016).

Considering the importance of AAs in T cell fate decision (Ren et al. 2016c, 2017a, b), it is not surprising that AAs regulate intestinal T cell response. For example, during ETEC infection, intestinal GABA promotes the expression of IL-17 in the jejunum of both mice and piglets (Ren et al. 2016d, 2019b). In addition to conventional T-cells, AAs also modulate the intestinal un-conventional T-cell response. For example, in the DSS-treated mice, glutamine administration increases the proportion of small-intestinal IEL γδ-T cells but decreases the expression of genes responsible for immunomodulation in IEL γδ-T cells, such as *Ifn-γ*, *Tnf-α* and *Il-17* (Pai et al. 2014). Similarly, glutamine decreases the percentage of IEL γδ-T cells, and regulates the mRNA expression of *Bcl-xl*, *Il-7 receptor* and *Reg3g* in IEL γδ-T cells in mice with ischemia/reperfusion injury (Pai et al. 2015). Furthermore, dietary supplementation with L-tryptophan (0.1 g/kg body weight per day) to mice with DSS-induced inflammation reduced the abundances of macrophages and neutrophils in the colon and improved colonic immune responses partly through attenuating the activation of toll-like receptor 4 (TLR4)-STAT3 signaling and nucleus p-65 (Wang et al. 2020). Thus, dietary AAs play an important role in the activation and function of intestinal immune cells (e.g., IgA-producing B cells, macrophages and T cells, Table 8.2). However, the influences of AAs on the number and function of other types of intestinal immune cells, such as M cell, dendritic cells and neutrophils, need further investigation. Besides the mTOR signaling, whether AAs affects the fate of intestinal immune cells through other cellular signaling molecules (such as nitric oxide, kynurenine, glycine, glutamate and hydroxyproline) remain to be determined (Hou and Wu 2018; Li and Wu 2018; Wang et al. 2013, 2020; Wu et al. 2019b).

Table 8.2 Effects of amino acids on intestinal immunity

Amino acids	Models	Effect on intestinal immunity	References
Tryptophan	Acetic acid-treated mice	Tryptophan supplementation inhibits the colonic mRNA expression of IL-22.	Chen et al. (2018a)
	DSS- treated mice	Tryptophan supplementation reduces the colonic mRNA levels of IL-6, TNF-α, IL-1β, CCL2, CXCL1 and CXCL2.	Islam et al. (2017)
Methionine	Chemotherapy-induced intestinal mucositis rats	Methionine supplementation increases the intestinal mRNA levels of IL-10.	Wu et al. (2019a)
Glycine	Rodent postoperative inflammatory ileus	Glycine treatment reduces the mRNA levels of IL-6 and TNF-α in the rat small intestinal muscularis.	Stoffels et al. (2011)
Valine	Normal grass carp	Valine deficiency down-regulates mRNA levels of IL-10 and TGF-β1, but up-regulates the mRNA levels of TNF-α and IL-8 in the small intestine.	Luo et al. (2014)
Phenylalanine	Normal grass carp	Phenylalanine supplementation increases the mRNA levels of IL-10 and TGF-β1 in the intestine.	Feng et al. (2015a)
Leucine	LPS-treated chicken embryos	Leucine supplementation decreases IgA production and mRNA level of IL-6 in small intestine.	Liu et al. (2018)
	Normal grass carp	Leucine supplementation down-regulates the mRNA levels of TNF-α and IL-8 in the mid and distal intestine.	Jiang et al. (2015)
Serine	Early-weaned piglets	Serine supplementation decreases both cytokine secretion and mRNA levels of IL-1β, IL-6, IL-8 and TNF-α in the small intestine.	Zhou et al. (2018)
	LPS-treated mice	Serine treatment reduces the mRNA levels of IL-1β, TNF-α, IL-6, IL-8 and IL-10 in the ileum.	Zhou et al. (2017)
Cysteine	LPS-treated piglets	Cysteine supplementation down-regulates the mRNA levels of TNF-α, IL-6 and IL-8 in the jejunum and ileum.	Song et al. (2016)
	DSS-treated piglets	Cysteine supplementation reduces the colonic mRNA levels of TNF-α, IL-6, IL-12p40 and IL-1β.	Kim et al. (2009)
Asparagine	LPS-treated piglets	Asparagine supplementation down-regulates the intestinal TNF-α secretion.	Chen et al. (2016)
Glutamine	Normal mouse	Glutamine supplementation increases ileal mRNA levels of IL-5, IL-6, IL-13 and TGF-β.	Wu et al. (2016)
	DSS-treated mouse	Glutamine administration increases the proportion of small-intestinal IEL γδ-T cells but decreases the mRNA levels of IFN-γ, TNF-α and IL-17 in IEL γδ-T cells.	Pai et al. (2014)
	Normal mouse	Glutamine supplementation enhances mRNA levels for IL-1β, IL-17 and TNF-α in the ileum.	Ren et al. (2014b)
	Soybean meal-induced enteritis turbot	Glutamine decreases the infiltration of leucocytes in the lamina propria and submucosa, as well as the mRNA levels of IL-8, TNF-α and TGF-β in the intestine.	Gu et al. (2017)
Threonine	LPS-treated chicken	Threonine administration reduces mRNA levels of the jejunal IFN- γ and ileal IL-1β.	Chen et al. (2018c)
	IUGR weanling piglets	Threonine supplementation reduces the ileal mRNA level of TNF-α, and increases the production of Muc2 and SIgA, as well as the density of goblet cells.	Zhang et al. (2019)
Aspartate	Normal mouse	Aspartate supplementation decreases the ileal mRNA levels of IL-17, IFN-γ and Muc2.	Bin et al. (2017)
Glutamate	LPS-treated Jian carp	Glutamate treatment suppresses the mRNA levels of IL-1β, IL-8 and TNF-α, but enhances the mRNA levels of IL-10 in the intestine.	Jiang et al. (2017a)

(continued)

Table 8.2 (continued)

Amino acids	Models	Effect on intestinal immunity	References
Arginine	*Clostridium perfringens* infected chickens	Arginine supplementation elevates jejunal mRNA levels of IFN-γ, IL-10 and NOD1.	Zhang et al. (2017)
	Normal mouse	Arginine supplementation increases the ileal mRNA levels of IL-1β, TNF-α and IFN-γ, and promotes the production of SIgA, mucins, and Paneth antimicrobials in the jejunum and ileum.	Ren et al. (2014a)
	LPS-treated weaned pigs	Arginine administration reduces the mRNA levels of jejunal IL-6, and jejunal and ileal TNF-α.	Liu et al. (2008)
γ-aminobutyric acid	Normal weaned piglets	γ-aminobutyric acid supplementation inhibits the intestinal mRNA levels of IL-22, IL-1, IL-18 and Muc1, but increases the mRNA levels of IFN-γ, IL-4 and IL-10.	Chen et al. (2019b)
	ETEC infected mouse or piglets	Intestinal γ-aminobutyric acid promotes the mRNA level of IL-17 in the jejunum.	Ren et al. (2016d)

8.8 Conclusion

The intestine interacts with a diverse community of antigens and bacteria, and has evolved with a complex defense system, including the indigenous intestinal microbiota, epithelial layer and lamina propria. Dietary intakes of AAs profoundly affect this defense system that involves not only luminal microbes but also intestinal epithelial cells, TJs, globet cells, Paneth cells and immune cells (e.g., macrophages, B cells and T cells). It is imperative to explore the roles of AAs on the function of other components of the intestinal defense system, such as tuft cells, enteroendocrine cells and intestinal innate lymphoid cells. Through modulation of the intestine immune and anti-inflammatory systems, AAs can control the progression of various intestinal diseases, such as intestinal infection and intestinal colitis. However, we eagerly await further investigations of the new roles of AAs in intestinal physiology and pathology, and more evidence about the benefits of manipulating AA metabolism for mitigating intestinal diseases. In practice, adequate intakes of dietary AAs, particularly functional AAs (Wu 2010), are crucial for maintaining the integrity and function of the intestine and the whole-body in humans and other animals.

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Conflict of Interest The authors declare that they have no conflict of interest.

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9 Maternal Nutrient Restriction and Skeletal Muscle Development: Consequences for Postnatal Health

Camila Sandoval, Guoyao Wu, Stephen B. Smith, Kathrin A. Dunlap, and M. Carey Satterfield

Abstract

Severe undernutrition and famine continue to be a worldwide concern, as cases have been increasing in the past 5 years, particularly in developing countries. The occurrence of nutrient restriction (NR) during pregnancy affects fetal growth, leading to small for gestational age (SGA) or intrauterine growth restricted (IUGR) offspring. During adulthood, SGA and IUGR offspring are at a higher risk for the development of metabolic syndrome. Skeletal muscle is particularly sensitive to prenatal NR. This tissue plays an essential role in oxidation and glucose metabolism because roughly 80% of insulin-mediated glucose uptake occurs in muscle, and it represents around 40% of body weight. Alterations in myofiber number, hypertrophy and myofiber type composition, decreased protein synthesis, lower mitochondrial content and activity of oxidative enzymes, and increased accumulation of intramuscular triglycerides are among the described programming effects of maternal NR on skeletal muscle. Together, these features would add to a phenotype that is prone to insulin resistance, type 2 diabetes, obesity, and metabolic syndrome. Insights from diverse animal models (i.e. ovine, swine, and rodent) have provided valuable information regarding the molecular mechanisms behind those altered developmental pathways. Understanding those molecular signatures supports the development of efficient treatments to counteract the effects of maternal NR on skeletal muscle, and its negative implications for postnatal health.

Keywords

Maternal nutrient restriction · SGA · Skeletal muscle · Metabolic syndrome

9.1 Introduction

Long-term maternal nutrient restriction (NR) during pregnancy impairs fetal growth, leading to intrauterine growth restriction (IUGR) or small for gestational age (SGA) offspring. In human medicine, intrauterine growth restriction (IUGR) has been defined as the offspring placed below the tenth percentile of fetal weight distribution at birth, and is typically associated to asymmetric growth (Goldenberg and Cliver 1997). In livestock species, maternal nutrient restriction is also a prevalent cause for IUGR, which have been defined as an impairment in gestational develop-

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ment of a fetus or its parts (Wu et al. 2006). A similar concept is small for gestational age (SGA) offspring, which is a broader classification and refers to fetuses that are smaller than expected for the species at a given gestational age (Goldenberg and Cliver 1997). IUGR or SGA offspring present a higher perinatal mortality and increased risk of metabolic syndrome during postnatal life (Barker et al. 1989).

Skeletal muscle, which represents about 40–45% of body weight in the young and adult, respectively (Wu 2018), is among the most sensitive tissues to maternal NR (Desai et al. 1996), and it plays an essential role in metabolic dysregulation due to its prominence in glucose and oxidative metabolism (Brown 2014). In addition, skeletal muscle is the major site for initiating the catabolism of branched-chain amino acids to synthesize glutamate, alanine and glutamine in mammals (Hou and Wu 2018; Wu 2013). Both alanine and glutamine participate in the interorgan metabolism of nitrogen and carbons. Particularly, alanine is a major glucogenic precursor in the liver, whereas glutamine is used by the small intestine of many mammals (including sheep, swine and humans) to synthesize citrulline (Wu and Morris Jr. 1998). The latter is either converted locally into arginine in enterocytes or taken up by extra-intestinal tissues and cells (e.g., kidneys, endothelial cells, and macrophages) to generate arginine, the nitrogenous precursor of nitric oxide (a major vasodilator, a neurotransmitter, a signaling molecule, and a killer of pathogens), creatine (crucial for energy metabolism), and polyamines (essential for DNA and protein syntheses) in animals (Dai et al. 2013; Wang et al. 2014; Wu et al. 2016, 2018). Therefore, skeletal muscle plays an important role in both growth and health of individuals.

Using animal models to understand the effects of maternal undernutrition in skeletal muscle growth and metabolism provides valuable information for translational research as well as agricultural performance. This chapter will discuss insights from the sheep, pig, and rodent models regarding the effect of maternal nutrient restriction (NR) on fetal skeletal muscle and its potential implications for postnatal health.

9.2 Maternal Undernutrition and SGA Offspring

Worldwide estimations indicate that around 821 million people are undernourished. Famine and undernutrition cases have continuously increased since 2014 and are a public health concern primarily in developing and low-income countries (FAO 2017) with the majority of cases occurring in Africa and Asia, followed by Latin America and The Caribbean (FAO 2017). The consequences of undernutrition are ample and include a higher predisposition for disease, and in extreme situations, death. This scenario becomes particularly challenging during pregnancy, when the female experiences a physiological increase in nutrient requirements to support herself as well as the needs of her developing fetus and placenta. Maternal undernutrition during pregnancy results in SGA offspring, with more than 20 million cases reported annually (UNICEF 2004).

Individuals born as IUGR or SGA are more prone to suffer perinatal mortality and experience increased risk for hypertension (Gennser et al. 1988), obesity (Fernandez-Twinn and Ozanne 2006), type 2 diabetes (Rich-Edwards et al. 1999), heart disease (Barker et al. 1989) and metabolic syndrome (McMillen and Robinson 2005). Epidemiological studies in the field of fetal programming have suggested the thrifty phenotype hypothesis (Hales and Barker 1992), which suggests that early life nutrient deficiency leads to a programming effect that would support immediate survival. However, in a postnatal scenario of normal or excessive nutrient availability, these adaptations would lead to type 2 diabetes, obesity, and other dysregulations associated with metabolic syndrome (Gluckman et al. 2005; Symonds et al. 2009; Hyatt et al. 2011).

The use of animal models for the study of maternal NR on programming of fetal growth and metabolism has provided supporting evidence for the initial epidemiological studies. A decrease in fetal weight has been a seminal finding of these studies (Osgerby et al. 2002; Kwon et al. 2004; Gao et al. 2008; Lassala et al. 2010; Satterfield et al. 2010). Results from our group show that impairment of fetal growth is corre-

lated to reduced concentration of polyamines and amino acids in maternal and fetal plasma, as well as fetal allantoic and amniotic fluids (Kwon et al. 2004). Lower plasma levels of insulin like growth factor 1 (IGF1) and insulin have also been reported in fetal plasma as a consequence of maternal NR (Osgerby et al. 2002), and both factors play an essential role in stimulation of fetal growth (Fowden et al. 1989; Baker et al. 1993).

Growth and metabolism in several fetal organs are affected by maternal NR (Osgerby et al. 2002; Vonnahme et al. 2003; Zhu et al. 2004, 2006; Costello et al. 2008; George et al. 2012; Lloyd et al. 2012; Satterfield et al. 2013; Shukla et al. 2014). Among them, skeletal muscle is particularly susceptible to maternal NR during fetal development because of nutrient prioritization to vital organs such as the brain (Desai et al. 1996)

9.3 Overview of Fetal Skeletal Muscle Development

Skeletal muscle development and growth during the fetal stage are accomplished by both cellular hyperplasia and fusion to originate myofibers (myogenesis), and hypertrophy, which continues postnatally. Myogenesis can be divided into primary and secondary myogenesis. During primary myogenesis, myoblasts fuse to form a primary myotube which will become a primary myofiber. A small percentage of myofibers are formed in this process, which starts during the first third of pregnancy (Maltin 2008). Secondary myogenesis occurs during the second third of pregnancy and accounts for the majority of myofiber formation (Maltin 2008).

Once the majority of myofibers are formed, fetal muscle growth continues through hypertrophy which starts around the second half of pregnancy and remains as an active process postnatally. In the sheep, it has been shown that myofiber area begins to increase around gestational day (GD) 85 (term ~147 days), which was caused by the addition of myonuclei between GD 85 and 100, while myoblast proliferation was completed by GD 100, and was followed by an increase in myofiber size, likely due to intracellular protein deposition (Wei et al. 2014). Since hypertrophy continues during postnatal life, prenatal alterations in myofiber size due to maternal NR have the potential to be compensated postnatally. However, persistent reductions in muscle mass and a tendency to increased adipose tissue have been demonstrated in adult sheep after prenatal NR (Ford et al. 2007), and similarly, decreased muscle mass persists until adulthood in low-birth-weight humans (Kensara et al. 2005).

Protein deposition is essential for muscle growth and hypertrophy (Yao et al. 2008) and is dependent upon an increase in the net balance of protein synthesis and protein degradation (Brown 2014). A central regulator of protein deposition is mechanistic target of rapamycin (MTOR), particularly MTOR complex 1, which is associated with regulatory associated protein of MTOR complex 1 (RPTOR) (Kim et al. 2002) and is activated by phosphorylation of its serine 2448 residue. Insulin, IGF1, AKT1, and amino acids (e.g., leucine, arginine, glutamine and glycine) have a stimulatory effect on MTOR complex 1 activity (Sun et al. 2016; Yao et al. 2008; Yoon 2017). In contrast, the activity of MTOR complex 1 is inhibited by glucocorticoids (Shimizu et al. 2011), protein kinase AMP-activated catalytic subunit alpha 2AMP-dependent kinase (PRKAA2) (aka AMPK), and myostatin (Rodriguez et al. 2014) (Fig. 9.1). As activation of MTOR is nutrient-dependent, severe prenatal undernutrition has the potential to decrease protein deposition in the fetus, and produce a reduction in lean mass content in the body.

9.4 Maternal Nutrient Restriction and Developmental Programming of Skeletal Muscle

9.4.1 Role of Skeletal Muscle in Whole-Body Metabolism

Skeletal muscle plays an essential role in locomotion and structural support, but it is also

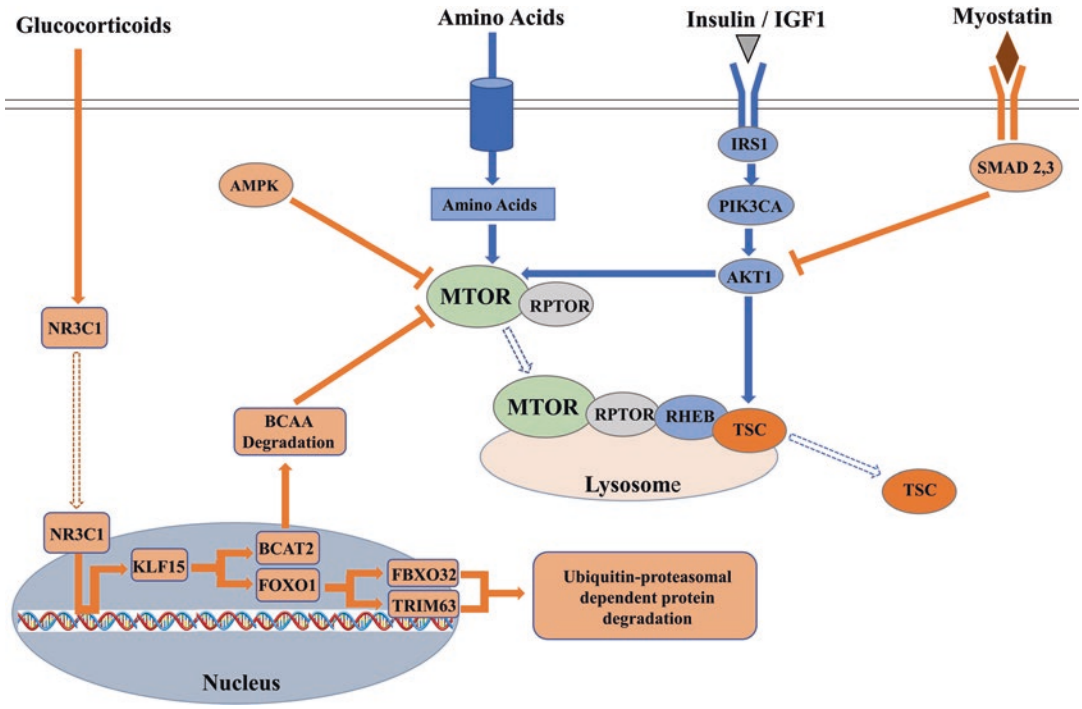

Fig. 9.1 Major regulatory pathways in skeletal muscle protein deposition. Protein deposition depends on the rate of protein synthesis and degradation. Pathways that stimulate protein synthesis in skeletal muscle are shown in blue. Amino acids (primarily the branched-chain amino acid leucine, and arginine) induce translocation of MTOR complex 1 to the lysosome, where the complex is activated by RHEB. TSC has inhibitory activity over RHEB. Insulin and IGF1 activate *AKT1* which activates RHEB by inducing its separation from the inhibitory factor TSC. Pathways that inhibit protein synthesis or stimulate protein degradation are shown in orange. Myostatin inhibits MTOR through inactivation of AKT1. Glucocorticoids bind to their receptor (NR3C1) to induce expression of KLF15. It is suggested that this decreases activation of MTOR through increased breakdown of branched-chain amino acids (BCAA) via BCAT2. KFL15 upregulation would also increase protein degradation through upregulation of the ubiquitin ligases FBXO32 and TRIM63. PRKAA2 (aka AMPK) also has an inhibitory effect on MTOR when AMP is increased in the cell (not shown) (Based on the data from Shimizu et al. 2011; Yoon 2017)

involved in several functions that regulate amino acid and energy metabolism. This tissue is highly abundant in free glutamine, glutamate, alanine, glycine, and taurine (Wu and Thompson 1990; Wu et al. 1991; Flynn and Wu 1996). Examples are, the capacity for oxidation of fatty acids, glucose, and some amino acids; storage of glycogen (Argilés et al. 2016), and support of gluconeogenesis in liver and kidney through the release of the amino acids, alanine and glutamine (Marliss et al. 1971; Garber and Missouri 1976). Skeletal muscle is essential in the regulation of glucose metabolism because about 80% of insulin-induced glucose uptake occurs in this tissue (Ferrannini et al. 1985; DeFronzo and Tripathy 2009). Skeletal muscle represents 45% of body mass in adult organisms (Janssen et al. 2000; Wu 2018), so any alteration in muscle mass or metabolism will significantly impact whole-body metabolism (Brown 2014). As example, it has been shown that insulin resistance at the skeletal muscle level is one of the primary metabolic alterations leading to type 2 diabetes in humans (DeFronzo and Tripathy 2009).

Solute carrier family 2 member 4 (SLC2A4) is the major glucose transporter in skeletal muscle, and its action is insulin-dependent. The abundance and activity of this transporter are essential for insulin-mediated glucose uptake (Scheepers et al. 2004). SLC2A4 proteins are stored in cytoplasmic vesicles and translocated to the plasma membrane by activation of the PI3K/AKT1 pathway after

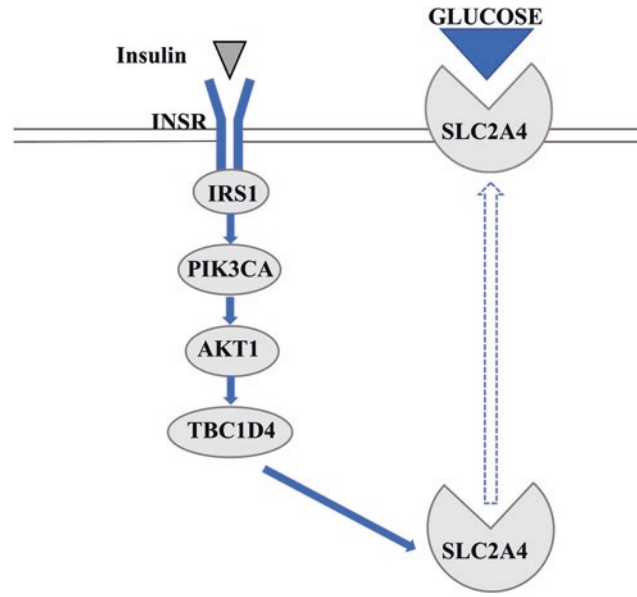

Fig. 9.2 Insulin-mediated SLC2A4 translocation. Around 80% of insulin-mediated glucose uptake occurs in skeletal muscle. Insulin binds to its receptor (INSR) to activate the downstream target *AKT1* which will activate TBC1D4 to induce SLC2A4 translocation from cytoplasmic vesicles to the sarcolemma. Because of a high level of homology, IGF1 can also activate this pathway through its receptor (IGF1R) or binding to INSR (not shown)

binding of insulin to insulin receptor (INSR) (Fig. 9.2) (Kohn et al. 1996; Taniguchi et al. 2006). IGF1 can also trigger the activation of this pathway by binding to its receptor or to INSR (Mora et al. 1995; Belfiore et al. 2009). Muscle contraction can also stimulate SLC2A4 translocation, which becomes relevant during postnatal life (Gao et al. 1994). Upregulation of SLC2A4 in skeletal muscle begins late in fetal life and continues postnatally when this glucose transporter reaches maximum functionality (Stuart et al. 2000).

Another factor that influences the metabolic characteristics of skeletal muscle is myofiber type composition, which impacts glucose metabolism and fatty acid oxidation (Mortensen et al. 2010). Type I myofibers are primarily oxidative and more sensitive to insulin than type II myofibers. Thus, its proportion shows a positive correlation with fatty acids and glucose oxidation, and with insulin-mediated glucose transport and whole-body insulin sensitivity (Lillioja et al. 1987; Fisher et al. 2017). Several studies have demonstrated that maternal NR impairs skeletal muscle growth, insulin sensitivity, and energetic metabolism (Table 9.1), which in addition to other systemic alterations, leads to a phenotype of increased risk for metabolic syndrome.

9.4.2 Prenatal Programming of Skeletal Muscle and Consequences for Postnatal Health

9.4.2.1 Ovine Model

Decreased number of secondary myofibers has been found in longissimus dorsi at GD 78 after 50% maternal NR between GD 28 and 78. Similarly, a peri-conception treatment of 50% NR applied from 18 days before ovulation to 6 days after ovulation found a tendency for decreased myofiber number in sheep semitendinosus muscle at GD 75 (Quigley et al. 2005). The study of Zhu et al. (2004) also found a decrease in myofiber area in longissimus dorsi of fetuses from NR dams which was associated with a reduction in MTOR and RPS6KB1 protein phosphorylation. Another model of 50% NR from GD 85 to 115 found a decrease in weight of longissimus dorsi in 14-day-old lambs (Fahey et al. 2005). Decreased muscle mass can be partially compensated postnatally as hypertrophy continues as an active process. However, having a lower number of myofibers limits a complete compensation because myofiber formation is not an active process under normal postnatal conditions.

Table 9.1 Summary of selected studies indicating the effect of maternal NR on fetal skeletal muscle features in different animal models

Model	Treatment	Gestational day	Effect	References
Ovine	50% NR	28 to 78	Reduced secondary myofiber number, smaller myofiber area, and downregulation in MTOR signaling at GD 78.	Zhu et al. (2004)
	50% NR	85 to 115	Reduced muscle mass in 14-day-old lambs.	Fahey et al. (2005)
		30 to 70	Increased type I myofiber content in 14-day-old- lambs	
	50% NR	28 to 78	Increased adipose tissue, tendency towards reduced muscle mass, hyperglycemia and reduced insulin secretion after GTT[a] in 280-day-old lambs.	Ford et al. (2007)
	50% NR	104 to 127	Upregulation of mRNA expression of *SLC2A4*, *INSR*, and *IGF1*, and reduced type I myofiber content at GD 127.	Costello et al. (2008)
	50% NR	28 to 78	Increased type IIb myofiber content, decreased activity of CPT1B, and increased IMTG[b] in 8-month-old lambs	Zhu et al. (2006)
Swine	6% crude protein	0 to Term	Decreased muscle mass, smaller myofiber area, upregulation in MSTN signaling, and downregulation in MTOR signaling in 35-day-old piglets.	Liu et al. (2015)
	Reduced digestible energy (11.24 MJ/kg)	0 to 90	Downregulation of mRNA expression of genes involved in mitochondrial signaling (*PPARGC1A*, *NRF1*, *TFAM*, *ATB5B*, *SIRT1*, and *CS*), and reduced mitochondrial DNA content at GD 90.	Zou et al. (2016)
	75% NR	0 to Term	Downregulation of mRNA expression of *SLC2A4,* and increased area under the curve in GTT in 6-week-old piglets.	Wang et al. (2016)
	Uterine crowding (naturally occurring NR)	0 to Term	Increased content of proteasome, a major system for protein degradation in skeletal muscle.	Wang et al. (2008)
Rat	50% protein-Isocaloric diet	0 to Term	Increased mRNA and protein levels of SLC2A4, and histone epigenetic modifications in SLC2A4 promotor zone in 38-day-old female offspring	Zheng et al. (2012)
	50% protein-Isocaloric diet	2 to Term	Upregulation in mRNA expression and protein content of C/EBPβ, and increase in histone acetylation at C/EBPβ promotor region in 38-day old female offspring	Zheng et al. (2011)
Mouse	50% NR	12.5 to 18.5	Reduced mitochondrial content, and resistance to weight loss in 14-week-old offspring.	Beauchamp et al. (2015)

[a]GTT = Glucose tolerance test
[b]IMTG = Intramuscular triglycerides

Impaired fetal growth after prenatal nutrient restriction is usually followed by compensatory growth during postnatal life (De Blasio et al. 2007). However, in the long term, this compensatory growth will favor adipose tissue deposition instead of muscle growth. For example, 280-day-old lambs born to dams that were subjected to 50% NR from GD 28 to 78 were heavier than controls, had increased renal and pelvic adipose tissue, and a tendency for decreased weight in longissimus dorsi and semitendinosus muscles. This study also found evidence of hyperglycemia and altered insulin secretion after a glucose tolerance test (Ford et al. 2007). Accordingly, 1-year-old offspring born to sheep under 50% NR from GD 110 to term, showed evidence of glucose intolerance as indicated by increased areas under the curve for glucose and insulin (Gardner et al. 2005).

Indicators of alterations in glucose and insulin metabolism have also been found in skeletal muscle at the fetal stage after maternal nutrient restriction. A sheep model of 50% NR from GD 104 to 127 produced upregulation of *SLC2A4*, *INSR*, and *IGF1* mRNA in fetal triceps brachii muscle at GD127 (Costello et al. 2008). These results suggest a metabolic programming effect that could be partly responsible for the compensatory growth that SGA animals experience early in postnatal life. The authors also suggested that an initial upregulation in insulin receptor may play a role in the development of metabolic diseases later in postnatal life (Costello et al. 2008).

Maternal nutrient restriction has also been shown to alter myofiber type composition in skeletal muscle. A 50% NR from GD 28 to GD 78 has been associated with increased content of type IIb myofibers in longissimus dorsi of 8-month-old lambs (Zhu et al. 2006). This study also found decreased activity of the enzyme carnitine palmitoyltransferase-1, which is involved in fatty acid oxidation, and accordingly, intramuscular triglyceride (IMTG) content was increased. These findings suggest a metabolic programming in skeletal muscle that would impair oxidative capacity and insulin sensitivity, as type II myofibers are primarily glycolytic and less insulin sensitive than type I myofibers (He et al. 2001). Accumulation of IMTG has also been recognized as a cause for disruption in insulin signaling, and insulin resistance in skeletal muscle (Corcoran et al. 2007).

Contradictory results have been found in the longissimus dorsi of 14-day-old lambs in which an increase in type I myofibers was found after 50% NR from GD 30 to 70 (Fahey et al. 2005). The difference in offspring age at which these two studies were conducted may be a cause for these conflicting results. However; the results of Zhu et al. (2006) are supported by the study of Costello et al. (2008), in which a sheep model of 50% NR from GD 104 to 127 was shown to reduce type I myofiber content in fetal triceps brachii muscle at GD127. Myofiber type composition conserves a certain level of plasticity during postnatal life in response to some stimuli such as exercise. Thus, more research is needed to confirm the long-lasting effect of myofiber type programming during fetal development (Brown 2014).

Findings from our group showed that administration of arginine to 50% NR ewes was successful in increasing fetal weight (Lassala et al. 2010). Similarly, administration of sildenafil citrate from GD 28 to 115 to ewes under 50% NR was effective in increasing fetal weight and total amino acids and polyamines in amniotic and allantoic fluids, and fetal serum (Satterfield et al. 2010). Amino acids are building blocks for protein synthesis (Wu 2013), and particularly leucine and arginine stimulate MTOR activity (Yao et al. 2008; Davis et al. 2010), enhancing protein deposition and skeletal muscle growth. Arginine also stimulates myoblast proliferation (Kalbe et al. 2013) and fusion (Long et al. 2006), likely supporting myofiber formation. These results represent potential treatments to mitigate the effects of maternal NR on skeletal muscle in the sheep model (Fig. 9.3).

9.4.2.2 Swine Model

A model of restricted protein (6% dietary crude protein) throughout pregnancy led to decreased muscle mass and myofiber area, upregulated MSTN signaling, and downregulated MTOR signaling in longissimus dorsi muscle of 35-day-old piglets (Liu et al. 2015). Moreover, a model of maternal low-energy diet (11.24 MJ/Kg of digestible energy) from mating to GD 90 was found to downregulate mRNA expression of *PPARGC1A*, *NRF1*, *TFAM*, *ATB5B*, *SIRT1,* and *CS*, which are regulators of mitochondrial biogenesis (Zou et al. 2016). Mitochondrial DNA content was also reduced, indicating a negative programming in oxidative capacity of skeletal muscle after prenatal NR (Zou et al. 2016). Decreased oxidative capacity in skeletal muscle may lead to IMTG accumulation, which is associated with the onset of insulin resistance (Corcoran et al. 2007). Accordingly, a model of 75% NR throughout pregnancy, followed by a postnatal cafeteria feeding up to 6 weeks, resulted in an impaired ability to clear blood glucose and was associated with a downregulation of *SLC2A4* mRNA in skeletal muscle (Wang et al. 2016).

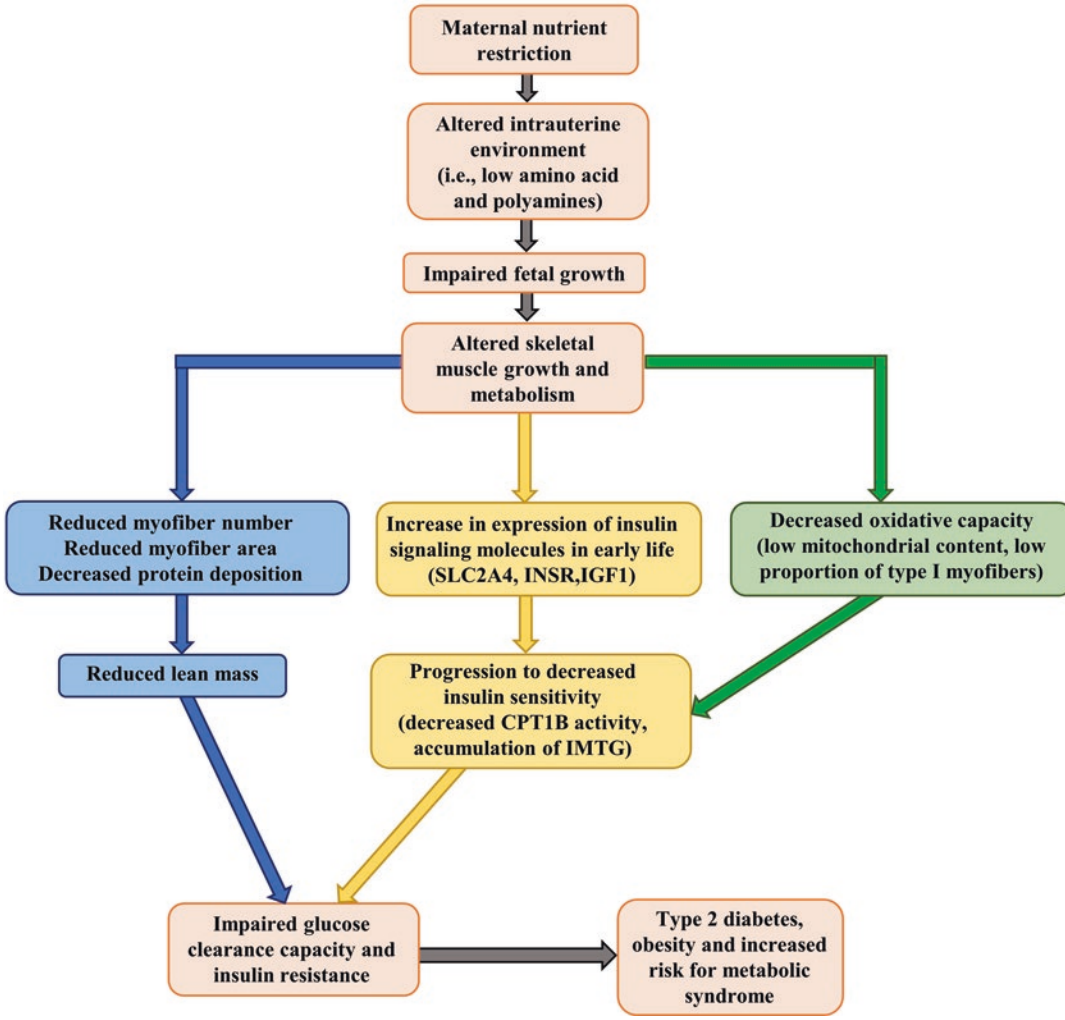

Fig. 9.3 Suggested model for the effects of maternal NR on fetal skeletal muscle growth and metabolism and consequences for postnatal health. Three major pathways of programming have been described in studies from ovine, swine, and rodent models. They are decreased muscle mass (blue), decreased oxidative capacity (green), and accumulation of IMTG and decreased insulin sensitivity (yellow). The additive effect of those programming trajectories would lead to impaired glucose clearance capacity, insulin resistance at the skeletal muscle level, and progression to type 2 diabetes, obesity, and metabolic syndrome

In addition to dietary NR, the pig model presents naturally occurring IUGR fetuses which suffer nutrient restriction because of uterine crowding (Wu et al. 2006). Using this model, the skeletal muscle proteome of IUGR piglets showed higher content of proteasome, a major system involved in protein degradation in skeletal muscle, indicating an upregulation of ubiquitin-dependent protein degradation in these animals (Wang et al. 2008). Enhanced protein degradation would lead to decreased muscle mass, which, in addition to impaired oxidative metabolism, may enhance the postnatal risk of metabolic syndrome. Through genetic and epigenetic changes, underdevelopment of fetal skeletal muscle has negative impacts on the postnatal growth and health of offspring (Ji et al. 2016, 2017).

Emerging evidence shows that glycine enhances MTOR activity and inhibits expression of genes involved in ubiquitin-dependent protein

degradation (*FBXO32* and *TRIM63*) in C2C12 myoblasts (Sun et al. 2016). Because the content of glycine is low in all plant-source foods (Hou et al. 2019), endogenous synthesis from amino acids or dietary provision of glycine plays an important role in stimulating muscle protein synthesis and animal growth (Li and Wu 2018, 2020; Wu et al. 2019). Leucine supplementation in neonatal pigs receiving a low-protein diet has been effective to enhance protein synthesis in longissimus dorsi muscle (Yin et al. 2010). Arginine supplementation in neonatal pigs was effective in increasing MTOR signaling and protein synthesis in skeletal muscle (Yao et al. 2008). Also, arginine supplementation in adult pigs had a beneficial effect on metabolic profiles in skeletal muscle and adipose tissue (Tan et al. 2011). These insights from the pig model are promising treatment alternatives to enhance muscle growth and metabolic profiles. However, their efficiency in a prenatal NR context remain to be determined.

9.4.2.3 Rodent Models

A rat model of 50% protein restriction and isocaloric diet throughout pregnancy showed increased mRNA and protein levels of SLC2A4 within gastrocnemius muscle in 38-day-old female offspring. Histone epigenetic modifications in the promoter region of *SLC2A4* were also found in female offspring from this study (Zheng et al. 2012). An early-life upregulation in insulin-responsive molecules has been suggested to happen before progression to metabolic diseases in postnatal life (Costello et al. 2008; Muhlhausler et al. 2009), and the discussed results indicate a potential sex-specific programming in glucose metabolism in skeletal muscle.

An upregulation in mRNA expression and protein content of *Cebpb* was found in gastrocnemius muscle of 38-day-old female offspring using a rat model of 50% protein restriction and isocaloric diet from GD 2 to term. An increase in histone acetylation was found at the promoter region of *Cebpb* in those females (Zheng et al. 2011). *Cebpb* is a transcription factor involved in the regulation of genes related to energy homeostasis, and one of its effects is the stimulation of adipogenesis by induction of fibroblast differentiation to adipocytes. These results support a programming effect towards increased intramuscular fat accumulation, which is correlated with insulin resistance and type 2 diabetes.

A mouse model of 50% NR from GD 12.5 to 18.5 reduced the mitochondrial content in tibialis anterior muscle and increased the levels of carcass adiposity in 14-week-old offspring (Beauchamp et al. 2015). This study also showed resistance to weight loss in offspring from NR dams, as these animals lost 50% of weight compared to control after a 40% caloric restriction from postnatal week 10−14. A decreased oxidative capacity and resistance to lose weight would further enhance the risk of metabolic disease. Interestingly, dietary supplementation with watermelon juice, which is a source of citrulline, increases arginine availability and reduced adipose tissue accretion, serum glucose concentrations and free fatty acids in a rat model of non-insulin dependent diabetes (Wu et al. 2007). Additionally, leucine supplementation to diet-induced obese mice was successful in activating genes involved in mitochondrial biogenesis and preventing mitochondrial dysfunction (Li et al. 2012). These results represent potential treatments to counteract the enhanced risk for metabolic disease once a stage of disease is already present. However, their effectiveness in individuals that have experienced maternal NR remains to be tested.

9.5 Concluding Remarks

Several studies have demonstrated that maternal nutrient restriction is a cause for SGA or IUGR offspring, which was epidemiologically correlated with increased risk of metabolic syndrome. Insights from diverse animal models have provided the molecular basis for the developmental trajectories that are induced by nutrient scarcity and lead to postnatal metabolic dysregulation. Seminal results collected from ovine, swine, and rodent models indicate that muscle mass, oxidative capacity, and insulin sensitivity are the major features affected by prenatal NR. These would

contribute to the onset of insulin resistance at the skeletal muscle level, with progression to a whole-body effect and type 2 diabetes, and obesity. Current data provide promising treatment alternatives to counteract these effects and minimize the negative consequences of prenatal NR in postnatal health. However, the specific windows for intervention, and conclusive results from NR models are still needed.

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10 Metabolism of Amino Acids in the Brain and Their Roles in Regulating Food Intake

Wenliang He and Guoyao Wu

Abstract

Amino acids (AAs) and their metabolites play an important role in neurological health and function. They are not only the building blocks of protein but are also neurotransmitters. In the brain, glutamate and aspartate are the major excitatory neurotransmitters, whereas γ-aminobutyrate (GABA, a metabolite of glutamate) and glycine are the major inhibitory neurotransmitters. Nitric oxide (NO, a metabolite of arginine), H_2S (a metabolite of cysteine), serotonin (a metabolite of tryptophan) and histamine (a metabolite of histidine), as well as dopamine and norepinephrine (metabolites of tyrosine) are neurotransmitters to modulate synaptic plasticity, neuronal activity, learning, motor control, motivational behavior, emotion, and executive function. Concentrations of glutamine (a precursor of glutamate and aspartate), branched-chain AAs (precursors of glutamate, glutamine and aspartate), L-serine (a precursor of glycine and D-serine), methionine and phenylalanine in plasma are capable of affecting neurotransmission through the syntheses of glutamate, aspartate, and glycine, as well as the competitive transport of tryptophan and tyrosine across from the blood-brain barrier. Adequate consumption of AAs is crucial to maintain their concentrations and the production of neurotransmitters in the central nervous system. Thus, the content and balance of AAs in diets have a profound impact on food intake by animals. Knowledge of AA transport and metabolism in the brain is beneficial for improving the health and well-being of humans and animals.

Keywords

Amino acids · Center nervous system · Neurotransmission · Brain · Food intake

10.1 Introduction

The brain, which is contained within the head and protected by its skull bones, is a highly complex organ and is the central commander of the body (Hellier 2014). Amino acids (AAs) are selectively transported from the blood into the brain, where they undergo active metabolism in the brain to maintain its normal structure and function. As shown in Table 10.1, neurotransmitters (chemical messengers) in the central nervous system (CNS) are AAs, low-molecular-weight metabolites of AAs, oligopeptides of AAs, or other nitrogenous metabolites (Dingledine and

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Table 10.1 Neurotransmitters or neuromodulators in the central nervous system of animals

Neurotransmitter	Precursor(s)	Function
Acetylcholine	Choline and acetyl-CoA	Neurotransmitters and neuromodulators in the brain; modulation of arousal, attention, memory and motivation
γ-Aminobutyrate	Glutamate (ultimately BCAAs)	The principal inhibitory neurotransmitter in the brain; a major inhibitory neurotransmitter in the spinal cord (50% sharing with glycine); regulation of food intake
Aspartate	Glutamine, glutamate, BCAAs	A major excitatory neurotransmitter in the brain
Carbon monoxide (CO)	Heme (ultimately glycine)	A neurotransmitter and a neuromodulator in the brain; modulation of synaptic plasticity and neuronal activity; modulation of LTP
Dopamine	Tyrosine	A neurotransmitter in the brain; modulation of learning, motor control, reward, emotion, and executive function; inhibition of food intake
Epinephrine	Tyrosine	A neurotransmitter in the brain; enhancer of memory formation processes
Glutamate	Glutamine, BCAAs, and	The primary excitatory neurotransmitter in the brain; primary mediator of possibly ammonia plus α-KG neuron system plasticity
Glycine	Serine, 4-hydroxyproline	A major inhibitory neurotransmitter in the spinal cord & lower brainstem; a co-agonist with glutamate at NMDA receptors
Hydrogen sulfide	Cysteine	A neurotransmitter and a neuromodulator in the brain; modulation of (H_2S) synaptic plasticity and neuronal activity; facilitating the induction of hippocampal LTP; potentiating the activity of NMDA receptors
Histamine	Histidine	A neurotransmitter in the brain and spinal cord; inhibition of food intake; promotion of wakefulness; control of motivational behavior
Nitric oxide	Arginine	A neurotransmitter and a neuromodulator in the brain; modulation of (NO) synaptic plasticity and neuronal activity; inhibiting the activity of NMDA receptors at physiological levels
Non-opioid NPs[a]	Amino acids	Neurotransmitters and neuromodulators in the brain; modulation of analgesia, hypothermia, and locomotion; involved in regulation of dopamine pathways
Norepinephrine	Tyrosine	A neurotransmitter in the brain; modulation of emotion, sleep, attention, focus and learning in the brain; modulation of response to the ANS
Opioid peptides[b]	Amino acids	Neurotransmitters and neuromodulators in the brain and spinal cord; modulation of pain perception, analgesia, and euphoria; modulation of the actions of other neurotransmitters
Serotonin	Tryptophan	A neurotransmitter in the brain and gastrointestine; modulation of neuropsychological processes and neural activity; in the brain, inhibition of food intake; in the small intestine, endocrine cells synthesize and release serotonin to stimulate gastrointestinal motility and food intake

ANS autonomic nervous system, *α-KG* α-ketoglutarate, *LTP* long-term potentiation, *NMDA* N-methyl-D-aspartate receptor (a glutamate receptor and an ion channel protein present in *nerve cells*), *NPs* neurotransmitter peptides

[a]Including (1) neurotensin (a 13 amino acid neuropeptide that plays a role in regulating the release of luteinizing hormone and prolactin from the anterior pituitary gland and has a significant interaction with the dopaminergic system in the brain; and (2) cholecystokinin (CCK) that is produced by endocrine cells of the small intestine and acts as a neurotransmitter and a neuromodulator in the gut and brain. CCK is composed of different numbers of amino acid residues (e.g., CCK58, CCK33, CCK22 and CCK8), depending on the post-translational modification of its 150-amino acid precursor, preprocholecystokinin

[b]Methionine-enkephalin (a pentapeptide), leucine-enkephalin (a pentapeptide), and related neuropeptides (e.g., endorphins) are generated from the degradation of proteins, and serve as endogenous opioid neurotransmitters. Opiates (e.g., morphine; naturally present) and opioids (e.g., heroin; synthetic substances) mimic the effects of the neuropeptides

McBain 1999; Smith 2000). For example, γ-aminobutyrate (GABA) is synthesized from glutamate, nitric oxide (NO) from arginine, serotonin from tryptophan, histamine from histidine, and norepinephrine from tyrosine (Wu 2013). The availability of AAs in plasma can affect the

uptake of neutral and basic AAs by the brain, as well as their concentrations and, therefore, the generation of neurotransmitters in the CNS. These processes are also influenced by the integrity of the blood-brain barrier (BBB) and complex interactions among AAs (Fernstrom 2013; Tran et al. 2019). Thus, the dietary content and balance of AAs have a profound impact on the nutrition, growth, development, and health of humans and animals. This article highlights the metabolism of AAs in the brain and their roles in regulating food intake of mammals.

10.2 Anatomy of the Brain

The brain consists of three specialized parts: the cerebrum (the largest part), the brainstem, and the cerebellum (Hellier 2014). The cerebrum is divided into two broadly similar cerebral hemispheres that are connected via commissural nerve tracts, with the cerebral cortex (an outer layer of grey matter) covering the core of white matter. Each hemisphere is divided into four lobes (frontal, temporal, parietal, and occipital) with different functions, with the frontal lobe for thought, the temporal lobe for auditory and visual memories as well as language, the parietal lobe for sensory information (including spatial sense and navigation), and the occipital lobe for vision. The brainstem (a stalk) connects: (1) the cerebrum to the spinal cord, and (2) the cerebellum by pairs of tracts. The cerebellum, which is smaller than the cerebrum in humans and many other animals, plays an important role in motor control. The cerebrum, brainstem, cerebellum, and spinal cord are covered for protection by a layer of three membranes (meninges): the dura mater, the arachnoid mater, and the pia mater (Dasgupta and Jeong 2019). Cerebrospinal fluid is located between the arachnoid mater and the pia mater.

Neurons (nerve cells) and glial cells (also known as glia or neuroglia) are the major cell types in the brain (Hellier 2014). The adult human brain is estimated to contain 86 billion neurons [16 billion (19%) in the cerebral cortex and 69 billion (80%) in the cerebellum] and an approximately equal number (85 billion) of glial cells (Azevedo et al. 2009; von Bartheld et al. 2016). The neuron has a cell body (containing all the components of the animal cell), dendrites (thin, branching structures from the cell body), and an axon (also known as a nerve fiber that projects usually with numerous branches). A myelinated axon is wrapped in an insulating sheath of myelin (a mixture of proteins and phospholipids), which serves to greatly increase the speed of signal propagation. The junction between two neurons is called a synapse. The types of neuron include: (1) sensory neurons in the sense organs; (2) motor neurons, which are efferent *neurons* that originate in the spinal cord and form synapses with skeletal muscle to control muscle contraction; and (3) inter-neurons that connect neurons within the central nervous system (Finlay and Darlington 1995). Neurons play an important role in neurotransmission.

There are four major types of glial cells in the CNS: astrocytes, oligodendrocytes, microglia, and ependymal cells (Verkhratsky et al. 2019). Astrocytes connect neurons to the CNS vasculature through the extended astrocyte end feet that attach to the basement membrane surrounding the endothelial cells and pericytes. The latter function as phagocytes. In humans, a single astrocyte can interact with up to 2 million neurons at a time (Hellier 2014). Although glial cells do not participate in neurotransmission, together they function to: (1) maintain the composition of the specialized extracellular environment surrounding the neurons within narrow limits that are optimal for normal neuronal function; (2) form myelin; (3) modulate (depress or enhance) synaptic function; and (4) support and protect neurons both physically and metabolically. For example, the astrocyte, which is most abundant among the glial cells, acts to (1) hold the neurons together; (2) help to form the blood-brain barrier; (3) transfer nutrients from blood to neurons; (4) take up and degrade locally released neurotransmitters; and (5) enhance synapse formation (Trujillo-Estrada et al. 2019).

The BBB separates the circulating blood from the brains, consists of endothelial cells of the capillary wall, the astrocyte end-feet, and pericytes (Daneman and Prat 2015). The endothelial

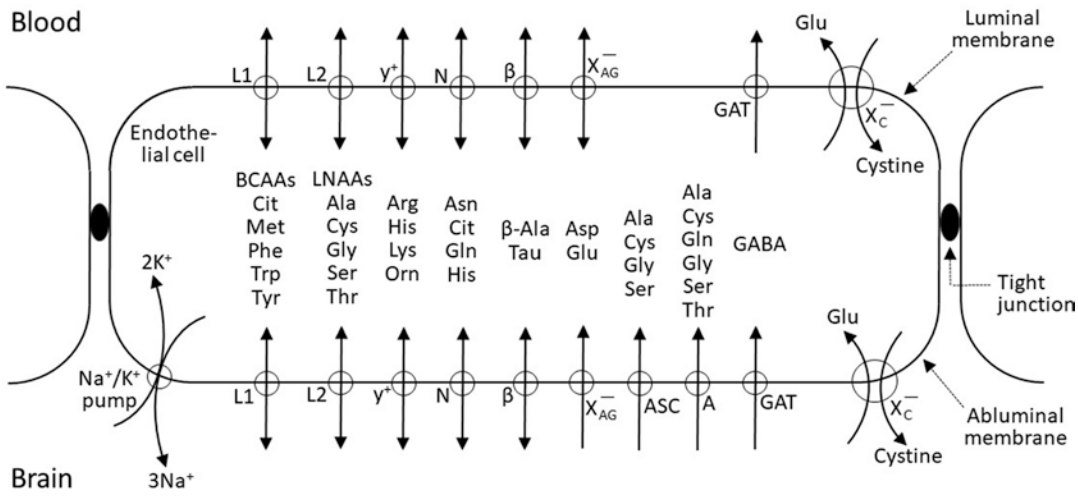

Fig. 10.1 Transport of amino acids (AAs) across the blood-brain barrier (BBB). The BBB consists of two polarized membranes: luminal membrane (blood side) and abluminal membrane (brain side). Nutrients in the blood must cross both membranes to enter the brain. Three classes of Na^+-independent facilitative AA transporters for large neutral amino acids (L1), cationic AAs (y^+), and acidic AAs (x_G^-), as well as one Na^+-dependent AA transport for neutral AAs with a side-chain NH_2 group (N) exist on the luminal membrane. L1 and y^+ are present in both membranes. In contrast, the *ASC* and imino systems are absent from the luminal membrane of the endothelial cells in the BBB. Thus, large neutral AAs (e.g., branched-chain AAs, phenylalanine, tyrosine, and tryptophan) and basic AAs (e.g., arginine, lysine, histidine, and ornithine) in the blood readily cross the BBB into the brain. Small neutral AAs (e.g., alanine, glycine, serine, and cysteine) in the blood readily cross the BBB into the brain, but the BBB restricts the entry of physiological concentrations of proline and hydroxyproline from the blood into the brain. The abluminal membrane of the endothelial cells in the BBB contain Na^+-dependent AA transport systems ASC (for small AAs), A (for alanine, serine, glycine, cysteine, threonine and proline), N (for glutamine, citrulline, asparagine and histidine), and EAAT (for acidic AAs and cysteine) for effluxes from the brain into the blood

cell has two polarized membranes: luminal membrane (facing the blood) and abluminal membrane (facing the brain; Fig. 10.1). Nutrients in the blood must cross both membranes to enter the brain. Except for branched-chain AAs (BCAAs), methionine, phenylalanine, and tryptophan, the concentrations of AAs in the brain are generally much greater than those in plasma (Jobgen et al. 2009; Sase et al. 2013; Ajinkya et al. 2016). The BBB allows for the entry of basic AAs (e.g., arginine, lysine, histidine and ornithine), large neutral AAs (e.g., BCAAs, citrulline, tryptophan, tyrosine, phenylalanine, and methionine), AAs with a side-chain NH_2 group (e.g., glutamine and asparagine), and small neutral AAs (alanine, serine, glycine, threonine, cysteine, and β-alanine) from the blood into the brain at different rates, and vice versa (Boado et al. 1999; Bagga et al. 2014; Barar et al. 2016). In contrast, the BBB restricts the entry of physiological concentrations of both acidic AAs (glutamate and aspartate) and GABA from the blood into the brain (Dingledine and McBain 1999; Smith 2000; Hawkins et al. 2006). Interestingly, proline undergoes efflux from the brain into the blood via SNAT2 (the transporter system A for small neutral AAs) in the abluminal membrane and a yet unidentified transporter in the luminal membrane of the endothelial cell in the BBB, but influx of physiological concentrations of proline from the blood into the brain is limited (Benrabh and Lefauconnier 1996; Takanaga et al. 2002; Langen et al. 2005). In addition, glucose, lactate, pyruvate are readily transported from the blood into the brain via specific transporters, whereas the BBB does not allow for the passage of long-chain saturated fatty acids from the blood into the brain (Kubo et al. 2015; Wu 2018). Of note, a small amount of

long-chain unsaturated fatty acids in the blood can cross the BBB into the brain, but gaseous molecules (e.g., O_2 and CO_2) rapidly diffuse through the BBB (Wu 2018). This indicates that the movement of substances (including AAs) between the blood and the brain is strictly regulated to ensure proper neuronal function and optimal health.

10.3 Neurotransmission in the Brain

Neurons connect to form neural pathways (circuits) and communicate via neurotransmitters (Table 10.1). When a neuron generates an electrical signal (also called an action potential) that travels along its axon to reach a synapse, the action potential causes the release of a neurotransmitter into the cleft of the synapse for uptake by target cells (Boto and Tomchik 2019). The neurotransmitter binds to the membrane receptor of the target cells to alter their electrical activity. An excitatory or inhibitory neurotransmitter affects trans-membrane ion flow to increase or decrease the action potentials of target cells, respectively. The target cells are nearby neurons, or other cell types (e.g., skeletal muscle, heart, and smooth muscle). For example, when an excitatory neurotransmitter is released by a neuron, the chemical molecule generates an action potential in the receiving neuron to exert an excitatory effect. Conversely, an inhibitory neurotransmitter released by a neuron reduces the action potential of the nearby receiving neuron below the threshold potential, such that the receiving neuron will not be excited. Most neurotransmitters are inactivated by high-affinity uptake into nerve terminals (Snyder 2017). Abnormalities of neurotransmission negatively affect mood (e.g., anxiety, depression), behavior, reasoning, locomotion, food intake, and sleep cycle, while increasing risks for Alzheimer's disease, schizophrenia, Parkinson's disease, Huntington's disease, and epilepsy (Fernando-Valenzuela et al. 2011). The following sections highlight AA transmitters as well as their metabolism and function in the brain.

10.4 Glutamate, Glutamine, GABA, and Aspartate

Glutamate is the principal excitatory neurotransmitter in the brain (Raevskii 1986). The excitatory actions of glutamate are terminated through its removal from the synaptic cleft by neuronal presynaptic and astrocyte reuptake systems. Thus, normal brain functions depend on the cooperation of different cell types to provide neurons with sufficient glutamate. As shown in Table 10.2, the concentration of glutamate in the brain is particularly high relative to its concentrations in plasma. Although the glutamate released from synaptic vesicles passes through the synaptic cleft to postsynaptic neurons, the glutamate

Table 10.2 Concentrations of free amino acids and glutathione in the brain and plasma of rats

Amino acid	Brain[a]	Plasma[b]
Alanine	1.00–1.35	0.37–0.45
β-Alanine	0.07–0.09	0.009–0.013
γ-Aminobutyrate	3.50–4.06	0.002–0.0025
Arginine	1.09–1.32	0.16–0.22
Asparagine	0.22–0.25	0.05–0.06
Aspartate	3.50–4.46	0.02–0.03
Citrulline	0.11–0.12	0.06–0.08
Cys + Cystine	0.33–0.41	0.15–0.22
Glutamate	9.41–14.3	0.06–0.09
Glutamine	5.64–6.82	0.53–0.66
Glycine	1.41–2.03	0.21–0.35
Histidine	1.30–1.97	0.05–0.08
Isoleucine	0.07–0.09	0.06–0.08
Leucine	0.14–0.22	0.13–0.16
Lysine	0.83–1.30	0.28–0.31
Methionine	0.06–0.09	0.03–0.06
Ornithine	0.13–0.15	0.03–0.06
Phenylalanine	0.08–0.09	0.05–0.08
Proline	0.84–0.90	0.31–0.39
Serine	1.23–1.42	0.24–0.31
Taurine	7.46–8.97	0.37–0.43
Threonine	0.57–0.73	0.19–0.25
Tyrosine	0.30–0.50	0.06–0.09
Tryptophan	0.06–0.08	0.07–0.09
Valine	0.14–0.19	0.12–0.16
Glutathione	3.20–4.25	0.004–0.005

Data are expressed as mM

[a]Adapted from Sase et al. (2013, 2016). Values were calculated on the basis of water content (70%) in the brain

[b]Adapted from Jobgen et al. (2009)

pools in the presynaptic and postsynaptic neurons remain constant in healthy individuals (Boldyrev 2000; Snyder 2017). This can be explained by active glutamate synthesis in the neurons and the intercellular glutamine-glutamate cycle between the neurons and astrocytes (Dingledine and McBain 1999; Hutson et al. 2001). In this glutamine-glutamate cycle (Fig. 10.2), neurons take up extracellular glutamine and hydrolyze it into glutamate by phosphate-activated glutaminase and then release the glutamate (Lee et al. 1998; Hediger and Welbourne 1999; Senkowska and Ossowska 2003), whereas astrocytes take up the glutamate and convert it into glutamine by glutamine synthetase (Norenberg and Martinez-Hernandez 1979; Storm-Mathisen et al. 1986). The glutamine is released from astrocytes into the interstitium to be taken up by the neurons. The sources of the extracellular glutamine for uptake by the neurons are: (1) the glutamine in the blood that crosses the BBB into the brain via the Na^+-dependent AA transport system N (Chaudhry et al. 1999); and (2) the glutamine synthesized by astrocytes. (Holten and Gundersen 2008). Due to its excitatory property, excessive glutamate in postsynaptic neurons is excitotoxic, resulting in brain injury, hypoxia, and epilepsy (Scheppach et al. 1991; Olney 2003; Waxman and Lynch 2005). Thus, glutamate readily exits the brain into the blood through the X_{AG}^- transporter and the Xc^- exchange (Hosoya et al. 1999).

In the brain, glutamate decarboxylase (GDC) catalyzes the decarboxylation of glutamate into GABA. This enzyme is localized in the grey matter of the CNS (specifically synaptosomes), and is inhibited by aspartate (Porter and Martin 1987). GABA is the primary inhibitory neurotransmitter in the brain (Porter and Martin 1984; Waagepetersen et al. 1999). In response to plasma membrane depolarization, the nerve terminals release GABA into the synaptic cleft, followed by its binding to GABA receptors on the post-synaptic cell membranes. The effect of GABA is terminated by its re-uptake by presynaptic neurons or nearby glial cells via specific and high-affinity transporters (Schousboe 2000). The enzyme for initiating the catabolism of GABA into succinate semialdehyde is GABA transaminase, which is present in the mitochon-

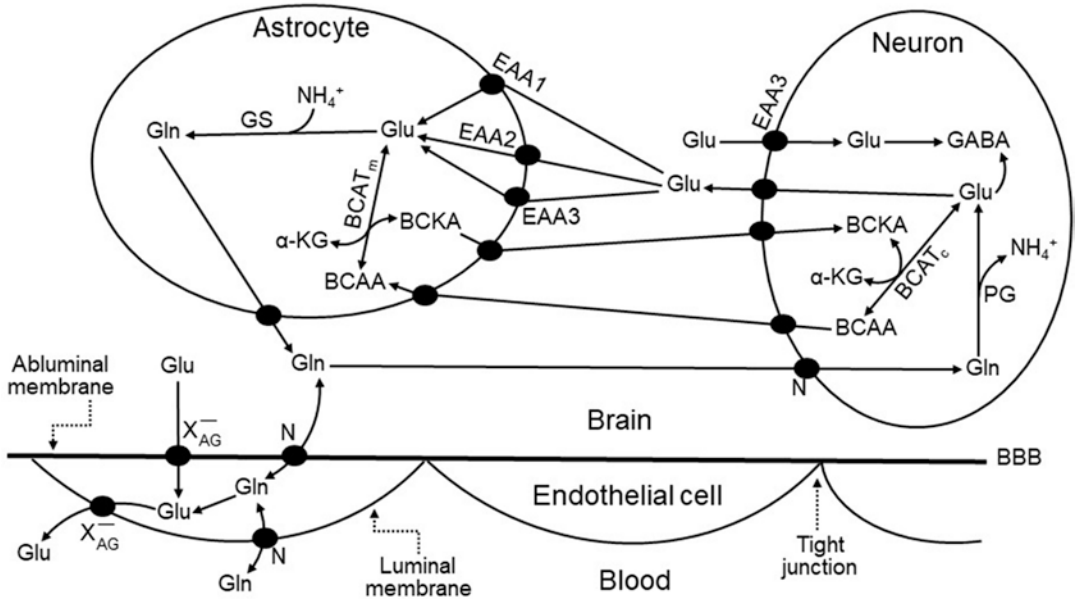

Fig. 10.2 Metabolism of glutamate and glutamine in the astrocytes and neurons of the central nervous system. Astrocytes are a sub-type of glial cells. *BBB* blood = brain barrier, *BCATc* branched-chain amino acid transaminase (cytosolic isoform), *BCATm* branched-chain amino acid transaminase (mitochondrial isoform), *BCKA* branched-chain α-keto acid, *BCKD* branched-chain α-keto acid dehydrogenase complex, *PAG* phosphate-activated glutaminase, *GS* glutamine synthetase, *α-KG* α-ketoglutarate

dria of grey matter in the brain and in other tissues (Sherif and Oreland 1992). Succinate semialdehyde is converted into succinate by succinate semialdehyde dehydrogenase, which is distributed in the CNS in a similar manner to that of GABA transaminase. The decarboxylation of glutamate to GABA, the transamination of GABA, and the dehydrogenation of succinate semialdehyde are collectively called the GABA shunt. As the precursors of GABA, the concentrations of glutamate and glutamine in the brain influence those of GABA in the brain. Because of its active synthesis, high concentrations of GABA are present in the brain of mammals although its concentrations in the plasma are exceedingly low (Table 10.2). As noted previously, in healthy humans and other animals, the BBB is impermeable to not only glutamate and aspartate, but also GABA in the blood and the endogenous cerebral GABA (Kuriyama and Sze 1971). However, GABA readily undergoes efflux from the brain to the blood to regulate its concentration in the CNS (Kakee et al. 2001).

Because of its active synthesis, aspartate (an excitatory neurotransmitter) is present at a high concentration in the brain relative to its concentration in plasma (Table 10.2). This is consistent with the finding that 90% of glutamate in the mitochondria of the brain undergoes transamination to generate aspartate (Banay-Schwartz et al. 1996; Holten and Gundersen 2008). The catabolism of aspartate is initiated by aspartate aminotransferase (Yoneda and Byori 2001). This enzyme is localized in the mitochondria and cytoplasm of neurons as distinct isoforms. The mitochondrial form of aspartate transaminase participates in the Krebs cycle, whereas the cytoplasmic form of the enzyme may play a regulatory role in glutamate and alanine syntheses (Yudkoff 1997). Because of its excitatory effect, excessive aspartate in postsynaptic neurons results in brain injury, hypoxia, and epilepsy (Waxman and Lynch 2005). Thus, aspartate readily exits the brain into the blood through the X_{AG}^- transporter in the luminal and abluminal membranes of the endothelial cell (Hosoya et al. 1999).

Dietary glutamate and aspartate are extensively catabolized by enterocytes of the small intestine in humans (Wu 1998) and animals, including fish (Jia et al. 2017; Li et al. 2020), swine (Hou and Wu 2018) and poultry (He et al. 2018). Therefore, only a small amount of the dietary glutamate and aspartate (e.g., about 5% in swine) enter the portal circulation. Thus, although plant- and animal-source proteins contain large amounts of both glutamate and aspartate (Li and Wu 2020; Wu et al. 2016; Hou et al. 2019), the concentrations of these two AAs in the plasma and brain of humans and animals are not changed after a meal or by their dietary supplementation within physiological levels (Fernstrom 1994; Rezaei et al. 2013). These findings are not consistent with a common misconception that ingestion of a small amount of monosodium glutamate as a flavor or aspartame (which contains aspartate) as a sweeter increases the concentrations of acidic AAs in the CNS, affects neurological function, and even causes brain damage in humans. Glutamate is classified as GRAS in the USA with an LD_{50} of 15.8 g/kg in the rat (Ataseven et al. 2016). Swine can tolerate oral administration of 2 g supplemental glutamic acid or 1.46 g supplemental monosodium glutamate per kg BW per day without adverse effects (Hou and Wu 2018). Due to a lack of experimental data, it is not possible to set an upper limit for dietary glutamate intake or supplementation for a NOAEL (no observed adverse effects level) in humans.

Ketogenic diets enhance the hepatic production of ketone bodies and have an antiepileptic effect (Wilder 1921; Wilkins 1937; Yudkoff et al. 2001; Freeman et al. 2006; Clanton et al. 2017). Ketone bodies replace some glucose as metabolic fuels in the brain (Yudkoff et al. 2001) and affect nitrogen metabolism in the whole body, including (1) the reduction of alanine and aspartate syntheses through an inhibition of glycolysis, (2) the stimulation of glutamine release by skeletal muscle (Thompson and Wu 1991), and (3) enhanced GABA generation in the brain (Erecinska et al. 1996). Addition of either acetoacetate or β-hydroxybutyrate to incubation medium increased glutamate concentration, as well as the

formation of GABA and its concentration in rat brain synaptosomes (Erecinska et al. 1996). These metabolic effects may contribute to the anti-epileptic function of high-fat diets or ketone bodies.

10.5 Glycine and Serine as Well as Creatine Synthesis

Glycine is the simplest AA in animals. It is a major inhibitory transmitter in the spinal cord and lower brainstem but not in the cerebral cortex (Snyder 2017). Glycine is also a co-agonist with glutamate at N-methyl-D-aspartate (NMDA) receptors in the cerebral cortex to further impact neurotransmission. L-Serine is the precursor of D-serine, which stimulates NMDA receptors that are essential for neurological function (including memory). Thus, when the concentration of L-serine is low in the brain, its conversion into D-serine by serine racemase (a vitamin B_6-dependent enzyme) is impaired, leading to reductions in neuronal plasticity and function as well as the associated memory capacities. Both glycine and serine readily cross the BBB through the LAT2 system for utilization by astrocytes and neurons (Holopainen and Kontro 1989; Bixel et al. 1993; Smith 2000). Thus, dietary supplementation with glycine or serine enhances their concentrations in the brain of rats (Shigemi et al. 2015). Interestingly, in a recent study with a mouse model of Alzheimer's disease where astrocytes have an impairment of L-serine synthesis due to defective glycolysis, Le Douce et al. (2020) reported that dietary supplementation with L-serine ameliorated cognitive deficits in the animals. This finding supports the notion that endogenous syntheses of so-called nonessential AAs (e.g., L-serine) are insufficient for meeting nutritional and physiological needs in mammals (Hou et al. 2015b; 2016b; Wu et al. 2013).

There are three metabolic pathways of glycine catabolism in the nervous tissues (Ashmarin et al. 1996): (1) reversible conversion of serine into glycine by serine hydroxymethyl transferase (SHMT), (2) oxidation of glycine to glyoxylate by glycine oxidase; and (3) decarboxylation of glycine to ammonia and CO_2 by glycine cleavage system in mitochondria. Glycine cleavage system is present exclusively in astrocytes (Sato et al. 1991; Bommacanti et al. 1996). The L-serine synthesized in astrocytes is released and taken up by neurons. L-Serine is converted into glycine in the neurons, and glycine is subsequently taken up by astrocytes. This pattern of glycine metabolism between astrocytes and neurons is called "the glycine-serine cycle" (Berl et al. 1977; Hamberger et al. 1977; Van den Berg et al. 1978; Westergaard et al. 1995; Sibson et al. 1997).

Previous studies using ^{13}C-nuclear magnetic resonance (NMR) spectroscopy identified ^{14}C-labeled creatine, serine, and glutathione in cell extracts and incubation medium when astrocytes were incubated with [2-^{13}C]glycine, suggesting that glycine is a precursor of creatine, serine, and glutathione synthesis in these cells (Bixel et al. 1993). This observation was confirmed by Dringen et al. (1998). As in the liver and kidneys, creatine is synthesized from glycine, arginine and S-adenosylmethionine (a metabolite of methionine) via L-arginine:glycine amidinotransferase (AGAT) and S-adenosylmethionine:N-guanidinoacetate methyltransferase (GAMT) in the rat brain (Braissant et al. 2001). AGAT and GAMT are expressed in neurons and glial cells, whereas creatine transporter-1 (CRT1) is present in neurons and oligodendrocytes throughout the brain but is absent from astrocytes. The endogenous synthesis of creatine in the CNS is physiologically significant, because (1) the permeability of the BBB for creatine is limited and creatine can cross blood-brain barrier only with a low efficiency (Béard and Braissant 2010); and (2) astrocytes lack CRT1 (Braissant et al. 2001). This indicates the importance of the balance and amount of dietary AAs for optimal neurological health and function.

10.6 Branched-Chain Amino Acids (BCAAs)

The transport systems for neutral AAs in the BBB are about 50% saturated with phenylalanine and leucine (Smith et al. 1987). Because BCAAs do not directly participate in neuronal activity, at

physiological concentrations of these AAs traffic within the brain without causing harm. BCAAs are amino group donors for glutamate and glutamine syntheses in the CNS (Yudkoff et al. 2005). Results of studies involving isotopic tracers indicate that about one-fourth of all glutamate nitrogen is derived from leucine via transamination with α-ketoglutarate (Yudkoff 1997). This reaction is catalyzed by BCAA transaminase (BCAT), with a branched-chain α-ketoacid (BCKA) being a product (Chaplin et al. 1976). In vivo studies with magnetic resonance spectroscopy have demonstrated that about 50% of all glutamate nitrogen in the brain comes from leucine (Kanamori et al. 1998). The significance of BCAAs as nitrogen donors for glutamate synthesis have also been established in other tissues, including skeletal muscle (Harper et al. 1984; Haymond et al. 1978), placentae (Self et al. 2004), and mammary glands (Li et al. 2009a).

There are two isoforms of BCAT in the CNS, with the mitochondrial form (BCATm) in astrocytes but the cytosolic form (BCATc) in neurons (Bixel et al. 2001). Immunocytochemical analyses have shown the absence of BCATc from astrocytes and of BCATm from neurons (Hall et al. 1993; Hutson et al. 2001). The second, committed step in the BCAA catabolism is the oxidative decarboxylation of BCKAs by the mitochondrial BCKA dehydrogenase complex (BCKD). BCKD is present in both astrocytes and neurons but its activity varies greatly among cell types. This enzyme is largely inactive in astrocytes but is active in neurons. Thus, after the blood-borne BCAAs enter astrocytes where they undergo transamination with α-ketoglutarate to form glutamate (which may be further amidated to glutamine), BCKAs (which are poorly oxidized in these cells) exit the astrocytes and are taken up by neurons for either transamination with glutamate to generate BCAAs and α-ketoglutarate or oxidative decarboxylation by BCKD (Shambaugh and Koehler 1981; Auestad et al. 1991; Bixel et al. 2001). In the neurons, the α-ketoglutarate may be reductively aminated with NH_4^+ to glutamate by glutamate dehydrogenase (a mitochondrial enzyme). Because leucine is the most abundant BCAA in the brain, this "glutamate buffering system" is referred to as "the leucine-glutamate cycle" (Fig. 10.2), which functions to maintain the concentrations of BCAAs, BCKAs, and glutamate in the CNS within physiological concentrations. The significance of such a metabolic cycle is epitomized by the maple syrup urine disease in patients with inborn BCKD mutations, leading to the accumulation of BCAAs in the brain, severe brain damage, and mental retardation (Yudkoff et al. 2005).

10.7 Sulfur-Containing Amino Acids

Interest in the metabolism and neurological function of sulfur-containing AAs originated from the early observation that a high concentration of cystathionine was present in the human brain (Tallan et al. 1958). Cystathionine is a condensation product of serine and homocysteine [catalyzed by cystathionine β-synthase (CBS), a vitamin B_6-dependent enzyme] in the transsulfuration pathway for methionine catabolism (Bao et al. 1998; Wu 2013). The activity of CBS can be allosterically enhanced by S-adenosylmethionine (Pey et al. 2013), and a deficiency of this enzyme induces hyperhomocysteinemia/homocystinuria to cause mental retardation in humans (Dutta et al. 2005). Because homocysteine (an oxidant) is a toxic molecule contributing to various diseases, its removal from the brain is of physiological significance. As in the liver (Kashiwamata and Greenberg 1970; Kashiwamata et al. 1970), CBS is essential for cysteine generation in the cerebral regions of the brain (Bao et al. 1998; Enokido et al. 2005). Cysteine is used via many metabolic pathways, such as the syntheses of protein, neuropeptids, and glutathione (a potent antioxidant) and taurine (Dringen and Hamprecht et al. 1996; Kranich et al. 1996; Vitvitsky et al. 2011). In the brain of non-carnivorous animals, the rate of conversion of methionine into cysteine is low as compared with their liver (Hayes and Sturman 1981). Although taurine can be formed from cysteine in neurons and astrocytes of non-carnivorous animals (Vitvitsky et al. 2011), the rate of taurine synthe-

sis by these cells and the brain is low as compared with their hepatocytes and liver (Hayes and Sturman 1981). To fulfill some of its physiological functions, cysteine is the substrate for the production of hydrogen sulfide (H_2S) by cystathionine γ-lyase (cystathionase) or CBS (Wu 2013). Of note, H_2S is a gasotransmitter in the CNS that affects protein activity through attachment to the sulfhydryl group of cysteine reside (called sulfhydration) in the protein (Snyder 2017). At physiological concentrations, H_2S is also a regulator of glutathione synthesis and a vasorelaxant in the vasculature (Li et al. 2009b). Thus, hypertension occurs in mice with the deletion of cystathionine γ-lyase (Yang et al. 2008).

Methionine and cysteine in the blood cross the BBB through specific AA transporters (Wu 2013). Methionine competes with large neutral AAs for Na^+-independent transporters [LAT1 (SLC7A5), LAT2 (SLC7A8), LAT3 (SLC43A1), and LAT4 (SLC43A2)] and Na^+-dependent transporters [B^oAT2 (SLC6A15) and B^oAT3 (SLC6A18). Thus, large neutral AAs can affect the concentration of methionine in the brain, and vice versa (Fernstrom 2013). In animals consuming AA balanced diets, methionine and cysteine are distributed evenly among different regions of the brain (Shaw and Heine 1965; Perry et al. 1971, 1972; Gaull et al. 1975) and their concentrations in the CNS do not change appreciably after normal feeding (Zeisel and Wurtman 1979). However, administration of high doses of methionine or cysteine alone can substantially increase the concentration of the test AA in the brain well above its physiological range (Anderson and Meister 1989; Daniel and Waisman 1969; Rubin et al. 1974). This explains, in part, why consumption of excessive methionine and cysteine is toxic to animals (Wu 2018).

10.8 Aromatic Amino Acids

Tryptophan is the precursor of serotonin, whereas tyrosine is the precursor of dopamine and norepinephrine (catecholamines) in the brain (Fernstrom 2013). The CNS lacks phenylalanine hydroxylase and, therefore, cannot convert phenylalanine into tyrosine (Karobath and Baldessarini 1972; Abita et al. 1974). This enzyme is present in the liver and kidneys to degrade phenylalanine and regulate the concentration of tyrosine in plasma (Wu 2013). Both serotonin and catecholamines are neurotransmitters in the brain (Table 10.1). Thus, there is growing interest in the metabolism of aromatic AAs in the CNS (Brosnan et al. 1984; Sperringer et al. 2017; Neinast et al. 2019). Because tryptophan and tyrosine are not synthesized by any cells in the brain, their uptake from the blood through the BBB is of enormous physiological significance. Tryptophan and tyrosine compete with BCAAs for passage through the BBB as noted previously. Therefore, the concentrations of tryptophan and tyrosine in the CNS are affected by dietary intakes of BCAAs and phenylalanine. In the brain, tryptophan is metabolized to 5-hydroxytryptophan by tetrahydrobiopterin-dependent tryptophan hydroxylase, followed by the conversion of 5-hydroxytryptophan into serotonin. Because tryptophan hydroxylase is not saturated with the normal physiological concentrations of tryptophan in the brain, the synthesis of serotonin is dependent on tryptophan availability (Feenstra and der Plasse 2010).

In neurons that use dopamine as a neurotransmitter, tyrosine is oxidized to DOPA by tetrahydrobiopterin-dependent tyrosine hydroxylase (Nagatsu et al. 1964; Kaufman and Kaufman 1985), and DOPA is subsequently decarboxylated by vitamin B_6-dependent DOPA decarboxylase to form dopamine (Wu 2013). Tyrosine hydroxylase is inhibited by catecholamines (Pogson et al. 1989). In neurons that use norepinephrine as a neurotransmitter, tyrosine-derived dopamine undergoes mono-oxygenation to norepinephrine by dopamine hydroxylase. Furthermore, in neurons that use epinephrine as a neurotransmitter, phenylethanolamine *N*-methyltransferase catalyzes the conversion of norepinephrine into epinephrine. Thus, the availability of tyrosine in the

CNS affects not only the rate of DOPA synthesis but also the overall production of catecholamines by neurons (Gibson 1986; During et al. 1988; McTavish et al. 1999).

10.9 Basic Amino Acids

Histidine is not synthesized by any cells of the CNS but is readily transported across the BBB (Fig. 10.1). In the brain, histidine is decarboxylated by histidine decarboxylase to generate histamine, which is a neurotransmitter and a neuromodulator (Snyder 2017). This enzyme is localized in the cytoplasm of nerve endings and is regulated via cAMP-dependent cell signaling (Moreno-Delgado et al. 2006). The newly synthesized histamine is stored in synaptic vesicles and released in response to K^+-induced depolarization. Once released, histamine interacts with its receptors, some of which have a postsynaptic neuronal localization. The activity of histidine decarboxylase in mammalian brain is relatively low (only about 10% of the activities of the enzymes that synthesize catecholamines and serotonin), which makes it technically difficult to determine its activity (Schwartz et al. 1970). The overall rate of histamine synthesis is dependent on the rate of histidine transport into neurons; and 2) the rate of histidine decarboxylation in the cells. The former step is not affected by prior depolarization of synaptosomes and is not rate-controlling for histamine production (Chudomelka and Murrin 1983). Administration of histidine either intraperitoneally or intraventricularly increases the activity of histamine decarboxylase and the concentration of histamine in the hypothalamus (Yoshimatsu et al. 2002). This histidine-induced lowering appetite is inhibited by supplementing BCAAs. Among different regions in the brain, the concentration of histamine is highest in the hypothalamus and lowest in the cerebellum, indicating the uneven distribution of this neurotransmitter in the brain (Ronnberg and Schwartz 1969).

Comparable amounts of arginine are present in synaptosomes and vesicles from several brain regions (Kontro et al. 1980). The metabolic pathways of arginine include: (1) NO synthesis by tetrahydrobiopterin-dependent NO synthase, with L-citrulline being a co-product (Snyder 2017); (2) conversion into ornithine and urea by arginase (Johnson and Roberts 1984); and (3) production of agmatine and carbon dioxide by arginine decarboxylase (Wu and Morris 1998). Note that arginine and citrulline in the blood enter the brain through the y^+ and LAT1 system, respectively, and vice versa (Fig. 10.1). NO is a neurotransmitter to maintain CNS function (Snyder 2017), whereas agmatine may play a role in learning and memory (McKay et al. 2002; Liu et al. 2008; Leitch et al. 2011) as well as mediating the production of NO and polyamines (Halaris and Piletz 2007). Ornithine can be further metabolized to yield proline, glutamate and polyamines (putrescine, spermidine, and spermine). The latter are essential for cell growth (including neurogenesis) and function (Wallace et al. 2003; Malaterre et al. 2004). As an inhibitor of arginase (Wu and Morris 1998), high concentrations of extracellular ornithine can spare arginine for NO generation. Likewise, extracellular proline and GABA can reduce their formation from ornithine and glutamate, respectively, by inhibiting pyrroline-5-carboxylate reductase (Yoneda and Roberts 1982) and ornithine aminotransferase (Yoneda et al. 1982), respectively. An emerging research area is arginine metabolism in the brain during the aging process (Liu et al. 2009; Gupta et al. 2012; Rushaidhi et al. 2012; Mazlan et al. 2017). The postrhinal cortex, entorhinal cortex, and cerebellum are most affected by age. Postrhinal cortex and entorhinal cortex are mainly involved in memory processing (Squire et al. 2004; Agster and Burwell 2009), whereas the cerebellum is responsible for motor control, emotion and cognition (Augustin et al. 2001; Schutter and van Honk 2005; Schmahmann and Caplan 2006). We reported that changes of hippocampal citrulline levels paralleled the early and late phases of retrieval in the Morris Water maze (Sase et al. 2013). These results indicate the complexity in the regulation of arginine metabolism in the CNS and the diverse roles of this AA in neurological function. Oral administration of up to 30 g L-arginine/day (in two or more divided

doses) to healthy adult humans for 3 months does not adversely affect their neurological function or physiological metabolites (McNeal et al. 2018).

In animals, lysine is catabolized via the saccharopine and pipecolate pathways (André et al. 2013; Wu 2013), as well as the homoarginine pathway (Hou et al. 2015a, 2016a). The saccharopine pathway is mainly present in the developing brain and extracerebral tissues, and the pipecolate pathway in the adult mammalian brain (Chang 1977; Hallen 2013; Roland et al. 2015). These two pathways converge at the level of Δ^1-piperideine-6-carboxylate, which is in equilibrium with its open-chain aldehyde form (α-aminoadipate δ-semialdehyde). The latter is metabolized via a series of enzymes to acetyl-CoA. A cerebral ketimine reductase, which catalyzes the reduction of Δ^1-piperideine-2-carboxylate to L-pipecolate, is identical to thyroid hormone-binding protein (μ-crystallin; Hallen 2013). L-pipecolate binds tightly to the P2 fraction membranes of mouse brain, indicating its neurochemical importance (Gutierrez and Giacobini 1985). In the homoarginine pathway, L-arginine:glycine amidinotransferase transfers the amidino group from L-arginine to the ε-amino group of L-lysine to form L-homoarginine, with the concentration of homoarginine in the rat brain being 1.05 nmol/g wet tissue (Hou et al. 2015a). There are suggestions that homoarginine contributes to neurological function, including behavior (Bernstein et al. 2015; Ajinkya et al. 2016). Because lysine interferes with NO synthesis by cells (Li et al. 2009b), lysine catabolism plays a role in maintaining neurological function.

10.10 The Role of Amino Acids in Regulating Food Intake

The amounts and balance of AAs in diets greatly affect the concentrations of neurotransmitters in the brain (Fernstrom 1990, 2013; Tews et al. 1984b) and food intake by animals (Wu 2018). An appropriate amount of some AAs (e.g., glycine, tryptophan, and glutamate) stimulates food intake. In contrast, an excess or deficiency of an AA (e.g., methionine, arginine, and leucine), particularly an AA that is not synthesized by animal cells, or an imbalance of AAs in diets, generally reduces food intake. For example, supplementing 0.2% tryptophan to a corn- and soybean meal-based, 19.5% crude-protein diet (containing 0.25% tryptophan) increases the feed intake of weanling piglets by 10% (Liang et al. 2018), whereas supplementing 2% glutamine to a corn- and soybean meal-based, 21% crude-protein diet (containing 1.82% glutamine) decreases the feed intake of weanling piglets by 18% (Wu 2018). Furthermore, rats prefer an AA balanced diet when compared with a diet deficient in one or more nutritionally essential AAs (Feurte et al. 1999; Gietzen and Magrum 2001). Clearly, animals increase the consumption of a low-protein diet to meet the minimum requirement for all proteinogenic AAs (Wu 2018). The underlying mechanisms are largely unknown but likely involve the metabolism of AAs, the production of neurotransmitters (including neuropeptides), and their signaling in the CNS (Michael 1987; Fernstrom 1994, 2013; Tran et al. 2019).

First, the concentrations of AAs, ammonia, glucose, and fatty acids in plasma affect food intake by animals (Holt et al. 1996; Morens et al. 2003; Wu 2018). Second, AAs influence the metabolism of the hypothalamus, which is the major region in the brain responsible for the control of food intake (Kelley et al. 2005). For example, whether administered intraperitoneally or intraventricularly into the hypothalamus, histidine dose-dependently increases the hypothalamic activity of histidine decarboxylase and the hypothalamic levels of histamine to inhibit food intake by rodents (Yoshimatsu et al. 1999, 2002; Kasaoka et al. 2004; Jørgensen et al. 2006; Gotoh et al. 2009). Interestingly, a histidine-induced decrease in appetite is attenuated by dietary supplementation with BCAAs (DiNicolantonio et al. 2018), suggesting an interaction between histidine and BCAAs or their metabolites (e.g., glutamate and GABA). Third, dopamine mediates anorexia (a lack or loss of appetite for food) in animals (Fibiger et al. 1973; Breese 1975). In support of this view, intraventricular injection of dopamine decreases the food intake of rats, and this effect of

dopamine is attenuated by co-administration with a dopamine antagonist into the perifornical hypothalamus (Leibowitz and Rossakis 1978, 1979). Fourth, serotonin (5-hydroxytryptamine) innervates the hypothalamus, whereby modulating its physiological activity (Saavedra et al. 1974; Palkovits et al. 1977). An increase in brain serotonin inhibits food intake, but a decrease in brain serotonin promotes hyperphagia and weight gain (Goldman et al. 1971; Singer et al. 1971; Lam et al. 2010). Similarly, intrahypothalamic injection of 5-hydroxytryptophan or tryptophan (precursors of serotonin) also suppresses food intake (Joyce and Mrosovsky 1964; Singer et al. 1971; Blundell and Leshem 1975; Goudie et al. 1976; Sugrue and Mireylees 1978; Blundell et al. 1980). Conversely, the anorectic effect of serotonin is blocked by the prior administration of the peripheral serotonin receptor antagonist, xylamidine (Clineschmidt et al. 1978). In the small intestine, serotonin is synthesized by enteroendocrine cells to promote gastrointestinal motility, which may explain why dietary supplementation with tryptophan enhances food intake by animals (Liang et al. 2018). Fifth, GABA regulates food intake by animals in a complex manner, depending on dose and anatomical site as well as dietary protein level. For example, Tews et al. (1984a) reported that supplementing GABA at 5%, but not 3%, of a low-protein diet depressed the food intake and growth of kittens. However, food intake is increased by microinjection of either 100 ng bicuculline methiodide (a GABA antagonist) into the anterolateral hypothalamus or 100 ng GABA into the ventromedial hypothalamus, but is decreased by microinjection of 100 ng GABA into the origin of the nigrostriatal dopamine neurons in the substantia nigra (Kelly et al. 1977). In contrast, microinjection of 100 ng GABA into the origin of the mesolimbic dopamine cells in the ventral tegmental area (VTA) does not affect feeding behavior (Kelly et al. 1977). These observations suggest that the GABAergic neurons in the lateral and ventromedial hypothalamus may serve as modulators of afferent inputs to feeding control neurons. There is evidence that dietary supplementation with GABA (75 and 100 mg/kg feed) for 35 days stimulated feed intake by broiler chickens exposed to cyclic heat stress (Chand et al. 2016).

10.11 Conclusion

Metabolism of AAs in the brain involves their transport across the BBB, catabolism (including the generation of low-molecular-weight neurotransmitters), and syntheses, as well as the production of proteins and oligopeptides (including neuropeptides). Physiological concentrations of AAs in plasma and the CNS are essential to maintain brain health and function. Some AAs or metabolites of certain AAs are neurotransmitters or modulators of neurotransmission (Table 10.1). The homeostasis of AAs in the CNS is regulated via tightly integrated metabolic pathways, including the glutamate-glutamine cycle, the glycine-serine cycle, the leucine-glutamate cycle, and the intracellular protein turnover. Glutamate, aspartate, histamine, serotonin, dopamine, and GABA in the brain are key regulators of neurological function. Some neurotransmitters (dopamine and serotonin) are often closely correlated with the aging process in that their concentrations in the brain decrease gradually with aging. Additionally, neurotransmitters are responsible for the regulation of food intake, movement control, and memory function, the adequate consumption of AAs (e.g., a proper mixture of animal- and plant-source proteins) is necessary for optimal health and well-being in humans and animals.

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Metabolism and Functions of Amino Acids in the Skin

11

F. Solano

Abstract

Amino acids are the building blocks of all proteins, including the most abundant fibrous proteins in the skin, as keratins, collagen and elastin. Sagging and wrinkled skin are features of chronic sun-damaged and aged uncared skin, and they are mainly associated with the deterioration of collagen and elastic fibers. The maintenance of skin structures by self-repair processes is essential to skin health. Thus, amino acids significantly impact the appearance of the skin. Amino acids are important nutrients required for (a) wound healing promotion and repair of the damaged skin; (b) acid-base balance and water retention in cellular layers, such as stratum corneum; (c) protection against sunlight damage; (d) maintenance of an appropriate skin microbiome. This review highlights the contribution of all proteinogenic amino acids and some related metabolites to the skin structures as constituents of the main cutaneous proteins or as signaling molecules for the regulation and determination of skin physiology.

Keywords

Amino acids · Skin · Epidermis · Dermis · Collagen · Elastin · Keratins · Melanin · Skincare

11.1 Introduction

Skin is a largest tissue in animals, with a surface of approximately 2 m^2 and around 4 kg in the adult human. It is a thin but wide tissue. In humans, its thickness goes from less than 1 mm on eyelids to more than 4 mm on the palms of hands and soles of feet. Skin is essential for general health as it is the first line of defense of the body against harmful external chemical, physical and biological agents in addition to other functions, such as the control of the corporal temperature. These agents include Vis-UV irradiation, chemical air pollutants, mechanical pressure, dehydration, as well as viral, bacterial and fungal infections. Skin cannot be considered just a physical barrier but it is also a dynamic tissue with its own metabolism and interactions between external and internal cells.

Anatomically, skin is a complex organ composed of two main compartments: epidermis and dermis, separated by the epidermal-dermal junction. Epidermis has several layers, and the most external one is the *stratum corneum* that constitutes an excretion system by losing old epidermal

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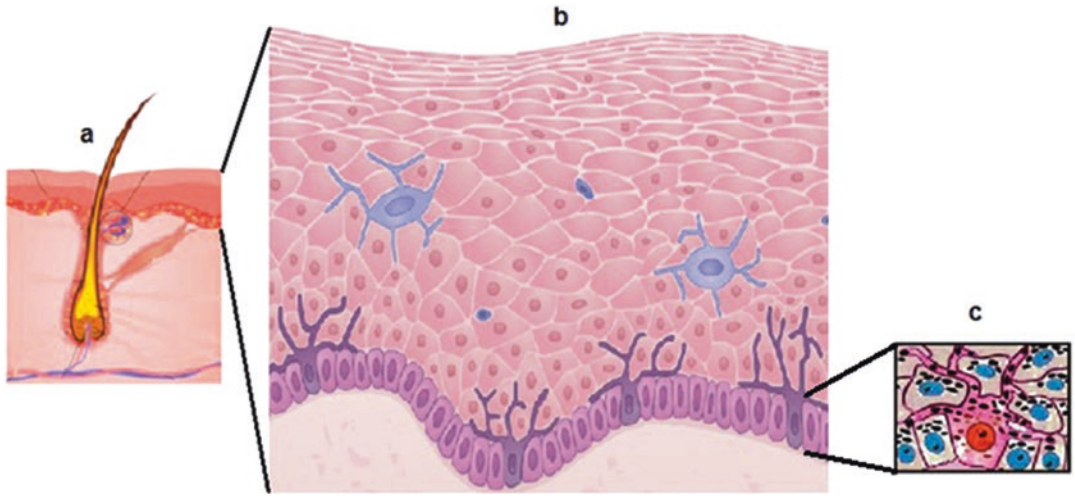

Fig. 11.1 Structure of skin. (**a**) Skin showing the three main parts, external epidermis, epidermal-dermal junction and dermis. Dermis contains fibroblast among extracellular matrix, with collagen and elastin as more abundant proteins; (**b**) Amplification of the epidermis layer showing abundant keratinocytes with some distribution of other cell types (Merkel and dendritic Langerhans cells). Melanocytes are also dendritic cells, but mostly found in the epidermal-dermal junction; (**c**) Detail of one melanocyte transferring melanosomes (melanin granules) to surrounding keratinocytes through its dendritic ramifications

cells with waste components. Epidermal cells are replaced about every month. The main cellular components of the epidermis are keratinocytes, and others include melanocytes, Langerhans cells, α-dendritic cells and Merkel cells (Fig. 11.1). Keratinocytes in the deepest layer of epidermis divide constantly and the new cells are pushed towards the external surface. Dermis contains fibroblasts as main cells among other components, such as blood vessels, touch and pain sensors, hair follicles, sweat and oil glands.

Keratinocytes synthesize high amounts of keratins, which are fibrous proteins providing the skin with a durable overcoat to protect from drying, mechanical damage and infection. Around 95% of epidermal proteins are keratins. Other important protein is filaggrin. Dermal fibroblasts are producers of two important proteins in the skin, collagen and elastin. Those proteins begin to decrease from age 25 in humans, and about 70% of the dry skin mass is collagen.

Keratinocytes and fibroblasts produce the essential proteins for cutaneous fibers. An unbalanced ratio of amino acids, the building blocks of proteins, will cause a reduction in protein synthesis in the skin (Li and Wu 2018). Those proteins prevent skin thinning, loss of skin elasticity and dehydration and minimize the conditions that lead to the appearance of sagging and wrinkles. Furthermore, deficit or dysfunction of these proteins lead to severe diseases, such as urticaria, xerosis, eczemas, thrush, itching and skin ulcers. In addition to internal requirements, epidermal keratinocytes need amino acids to synthesize some antimicrobial peptides to kill pathogens (Park et al. 2013), although at the same time skin provides an interface to host the epidermal common microbiota. The metagenomic DNA sequencing studies in the skin microbiota has been recently focused on *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus* (Byrd et al. 2018). Amino acid replacement is especially important in the skin where they are lost due to shedding of *stratum corneum* cells. This requirement of particular amino acids is related to: (1) their relative abundancy in the sequence of main skin proteins, and (2) their forming-capacity. As it is well known, human cells cannot synthesize all the proteinogenic amino acids (Wu 2013). From this point of view, amino acids had traditionally been classified as nutritionally essential or non-essential (Hou et al. 2015). Essential amino acids cannot be formed de novo, so that they must be obtained

from foods or supplements and any deficiency can result in health problems. The "non-essential" term means that the human cells can synthesize them de novo, but it does not mean that those amino acids are less important for skincare. Some of these non-essential (arginine, glycine and tyrosine) are also known as "conditionally essential" amino acids, meaning they cannot be formed sufficiently under certain conditions. The term "nutritional nonessential amino acids" is now considered as a misnomer in nutritional sciences and should no longer be used (Hou and Wu 2017).

The most common amino acids in skincare products are the 3 cationic ones (His, Lys and Arg), and 3 neutral ones that are especially abundant in collagen (Gly, Pro and Leu). Amino acids are not only needed for protein synthesis, but they play specialized roles in maintaining healthy skin. None of them can be considered as the most important one, as they are complementary to each other. For example, a combination of Lys and Arg can effectively treat certain skin injuries by accelerating would healing, while Pro and Leu can attenuate wrinkles when paired together. A mixture of four amino acids supplemented with other components are effective to decrease the time of wound healing (Corsetti et al. 2010). It should bear in mind that they act in combination with other components of the skin, including lipids (e.g., glycerin, ceramides and ω^3 fatty acids), polysaccharides (e.g., hyaluronate, vitamins D and E, and some inorganic ions such as Zn).

Thus, amino acids are active compounds used in cosmetic and therapeutic treatments for a number of skin diseases and just for hydrated and young skin maintenance. This review is devoted to amino acids and skin health. The specific roles of each of them will be described in the next paragraphs.

11.2 Histidine (His)

This amino acid has important roles in the skin. His is related to the formation of several biomolecules, not always proteins. This paragraph will be focused on a very important protein in the stratum corneum, filaggrin and a His-derivative, urocanic acid (UCA, Fig. 11.2). In fact, both molecules are correlated because filaggrin could be a reservoir of His for its transformation into UCA. Increased filaggrin expression and urocanic concentration have been found to be correlated in chronic spontaneous urticaria (Le Pharm et al. 2017). Other roles of His are related to the prevention against certain fungal infections, as the systemic candida, due to the synthesis of one antifungal histidine-rich glycoprotein that breaks the cell walls of fungal cells by specific interactions with ergosterol (Rydengård et al. 2008) or the formation of histamine in dermal mast cells, and an involvement in inflammatory and allergic responses. The formation of antimicrobial peptides or the role of histamine are out of the scope of this review.

11.2.1 Filaggrin

His plays an important role in preserving the moisture of stratum corneum. Filaggrin is one of the most important proteins specifically located in in granular keratinocytes and lower corneocytes of the stratum corneum (Hsu et al. 2011). Filaggrin aggregate with keratin intermediate filaments during terminal differentiation of mammalian epidermis and it is a component of the cornified cell envelope. Human filaggrin is synthesized as a large, insoluble, highly phosphorylated precursor containing more than 20 tandem copies around 50 residues long (P20930, www.expasy.org) especially rich in histidine, but also serine and arginine.

The stratum corneum maintains its hydration level at ~15% by retention of free amino acids that act as emollient factors (Jacobson et al. 1990; Seguchi et al. 1996; Kim et al. 2012). Many data suggest that selective proteolysis of filaggrin is the source of those amino acids. First, the composition of free amino acids in skin shows a high degree of similarity to the composition of filaggrin. Secondly, a decrease in the amount of amino acids contributes to the pathogenesis of xerotic skin conditions (Horii et al. 1989), and this is correlated with low filaggrin content in the epidermis. Thus, during terminal differentiation filaggrin acts as a natural moisturizing factor. It is phosphorylated and proteolytically cleaved by calpain-1 (Senshu

et al. 1996; Hsu et al. 2011). In addition, some His and Arg residues in filaggrin undergoes deimination, so that UCA and citrulline residues appear. Thus, filaggrin can be considered the major source of His in the stratum corneum (Koyama et al. 1984), and then this amino acid could be transformed to UCA, which is an important signal of stress conditions in the skin.

11.2.2 Urocanic Acid

UCA is produced by His deamination (Fig. 11.2). Significant amounts of this metabolite are found in blood, but interestingly it is mostly concentrated in skin, particularly in the *stratum corneum* (Tabachnick 1957). Due to that location and its light-absorbing properties, the initial studies of this chromophore indicated that it could play a role as a physiological sunscreen, as its main UVR absorption band around 260 nm is coincident with the absorption band of the bases contained at nucleic acids. This proposal was supported with the direct evidence of the photoprotective effect of UCA (Barresi et al. 2010). However, UCA content does not correlate with the pigmentation level or the minimal erythema doses. Therefore, the other roles of UCA were proposed, such as a buffering effect to maintain the relatively acidic pH of the stratum corneum (around 5.5) that contributes to the inhibition of pathogenic bacterial and fungal growth.

The interest in UCA has been increased in recent years and its two other possible physiological roles have been proposed, involving not only the skin but other tissues. For instance, it has been detected that the His-derived isomer t*rans*-UCA is photoisomerized to *cis*-UCA by exposure to UVR. Opposite to the trans-isomer, *cis*-UCA produces proapoptotic intracellular acidification and oxidative DNA damage, and triggers specific immunologic responses. Some authors describe immunosuppression (Kaneko et al. 2008) and others reported an IgE-mediated basophil activation (Le Pharm et al. 2017). Relative "beneficial" versus "detrimental" properties of UCA have been reported. Thus the formation of UCA in the stratum corneum is important, but its roles are complex and remain largely unknown (Gibbs and Norval 2011).

On the other hand, a novel metabolic pathway that transforms UCA to glutamate in neurons have been suggested (Zhu et al. 2018). The UV exposure of skin increases the levels of UCA, which then could be able to cross the blood-brain barrier and be transformed into glutamate by neuronal urocanase. If so, this mechanism reveals a new glutamate biosynthetic pathway that may contribute to some of the sunlight-induced neurobehavioral changes related to the ancient belief that sunlight exposure affects mood, learning, memory and cognition. The underlying mechanisms may involve diverse signaling molecules, such as melatonin, without a total satisfactory correlation (Goswami and Haldar 2015).

11.3 Lysine (Lys)

Lysine is one of the essential amino acids that have been extensively studied. Its recommended daily allowance is from 41 mg per kilogram of

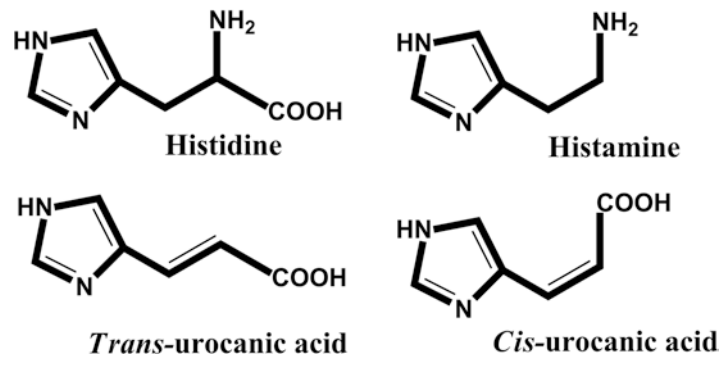

Fig. 11.2 Chemical structure of Histidine and derived molecules with relevance in the skin: histamine; trans-urocanic acid; cis-urocanic acid

body weight per day in adults to 89 mg/kg of body weight in children, although the estimated requirements are lower since its absorption is dependent on other nutrients in diets. Lysine is especially important for proper collagen and elastin functions. Once Lys is incorporated into skin proteins, and some post-translational modifications on the side chain make Lys residues essential for maturation of those proteins. Those modifications are catalyzed by different types of enzymes, lysyl hydroxylases and lysyl oxidases. In preprocollagen chains, some lysyl residues are intracellularly hydroxylated at the 5-carbon by vitamin-C dependent lysyl hydroxylases. Human collagen analysis shows that approximately 20% of the Lys residues are 5-hydroxylated (Veis and Anesey 1965). Subsequently some glycosyl transferases add carbohydrate moieties at the 5-hydroxy-Lysine residues. On the other hand, once the tropocollagen or proelastin are secreted to the extracellular matrix, some ε-amine groups of lysyl or 5-hydroxylysyl residues are oxidized to aminoadipic semialdehyde (also named al-lysine, Fig. 11.3) by lysyl oxidases (Lox), a specific type of copper amine oxidase present in the skin. The aldehyde group of al-lysine reacts with other ε-NH_2 groups of unaltered lysyl residues to form covalent cross-linked bonds among tropocollagen units, thereby providing tensile strength and insolubility to protein fibers. Other cross-linking reactions are possible, forming structures such as lysinonorleucine (two cross-linked chains), merodesmosine, (3 cross-linked chains) and desmosine or isodesmosine (4 cross-linked chains with the formation of a complete pyridinoline ring). Desmosine, which is much more abundant in elastin than in collagen, is primarily responsible for the elasticity and is commonly used as a marker for elastin.

It is worth to mention that Lys supplementation has been also proposed for prevention of acne and cold sore. Complex factors, such as hormone fluctuations and stress are involved in the appearance of acne. In fact, acne is the result of a combination of bacteria, oil (sebum) and dead skin cells trapped in hair follicles, clogging pores. There is no doubt that acne repair would require collagen turnover, so that adequate intakes of amino acids, along with other nutrients, may help to treat acne. However, there is no particular scientific evidence that Lys improves the lesions of this skin disorder.

Finally, unrelated of collagen production, a tripeptide containing Lys and His has been proposed as a skin moisture agent used in skincare products (Choi et al. 2012). Gly-His-Lys is a copper(II)-chelating motif occurring in some serum proteins that promotes the survival of basal

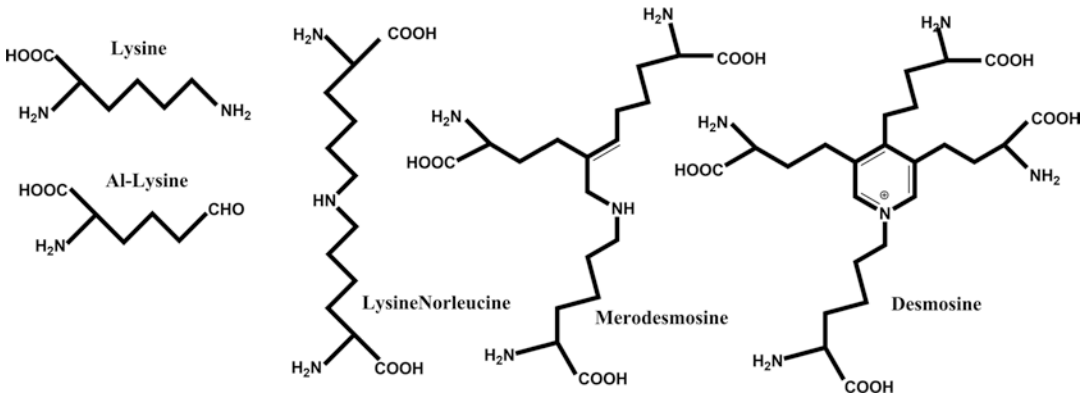

Fig. 11.3 Chemical structure of Lysine and the derivative aldehyde Al-Lysine formed by Lox action. During the process of cross-linking in collagen and elastin, the side chain of these amino acids can react to form Lysinenorleucine (1 Lys and 1 Al-Lys), Merodesmosine (1Lys and 2 Al-Lys) or Desmosine (1 Lys and 3 Al-Lys residues forming the pyridinoline ring). Isodesmosine is similar to desmosine, but the positions of the 4 side chains are 1,2,3 and 5

stem cells in the skin and the proliferation of keratinocytes with increased expression of integrin.

11.4 Branched-Chain Amino Acids: Isoleucine, Leucine and Valine

These branched-chain amino acids are nutritionally essential for humans (Wu 2013). Formation of keratins, collagen and skin proteins is affected by the availability of these nutrients, especially for replacement of damaged collagen. However, their specific effects on the skin have been rarely studied. Amino acid mixtures consisting of branched-chain amino acids (Ile, Leu and Val) plus Arg, Gln or Pro significantly increased the synthesis of dermal collagen in hairless mice submitted to UVR irradiation (Murakami et al. 2012). The stimulatory effect does not occur when the amino acids are supplied individually, but the presence of all the three branched-chain amino acids seems to be necessary for beneficial effects (Murakami 2014). Leu has also been used for attenuation of skin wrinkles in conjunction with Gly and Pro (Kawashima et al. 2013). Beyond the general effect as building blocks of the protein, recent data suggest that deficiencies of Leu and Ile reduce collagen synthesis in skin by suppressing the action of mTOR (Yamane et al. 2018). Thus, these amino acids could be involved in regulatory mechanisms, such as autophagy or transcription.

To strength muscles for exercise, a Leu metabolite HMB (β-Hydroxy-β-methylbutyrate) has been used as a dietary supplement. This substance has been proposed to promote wound healing, and therefore it has been added to some skincare products (Williams et al. 2002; Morfino et al. 2013). However, recent studies have shown that supplementation with Gln, Arg and HMB does not have any beneficial effect in enhancing healing of open wounds in rats (Bozkirli et al. 2015). Ile has been also used as a complement component of ceramides-based emollient cream for treatment of facial atopic eczema (Puviani et al. 2014), but the precise role of this amino acid is unclear.

11.5 Phenylalanine (Phe) and Tyrosine (Tyr)

Tyr and Phe are not very abundant in collagen, elastin or keratins, so that the correlation between the supply of these amino acids and the synthesis of skin proteins has not been reported. However, these aromatic amino acids are important as precursors of melanin (Fig. 11.4). Melanin absorbs the harmful sunlight UV and thus is the main cutaneous photoprotective pigment for avoiding DNA damage and skin cancer types (Brenner and Hearing 2008; Solano 2016; Fajuyigbe et al. 2018). Phe can be converted into Tyr by phenylalanine hydroxylase. This enzyme generates Tyr from essential Phe. Of note, Phe hydroxylase is activated in human melanocytes by ROS and the oxidant conditions created after UV exposure (Schallreuter et al. 2004). Then, Tyr is further oxidizes to melanin by tyrosinase (Solano 2014). Phe hydroxylase (a tetrahydrobiopterin-dependent enzyme) and tyrosinase (a copper enzyme, Solano 2018) are present in melanocytes, the specialized cells for melanin synthesis

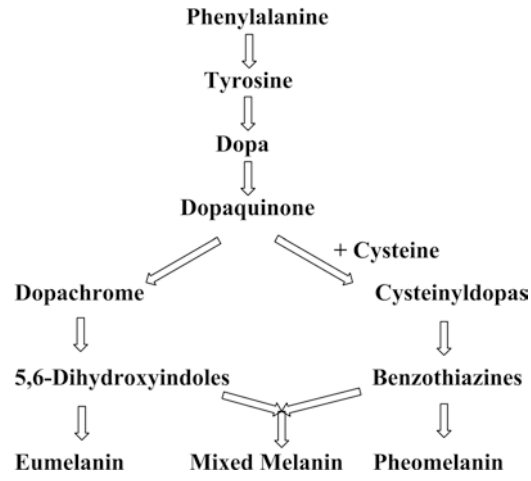

Fig. 11.4 Scheme of the melanogenesis pathway showing the role of Phenylalanine and Tyrosine as melanin precursors. Cysteine is also involved in pheomelanin formation

(Fig. 11.1). Melanogenesis takes place in a subcellular organelle of melanocytes called melanosomes (Slominski et al. 1988), and then melanosomes are transferred to keratinocytes (Serre et al. 2018). Freckles and moles are patches of skin with more melanocytes and therefore more melanin than the surrounding area.

The requirements of Phe and Tyr for melanin synthesis depends on the exposure to sunlight and the skin phototype. The amount and type of melanin determines the degree of tanning in each phototype and the skin color. Dark skin needs higher amounts of (eu)-melanin than fair skin. Fair skin contains predominantly pheomelanin, a type of melanin that is structurally different from the dark eumelanin. Pheomelanin formation needs lower amounts of Phe/Tyr but it needs Cys (Wu 2013; Solano 2014).

11.6 Tryptophan (Trp)

This is another aromatic amino acid that is not abundant in skin proteins. There are very few studies on the role of tryptophan in the skin health, but Trp is the precursor of melatonin, a hormone involved in skin protection against oxidative stress. Human skin contains all the enzymes necessary to transform Trp into melatonin, as the pineal gland does (Slomisnki et al. 2008). Absorption of sunlight in the skin, particularly UVA rays, induces the formation of photosensitizers (Wondrak et al. 2006). The endogenous chromophores in human skin serve as photosensitizers but are not well characterized. In addition to melatonin, the Trp derivative, 6-formylindolo[3,2-b]carbazole (FICZ) found in epidermal keratinocytes has been proposed as one possible UVA-photosensitizer. FICS may bind to a skin-occurring aryl hydrocarbon receptor (Syed and Mukhtar 2015), and could be formed as a consequence of spontaneous chemical damage of Trp residues in the skin proteins.

Impaired Trp transport is related to Hartnup disease. The mutated gene encoding a solute carrier protein is mainly expressed in the small intestine and renal tubules, causing malabsorption and neutral amino aciduria including Trp and other amino acids (Wan 2011). The Hartnup symptoms are photosensitivity and pellagra-like skin rash, suggesting that Trp deficiency affects skin integrity and function.

11.7 Sulphur-Containing Amino Acids: Methionine (Met) and Cysteine (Cys)

Oral ingestion of Met can be an endogenous source of Cys in keratins, and Cys plays an important role in the formation of the disulfide bridges present in the integumentary skin structures (Fraser et al. 1972; Miniaci et al. 2016). These sulfur-containing amino acids along with other sulfated compounds are the sources of sulfur for several functions. Adequate consumption of dietary Met and Cys is required to meet the body's requirements for the formation of skin structural polysaccharides and glycosaminoglycanes (Danzberger et al. 2018). Met can also be used as a zinc-vehicle rather than a sulfur-supplier for treating acne and inflammatory cutaneous lesions as antioxidant complexes (Sardana and Garg 2010).

11.8 Threonine (Thr)

The role of Thr in skin health has not been described in the literature. Thus, there are very few data concerning nutritional Thr requirements for the maintenance of the skin or any other tissue. Thr and Ser are appropriate amino acids for keeping the stratum corneum in a hydrated state. In proteins, Thr and Ser may be phosphorylated by a number of kinases. Several epidermal proteins must be phosphorylated for a proper structural function and control of skin metabolism.

11.9 Amino Acids that Are Synthesized in Animals (AASA)

These amino acids are synthesized de novo by humans (Wu et al. 2013), and are Arg, Gly, Pro, Ala, Ser, Asp, Glu, Gln and Asn. Tyr and Cys are formed from Phe and Met, respectively, as noted previously.

11.9.1 Arginine (Arg)

Arg can formed as an intermediate in the urea cycle in the mammalian liver, but there is no net synthesis of arginine by the liver (Wu and Morris 1998). There are no reports on the urea cycle in skin. In humans, arginine is synthesized from glutamine, glutamate and proline via the intestinal-renal axis. Arg is a conditionally essential amino acid in children and possibly in adults (Wu et al. 2009). Arg is one of the highly recommended amino acids to accelerate the wound healing of injured skin (Stechmiller et al. 2005) due to the production of nitric oxide (NO). In addition, poly-Arg has been recommended for topical application for treatment of frostbite injuries in frozen skin (Auerbach et al. 2014), although the role of this polypeptide in Arg storage in vivo has not been demonstrated.

NO is involved in the inflammatory and proliferative mechanisms of wound healing and has been proposed as an inducer of collagen synthesis in fibroblasts (Childress and Stechmiller 2002; Kim et al. 2012; Alexander and Supp 2014). However, other data indicate that Arg is not a key amino acid for skin-repairing processes. First, Arg is not contained in Vulnamin (Gly, Lys, Pro and Leu), one of the most efficient creams for skin ulcers treatment (Corsetti et al. 2010). Second, protein hydrolysates of some mollusks, such as mussels (*Mytilus galloprovincialis)* and hard-shell clam (*Rapana venosa)* are really effective in wound healing. *R. venosa* extracts contain higher amounts of Leu, Pro, Thr and Lys but not Arg than those of mussels, but are more efficient to reduce the time of wound (Badiu et al. 2010).

11.9.2 Glycine (Gly) and Proline (Pro)

Gly and Pro are by far the two most abundant amino acids in collagen, and therefore it is not surprising that they play an important role in the production of this protein (Albaugh et al. 2017). These amino acids are not only abundant constituents of collagens but also regulators of collagen synthesis (Wu et al. 2011). As collagen turnover is perhaps the most important factor to keep skin health, there is a huge amount of nutritional and dermatological studies around it. There are even some collagen-containing-creams used for topical application, although this approach is unfortunately useless as collagen cannot penetrate the skin due to its high molecular mass. Compared with meat (Wu et al. 2016), the content of both glycine and proline in plant-source foods for humans and animals is relatively low (Hou et al. 2019; Li and Wu 2020). The mean molecular mass of the tropocollagen unit is around 130 kDa, much higher than the 0.5 kDa limit for possible skin penetration (Bos and Meinardi 2000). It is much more effective for topical application mixtures of free amino acids containing Gly and Pro than collagen (Corsetti et al. 2010).

Pro, similarly to Lys, undergoes significant post-translational modifications during collagen maturation. A variable number of Pro residues in preprocollagen should be hydroxylated to 4-hydroxyproline (OHPro) by vitamin-C dependent intracellular prolyl hydroxylases (Wu et al. 2019). The HOPro residues are essential for tropocollagen stability due to the formation of hydrogen bonds inside trimers. Degradation of collagen generates OHPro, which is effectively used for glycine synthesis in mammals (including humans) (Wu et al. 2019) and is a potent antioxidant (Ji et al. 2018). Quantitative determination of collagen is difficult, but some methods have been developed for diagnosis of skin disorders based on acidic hydrolysis of a tissue sample and determination of a specific marker (Stoilov et al. 2018). Pyrrole-2-carboxylic acid derived from OH-Pro is used as a biomarker for collagen.

Due to the requirements for HOPro, the mostly used collagen-related products for skincare are short collagen-peptides obtained from partial

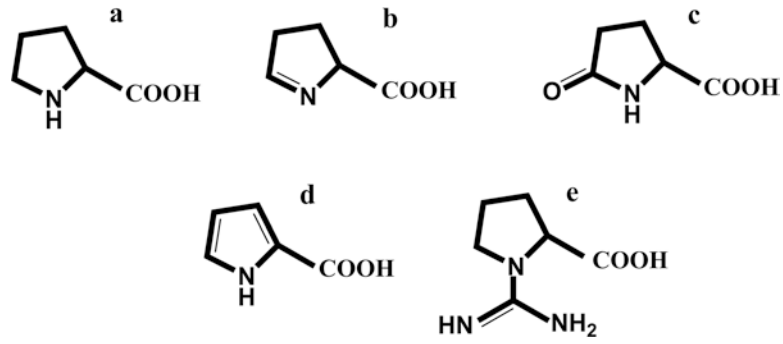

Fig. 11.5 Chemical structure of Proline and derived molecules with relevance in the skin. (**a**) Proline; (**b**) Pyrroline-5-carboxylic acid; (**c**) Pyroglutamic acid (or 5-oxo-Proline); (**d**) Pyrrole-2-carboxylic acid; (**e**) 1-Carbamimidoyl-Proline

hydrolysis. They are mixtures of low-molecular-weight short-peptides obtained from gelatins of several animal sources (Gomez-Guillen et al. 2011). These peptides are most studied than the free amino acids concerning their skin effects. Their beneficial action on human fibroblasts was described 40 years ago (Postlethwaite et al. 1978) and they are currently the most important nutrient to retard skin aging (Sibilla et al. 2015). Identification, traceability, half-life and skin effects of those peptides in human blood have been studied (Iwai et al. 2005; Zague 2008; Draelos 2010). The most effective peptides are Gln-Gly-Ala-Arg (Li et al. 2007), Gly-Pro-HOPro (Watanabe-Kamiyama et al. 2010) and Pro-HOPro (Shigemura et al. 2009). They are absorbed across intestinal brush-border membrane through peptide transporter 1 (Aito-Inoue et al. 2007) and then distributed in the human body. After oral ingestion, Pro-HOPro has a long half-life in human blood until reaching the skin. Inside fibroblasts, this dipeptide is easily hydrolyzed by intracellular prolidase to release the constituent amino acids. This enzyme plays an essential regulatory role in collagen turnover, recycling peptides derived from endogenous or exogenous collagen-degradation products. Several lines of evidence suggest that prolidase activity may be a step-limiting factor of this process during wound healing (Surazynski et al. 2008).

Recently, a double-blind, randomized clinical trial using collagen peptides demonstrated a significant increase in human skin elasticity after daily oral consumption of these products (Genovese et al. 2017). The measured indicators include human skin moisture and surface roughness Proksch et al. (2014). The skin elasticity is regulated, at least in part, by maintaining the hydration degree of the stratum corneum, presumably with the contribution of several amino acids. Another clinical trial (Kawashima et al. 2013) showed that topical treatment with a proline-derivative (1-carbamimidoyl-L-proline, Fig. 11.5) also improved skin elasticity in a number of Japanese women who had crow's feet lines on their faces. 1-Carbamimidoyl-L-proline would stabilize Pro due to the carbamimidoyl group, likely increasing the absorption of this derivative through the skin.

11.9.3 Other Amino Acids

Serine (Ser) and alanine (Ala) are moisturizing agents added to some skincare products. As for free amino acids, Ser and Ala play a general role in water retention in the stratum corneum. Ser is one of the most abundant amino acids in filaggrin, the main skin protein that helps to maintain pH and hydration at the stratum corneum (see above). Therefore, dietary sericin, a Ser-rich silk protein, has been proposed for improving skin hydration in atopic dermatitis (Kim et al. 2012).

Glutamine (Gln) has been proposed as a stimulator of collagen biosynthesis (Karna et al. 2001). Thus, this amino acid is another usual ingredient of diet supplements frequently proposed for improving wound healing because it inhibits protein breakdown (Williams et al. 2002; Morfino et al. 2013; Xi et al. 2012). High levels of Gln are needed for regulation of the acid-base

balance via renal ammonia genesis in the human body. When the intake of Gln decreases, this amino acid is obtained from muscle proteins hydrolysis, but also collagen and elastin, therefore decreasing the amount of those proteins. The mechanism for Gln to stimulate collagen synthesis remains unknown. It may be related to its interconversion in glutamate (Glu) and pyrroline-5-carboxylate (P5C) or pyroglutamic acid (Fig. 11.5) that allows the interconversion of Glu into Pro. Some of these molecules show higher stimulation of collagen biosynthesis than Gln (Karna et al. 2001). P5C induces the expression of prolidase, an enzyme involved in collagen metabolism. Thus, the most likely mechanism for Gln to enhance collagen biosynthesis could involve its conversion into Pro through the intermediate P5C. In most mammals (including humans), the conversion of Gln into P5C occurs exclusively in the enterocytes of the small intestine (Wu and Morris 1998), indicating a close link between gut and skin health.

Aspartic acid (Asp) and asparagine (Asn) are not considered important amino acids for skin health. However, as excitatory neurotransmitters, excessive Asp and Glu may contribute to nociception and inflammatory pain in peripheral tissues including the skin (Omote et al. 1998). On the other hand, Asp has been proposed as an aging index. In mammals, proteins are composed of L-amino acids except for glycine which has no L or L form. However, it was observed that the skin of proteins in old adults contained some D-Asp residues that resulted from the spontaneous racemization of L-Asp residues (Ritz-Timme et al. 2003). Recently, it has been demonstrated that this is a general feature of proteins (Fujii et al. 2018). Asp racemization can be correlated with long-lived proteins, and skin proteins show a low rate of turnover. The accumulation of D-Asp residues is a feature in aging elastin, and therefore the determination of the content of this racemic mixture is useful for assessing the *in vivo* turnover and degradation of skin elastin.

Asn is needed for cell growth. Deprivation of Asn with the bacterial enzyme asparaginase is used to inhibit cell proliferation (Durden 2016). This approach has been used for malignant cells and it might be also applied to the treatment of cell hyperproliferation of keratinocytes, as occurring in psoriasis.

11.10 Non-protein Amino Acids: Mycosporine-Like Amino Acids

These are natural products of the secondary metabolism in organisms living in marine and sunlight environments. The mycosporine family of amino acids consists of several members, but they are not alpha-amino acids. The most studied mycosporine is palythine (Fig. 11.6), extracted from corals and sea hares (Carignan et al. 2009; Kicklighter et al. 2011). These molecules are photostable and show strong UVR-absorbing properties that have evolved for protection against the chronic sunlight exposure, although they can also play other roles as chemical cues. However, the mechanisms behind the UV absorption and its photostability are largely unknown.

Concerning these ecologic photoprotectors, recent data indicate that very low concentrations of palythine can provide a significant protection to human keratinocytes exposed to UVA irradiation (Lawrence et al. 2018). In addition, when palythine is added after UV light exposure, it behaves as a potent antioxidant, reducing oxidative stress by scavenging ROS species. Those results suggest that they have an interesting potential as natural and biocompatible alternatives to currently UVR filters to protect human epidermis.

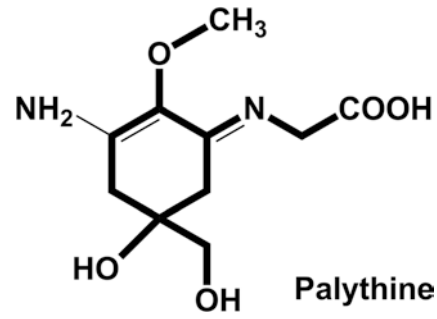

Fig. 11.6 Chemical structure of Palythine, one of the Mycosporine-like amino acids found in marine organisms

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Metabolism and Functions of Amino Acids in Sense Organs

12

Guoyao Wu

Abstract

Sense organs (eyes, ears, nose, tongue, and skin) provide senses of sight, hearing, smell, taste, and touch, respectively, to aid the survival, development, learning, and adaptation of humans and other animals (including fish). Amino acids (AAs) play an important role in the growth, development, and functions of the sense organs. Recent work has identified receptor-mediated mechanisms responsible for the chemosensory transduction of five basic taste qualities (sweet, sour, bitter, umami and salty tastes). Abnormal metabolism of AAs result in a structural deformity of tissues and their dysfunction. To date, there is a large database for AA metabolism in the eye and skin under normal (e.g., developmental changes and physiological responses) and pathological (e.g., nutritional and metabolic diseases, nutrient deficiency, infections, and cancer) conditions. Important metabolites of AAs include nitric oxide and polyamines (from arginine), melanin and dopamine (from phenylalanine and tyrosine), and serotonin and melatonin (from tryptophan) in both the eye and the skin; γ-aminobutyrate (from glutamate) in the retina; and urocanic acid and histamine (from histidine) in the skin. At present, relatively little is known about the synthesis or catabolism of AAs in the ears, nose, and tongue. Future research should be directed to: (1) address this issue with regard to healthy ageing, nasal and sinus cancer, the regulation of food intake, and oral cavity health; and (2) understand how prenatal and postnatal nutrition and environmental pollution affect the growth, development and health of the sense organs, as well as their expression of genes (including epigenetics) and proteins in humans and other animals.

Keywords

Amino acids · Health · Metabolism · Neurotransmitters · Receptors · Sensing

12.1 Introduction

A sense is a physiological response of the body to an external or internal stimulus, such as light, sound, odor, food, or touch. Humans and other animals have five sense organs (eyes, ears, nose, tongue, and skin) that possess sensory capabilities. They provide senses of sight, hearing, smell, taste, and physical contact, respectively, to aid the survival, development, learning, and adaptation of the organisms. Taste receptors for bitter,

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sweet and umami stimuli are G-protein-coupled receptors, taste receptors for sour and salty substances are H^+ and Na^+ ion channels, respectively (Lu and Wu 2016; Ye et al. 2015). Sense organs transmit information to the brain for integration and interpretation through the actions of neurotransmitters. The latter are either amino acids (AAs; e.g., glutamate, aspartate, and glycine) or their metabolites [e.g., nitric oxide (NO), γ-aminobutyrate (GABA), and serotonin; Fernstrom 2013). Sensory capacities differ among animal species and individuals due to different genomes and epigenetic alterations. AAs are necessary for the metabolism (e.g., protein synthesis) and functions of cells in sense organs. In those organs, the syntheses and catabolism of AAs are cell-, tissue-, and species-specific (Table 12.1). Abnormalities in the syntheses, catabolism, or dietary intake of AAs can disturb the homeostasis of sense organs and result in diseases (Wu 2020a). Recent advances in this field are highlighted in the current review.

12.2 Metabolism and Functions of AAs in Eyes

The retina is a thin layer of tissue that lines the inner surface of the back of the eyeball and is located near the optic nerve. This tissue consists of photoreceptor cells (rods and cones), as well as glial and neuronal cells to convert light energy into electrical impulses for initiating the vision process. AAs are essential for retinal maturation,

Table 12.1 Syntheses and catabolism of amino acids (AA) in sense organs

	Synthesis of AAs					Catabolism of AAs				
AA	Eye	Ear	Nose	Tongue	Skin	Eye	Ear	Nose	Tongue	Skin
Alanine	+	+	+	+	+	+	+	+	+	+
Arginine[a]	+	+	+	+	+	+	+	+	+	+
Aspartate	+	+	+	+	+	+	+	+	+	+
Asparagine	+	?	?	?	+	+	?	?	?	+
Cysteine[b]	–	–	–	–	–	?	?	?	?	?
Glutamate	+	+	+	+	+	+	+	+	+	+
Glutamine	+	?	+	?	+	+	?	?	?	+
Glycine[c]	+	?	?	?	+	+	?	?	?	+
Histidine	–	–	–	–	–	?	?	?	?	+
Isoleucine	–	–	–	–	–	+	+	+	+	+
Leucine	–	–	–	–	–	+	+	+	+	+
Lysine	–	–	–	–	–	?	?	?	?	+
Methionine[d]	–	–	–	–	–	?	?	?	?	+
Phenylalanine	–	–	–	–	–	?	?	?	?	+
Proline	?	?	?	?	+	?	?	?	?	+
OH-Proline	+	+	+	+	+	?	?	?	?	+
Serine	?	?	?	?	+	+	?	?	?	+
Threonine	–	–	–	–	–	?	?	?	?	?
Tryptophan	–	–	–	–	–	+	?	?	?	+
Tyrosine	?	?	?	?	+	?	?	?	?	+
Valine	–	–	–	–	–	+	+	+	+	+

"?" denotes the lack of data, "+" denotes the presence of the metabolic pathway; "–" denotes the lack of synthesis of an amino acid from precursors other than its α-ketoacid

[a]Synthesis of L-arginine from L-citrulline via argininosuccinate synthase and lyase, and catabolism of L-arginine via arginase and nitric oxide synthase

[b]Cys may be used for the synthesis of glutathione or conjugation with some biochemicals

[c]Synthesis of glycine from 4-hydroxyproline and serine in the skin, and catabolism of glycine via either conversion into serine by serine hydroxymethyltransferase or oxidative decarboxylation by the glycine cleavage system

[d]Partial catabolism of L-methionine by *S*-adenosylmethionine synthase to form *S*-adenosylmethionine

function, survival, and neurotransmission (Kalloniatis et al. 2013). There are reports on the syntheses of glutamate, glutamine, serine, glycine, alanine, aspartate, ornithine, and arginine, as well as the catabolism of those AAs, branched-chain AAs (BCAAs) and aromatic AAs, in the retinas of animals (see the sections below). However, little is known about the metabolism of other AAs in the retina. Many ocular diseases, including ischemia, retinal detachment, and retinal degeneration retinopathy, are associated with changes in concentrations of AAs in the retina (François 1972; Ripps and Shen 2012). Thus, AAs are essential for the health of the eyes and abnormal AA metabolism contributes to the dysfunction of this organ.

12.2.1 Metabolism of the Glutamate Family of AAs

Glutamate (the major excitatory neurotransmitter) is present in almost all retinal cells (including photoreceptor, bipolar, and ganglion cells) except for glial (Müller) cells that are virtually devoid of glutamate due to its active conversion into glutamine by glutamine synthetase (Pulido et al. 2007; Nivison-Smith et al. 2014). Both ionotropic and metabotropic glutamate receptors are dispersed throughout the retina (Brandstätter et al. 1998). Krebs (1935) discovered that the retinas of animals (cattle, pigs, rats, sheep, pigeons, domestic fowl, tortoises, and trout) synthesize glutamine from glutamate and ammonia, while enzymatically hydrolyzing glutamine into glutamate and ammonia. In this tissue, glutamate is also decarboxylated by glutamate decarboxylase (a cytosolic enzyme) to generate GABA (a major inhibitory neurotransmitter), which is localized in GABAergic and other amacrine cells. There is cell-specific metabolism of glutamine and glutamate in the retina, where glia cells synthesize glutamine from glutamate plus ammonia and then release glutamine. In contrast, photoreceptor cells of the retina take up glutamine and hydrolyze it into glutamate. The photoreceptor cells release the newly synthesized glutamate in exchange for the entry of cysteine (a precursor of glutathione) via the glutamate-cystine transporter (Xc^-) for glutathione and protein syntheses. These cells can also metabolize glutamate via its transamination with oxaloacetate and pyruvate to form aspartate (an excitatory neurotransmitter) and alanine, respectively, in response to physiological needs. Thus, there is an intercellular glutamine-glutamate cycle in the retina. In this tissue, asparagine synthetase converts aspartate and glutamine into asparagine and α-ketoglutaramate, with the latter being metabolized to ammonia and α-ketoglutarate by ω-amidase (Wu 2013).

The metabolism of glutamine and glutamate is necessary to maintain the retina in the healthy state. This view is supported by several lines of evidence. First, inhibiting glutamine synthesis through intravitreal injections of methionine sulfoximine alters the concentrations of AAs in the retina and causes retinal dysfunction (Bui et al. 2009). Second, oral administration of Vigabatrin (a synthetic γ-AA that is an inhibitor of GABA transaminase, a mitochondrial enzyme) to inhibit GABA degradation results in permanent peripheral visual field deficits, retinal electrophysiological changes, and other visual disturbances (Hilton et al. 2004). Third, the glutamate recycling is important for photoreceptor-to-bipolar cell neurotransmission in vivo (Bui et al. 2009). Fourth, glutathione protects the eyes from oxidative damage, and a deficiency of intracellular glutathione in the lens, cornea, retina and other tissues of the eyes is associated with ageing, cataract, diabetes, irradiation, and the administration of some drugs (Ganea and Harding 2006).

12.2.2 Metabolism of Serine and Glycine

Recent years have witnessed growing interest in the metabolism and function of serine in the retina, where this AA is synthesized from 3-phosphoglycerate (an intermediate of glucose metabolism via glycolysis) and glutamate (the donor of the amino group) (Gantner et al. 2019). Serine is converted into glycine by serine hydroxymethyltransferase that is present in both the cytosol and mitochondria. Glycine is used for the syntheses of glutathione and purines, while serving as a major inhibitory neurotransmitter. Glutathione is a potent antioxidant and plays an

important role in cellular redox signaling (Wu et al. 2004), whereas purines, such as cGMP, ATP and hypoxanthine, are required for phototransduction. In addition, serine is a substrate for the generation of glycerophospholipids, sphingosine, and ceramide (Wu 2018), which regulate the proliferation, survival, migration, neovascularization, inflammation and apoptosis of retinal cells (Simón et al. 2019). Results of genome-wide analyses indicate that key enzymes in pathways for serine and glycine metabolism have common variants associated with macular telangiectasia type 2, which is a neurovascular degenerative retinal disease (Scerri et al. 2017). Of particular note, emerging evidence from studies with humans shows that de no synthesis of serine is insufficient for the health of eyes, and an adequate amount of this AA must be provided to both children and adults to protect the retina from damage. For example, when serine is sufficient, serine palmitoyltransferase condenses serine and palmitoyl-CoA into 3-ketodihydrosphingosine, which is a precursor of sphinganine. However, when serine is deficient or a variant of serine palmitoyltransferase is expressed, this enzyme uses alanine as a substrate in addition to palmitoyl-CoA to produce 1-deoxysphinganine. The latter is highly toxic to photoreceptor cells and causes their death, resulting in retinal and peripheral neuropathies (Gantner et al. 2019).

$$\text{Serine} + \text{Palmitoyl} - \text{CoA}\left(\text{Serine Palmitoyltransferase}\right)$$
$$---> 3 - \text{Ketodihydrosphingosine}$$
$$---> \text{Sphinganine}$$

$$\text{Alanine} + \text{Palmitoyl} - \text{CoA}\left(\text{Low Serine or Variant Enzyme}\right)$$
$$---> 1 - \text{Deoxysphinganine}$$
$$---> \text{Deoxysphinganine}$$

12.2.3 Metabolism of Arginine, Proline and Ornithine

Arginine is degraded via arginase II (a mitochondrial enzyme) and NO synthase (a cytosolic enzyme) pathways in the retina. As in many extrahepatic tissues, arginase I (a cytosolic enzyme) is not expressed in either the retina or the cornea (Koshiyama et al. 2000). Arginase hydrolyzes arginine into ornithine and urea, whereas NO synthase [NOS, a tetrahydrobiopterin (BH4)- and NADPH-dependent enzyme) oxidizes arginine to generate NO and citrulline (Wu and Morris 1998). The latter is recycled into arginine by argininosuccinate synthase (requiring aspartate as a co-substrate) and argininosuccinate lyase in the retina and cornea. The arginine-derived ornithine is further metabolized to pyrroline-5-carboxylate (P5C) and putrescine, respectively, by ornithine aminotransferase and ornithine decarboxylase, which are expressed in both the retina and the cornea. P5C is converted into proline (required for collagen synthesis) by P5C reductase, and putrescine is sequentially converted into spermidine and spermine by spermidine synthase and spermine synthase. In addition, human retinal pigment epithelial cells can oxidize proline via proline oxidase to form P5C and then ornithine (Chao et al. 2017). Because arginase and NOS compete for the common substrate (i.e., arginine), a change in arginase activity can affect the rate of NO synthesis by cells in the eyes. Inflammatory cytokines induce the expression of these two enzymes to favor arginine utilization for polyamine and NO syntheses, whereas glucocorticoids enhance the expression of arginase but inhibits the expression of inducible NOS to reduce NO production. Furthermore, the uptake of arginine by the retina is upregulated by the photic stimulus to augment the provision of arginine to the retina (Sáenz et al. 2002).

Arginine metabolism is altered under a variety of pathological conditions, such as diabetes and an inborn deficiency of arginase or ornithine aminotransferase. For example, the retina from streptozotocin-induced diabetic rats (8 days and 4 months after the onset of diabetes) exhibited increases in both arginine uptake and total NOS activity, compared with normal (nondiabetic) rats (do Carmo et al. 1998). Because the activities of both arginase II and ornithine aminotransferase are enhanced in the retina of diabetic rats (Patel et al. 2013), the concentrations of arginine and ornithine in this tissue are decreased compared with normal rats (do Carmo et al. 1998). There is evidence that a deficiency of NO contributes to the development of diabetic retinopathy, a microvascular complica-

tion that is the leading cause of blindness in affected subjects (Patel et al. 2013). Oral administration of arginine to rats with type-I diabetes mellitus ameliorates retinal retinopathy (Savitskyi et al. 2017). Based on the finding that an intravenous infusion of arginine (1 g/min for 30 min) into healthy adults increases retinal and choroidal blood flow (Garhöfer et al. 2005), dietary supplementation with arginine may be effective in treating ocular diseases associated with endothelial dysfunction in diabetes or glaucoma.

Hyperornithinemia is harmful to the retina and results in the gyrate atrophy of the choroid and retina in humans (Kaiser-Kupfer et al. 1991). This is an autosomal recessive, chorioretinal dystrophy that begins in childhood and leads to blindness in the fourth to seventh decade of life. The primary defect is a deficiency of ornithine aminotransferase, which impairs the degradation of ornithine and causes the accumulation of ornithine. This disease also occurs in the gyrate atrophy of the choroid and retina animals with hyperornithinemia due to a deficiency of ornithine aminotransferase (Wang et al. 1995). Thus, correction of ornithine accumulation prevents retinal degeneration in a mouse model of gyrate atrophy of the choroid and retina (Wang et al. 2000). In clinical medicine, long-term (1.5 year) feeding of a diet containing 10–20 g of protein plus adequate amounts of nutritionally essential AAs can maintain plasma ornithine at 55–355 μM, inhibit chorioretinal degeneration, and improve vision in patients with a deficiency of ornithine aminotransferase (Valle et al. 1980). Similarly, long-term (5–7 years) reduction in plasma ornithine concentration through the restriction of dietary arginine intake slows retinal degeneration in children with an inborn deficiency of ornithine aminotransferase and gyrate atrophy (Kaiser-Kupfer et al. 1991).

12.2.4 Metabolism and Function of Taurine

Taurine (a β-AA) is synthesized in the liver of humans and most of other animals (Wu 2020b). This AA is neither synthesized nor degraded in the retina and cornea. These tissues take up taurine from the arterial blood so that its retinal concentration can be 20–35 mM. Taurine plays an important role in the regulation of osmolality, cell volume, muscular function, neurological activity, antioxidant defense, immune response, as well as the stabilization of proteins and lipids. In addition, through the formation of taurinechloramine, taurine contributes to immunomodulation in the body. Furthermore, taurine participates in the production of rod photoreceptors during development through interactions with glycine receptors and GABA receptors (Ripps and Sen 2012). Thus, a deficiency of dietary taurine results in retinal degeneration in cats (Hayes et al. 1975) and children (Geggel et al. 1985) that can be corrected with taurine supplementation. Interestingly, taurine depletion in the plasma and retina contributes to the retinal toxicity of Vigabatrin (an antiepileptic drug that may inhibit hepatic taurine synthesis) in humans that is characterized by cone damage and the loss of retinal ganglion cells (Krauss 2009). Results of animal studies have shown that Vigabatrin causes abnormalities of the photopic cone system, including marked gliosis and disorganization in the peripheral retina as well as smaller photoreceptor losses in central cone segments (Duboc et al. 2004). Taurine supplementation can reduce the retinal lesions in Vigabatrin-treated rats and mice (Jammoul et al. 2008).

12.2.5 Metabolism of BCAAs

Branched-chain AAs (BCAAs) are degraded in the retina via BCAA transaminase (present in both the cytosol and mitochondria) and branched-chain α-ketoacid (BCKA) dehydrogenase, a mitochondrial enzyme (Frayser and Buse 1978). The BCAA-derived glutamate and aspartate likely contribute to neurotransmission and the inter-cellular glutamine-glutamate cycle in the retina. Interestingly, the concentrations of BCAAs in the plasma and retinas of streptozotocin-induced diabetic rats are elevated due to increases in both whole-body protein breakdown and the retinal uptake of BCAAs, although the rates of retinal BCAA catabolism

are greater in diabetic animals, compared with the normal (nondiabetic) ones (Frayser and Buse 1978). It is unknown whether glutamate production contributes to retinal dysfunction in diabetic subjects.

Glycolysis is the exclusive source of energy for the retina (Wu 2018). There are reports that in nondiabetic subjects, BCAAs help to maintain the integrity and function of the retina by promoting glycolysis for ATP production (Hasegawa et al. 2018). The underlying mechanisms are unknown. These authors demonstrated that administration of BCAAs to mouse models of retinitis pigmentosa and glaucoma maintained adequate ATP concentrations in the retinal cells and attenuated retinal degeneration. This finding is novel and important, as retinitis pigmentosa and the more common glaucoma are leading causes of blindness worldwide (Bourne et al. 2013).

12.2.6 Metabolism and Function of Aromatic AAs

Studies with several species (e.g., cattle, chicks, rabbits and rhesus monkeys) have revealed that aromatic AAs (phenylalanine, tyrosine and tryptophan) can be catabolized in the intact retina. Physiologically important products of the phenylalanine and tyrosine catabolism in the dopaminergic, interplexiform amacrine cells of the retina include melanin and dopamine (Witkovsky and Dearry 1991; Gustincich et al. 1997), whereas those generated from tryptophan in the photoreceptor cells of the retina include serotonin and melatonin (Chanut et al. 2002; Tosini et al. 2012). Note that dopamine, serotonin and melatonin are neurotransmitters in the retina. The pathways for the degradation of aromatic AA are initiated by specific aromatic AA decarboxylases, such as phenylalanine hydroxylase for phenylalanine, tyrosine hydroxylase for tyrosine, and tryptophan hydroxylase for tryptophan (Parkinson et al. 1981; Fernstrom et al. 1989; Iuvone et al. 2000). Intravitreal administration of α-fluoromethyldopa (an irreversible mechanism-based inactivator of aromatic AA decarboxylase) rapidly reduces the concentrations of dopamine in the retinas of chickens and rabbits (Parkinson et al. 1981). Amacrine neurons generate serotonin, dopaminergic amacrine cells synthesize dopamine in the retina, and photoreceptor cells produce melatonin at high levels in the darkness (e.g., during the night) and lower levels in response to light (e.g., during the day). Dopamine receptors (D1 and D2) are distributed throughout the retina, and melatonin receptors (MT_1 and MT_2) are expressed in the retina (all layers of the neural retina and in the retinal pigmented epithelium) (Tosini et al. 2012). There is diverse expression of serotonin (5-HT) receptors in the mammalian retina (Popova and Kupenova 2017). Specifically, 5-HT1a is localized in photoreceptor cells, ganglions cells, and a population of cells in the inner nuclear layer, 5-HT5a is restricted to ganglion cells, and 5-HT1b is predominantly in the outer plexiform layer. Retinal dopamine, serotonin and melatonin are involved in neuromodulation as well as the survival and vision function of photoreceptor cells (Witkovsky 2004; Masson 2019). In addition, melatonin acts directly on ocular structures (the retina, ciliary body, lens and cornea) to mediate a variety of diurnal rhythms (including photoreceptor disc shedding, neuronal sensitivity, and intraocular pressure control) and physiological processes within the eye (Ostrin 2019). Thus, the metabolism of aromatic AAs in the retina is essential to the eye function.

12.3 Metabolism and Functions of AAs in the Nose

In humans and other vertebrates, the nose is the primary organ of smell and is also the entrance to the respiratory tract. This organ consists of the olfactory epithelium, which is a specialized epithellial tissue inside the nasal cavity that contains olfactory receptor cells (neurons). Olfactory receptors (also known as odorant receptors; members of the class A rhodopsin-like family of G protein-coupled receptors) are expressed in the plasma membrane of olfactory receptor cells, and are responsible for the detection of odorants that give rise to the sense of smell (Fleischer et al.

2009). Activated olfactory receptors trigger nerve impulses, which transmit information about odor to the central nervous system (brain). At present, little is known about AA metabolism in the nose. Chemuturi and Donovan (2006) reported that the nasal mucosa can metabolize dopamine via monoamine oxidase to form dihydroxyphenylacetic acid.

Extensive studies have shown that fish can sense the smell of AAs dissolved in water (Caprio 1978; Schiffman et al. 1981; Caprio and Byrd 1984). For example, in channel catfish, AAs (10–100 μM) act as ligands for olfactory receptors with high affinity and specificity. Interestingly, these receptors contain independent binding sites for acidic AAs (aspartate and glutamate); basic AAs (arginine and lysine); glutamine and small neutral AAs (glycine, alanine, serine, and possibly cysteine); and large neutral AAs (methionine, 3 BCAAs, and phenylalanine) and possibly cysteine (Caprio and Byrd 1984). Hammerhead sharks, which possess an expanded head and the enlarged olfactory epithelium, have a well-developed olfactory system. Work with hammerhead sharks (large carnivorous fish that have an enlarged olfactory epithelium) indicates that micromolar concentrations of cysteine evoke the greatest electro-olfactogram response among all 20 proteinogenic AAs (Tricas et al. 2009). In these aquatic animals, olfactory thresholds for detection differ among AAs: alanine (92 pM), cysteine (835 pM), aspartate (63.7 nM), methionine (13.6 nM), serine (216 nM), and proline (3.28 μM). These thresholds values for hammerhead shark are comparable or lower than those reported for other teleost and elasmobranch species (Tricas et al. 2009). Likewise, in humans, AAs contribute to the flavor of food by serving not only as taste stimuli (via a G-protein-coupled sensing mechanism) but also as olfactory stimuli (via ortho- or retronasal smell) (Laska 2014). In adult men and women, olfactory thresholds for AA detection are 10 μM for D-methionine and 80 μM for L-methionine; 220 μM for D-cysteine and 200 μM for L-cysteine; 75 μM for D-proline and 100 μM for L-proline (Laska 2014). Thus, the sensitivity of the nose to AAs varies greatly among animal species.

Some microbial metabolites, such as dimethylamine and trimethylamine with a strong odor), stimulate the olfactory bulb of fish and, therefore, are used as feed attractants (Harada 1985). Intestinal bacteria converts dietary choline, betaine and carnitine into dimethylamine and trimethylamine, which is subsequently metabolized into trimethylamine-*N*-oxide (an odorless substance) in the liver (Messenger et al. 2013). The presence of dimethylamine and trimethylamine in fishmeal contributes to the preference of fish for this high-protein feedstuff. Similarly, in humans, excessive production of trimethylamine due to increases in dietary protein intake as well as the number and activity gastrointestinal bacteria, along with a reduced ability to break down trimethylamine in the body, can result in fish odor (Mackay et al. 2011). Of note, trimethylaminuria (also known as fish odor syndrome) occurs in humans with mutations in the FMO3 gene (Treacy et al. 1998). This gene encodes for a hepatic enzyme named flavin-containing monooxygenase 3, which catalyzes the *N*-oxygenation of dietary or bacterial trimethylamine into trimethylamine *N*-oxide. A defect of this enzyme causes the accumulation of trimethylamine in the sweat, urine, and breath of affected individuals, giving rise to a strong fishy odor in humans.

12.4 Metabolism and Functions of AAs in the Tongue

The tongue is the muscular organ within the cavity of the vertebrate mouth and is almost completely covered by a mucosa. Much of the tongue's surface is covered by taste buds housed in numerous lingual papillae. Taste receptor cells are located on the taste buds of the tongue and connect with nerves that detect and transmit taste signals to the brain, giving rise to five distinct tastes (sweet, sour, bitter, salty, and umami). At present, little is known about AA metabolism in the tongue. However, there are reports that some AA metabolites in the tongue and the oral cavity, such as hydrogen sulfide (H_2S) and methyl mercaptan (methanethiol, CH_3-SH), are the sources of bad breath in humans (Porter and Scully 2006).

Furthermore, a dietary deficiency of isoleucine, leucine or phenylalanine causes a deformity of the tongue (i.e., the folding-back of the tip of the tongue) in chicks (Grau 1945) and young turkeys (Bragg 1953), indicating an important role for these AAs in the development of the tongue.

12.4.1 Tastes of AAs

Different AAs confer different tastes (Table 12.2). Generally speaking, small AAs [except for small sulfur AA (taurine and cysteine)] and β-alanine confer sweet taste, whereas most of the large L-AAs are bitter tastants. Many large D-AAs (including D-leucine and D-tryptophan except for D-lysine, D-proline and D-4-hydroxyproline), and some small D-AAs (including D-alanine and D-serine except for D-cysteine and D-threonine) have sweet taste. D-Arginine, D-lysine, D-cysteine, D-proline and D-4-hydroxyproline have a bitter taste, whereas β-alanine, L-cystine, L-methionine, and L-tyrosine are flat (lacking flavor) (Schiffman et al. 1981; Birch and Kemp 1989; Bachmanov et al. 2016). The taste of an AA may differ among animal species. For example, pigs (Hu et al. 2015) and rats (Tsubuku et al. 2004) tolerate very well crystalline L-arginine (in either the base or HCl form) supplemented into their pellet diets, but humans are very sensitive to the unpleasant taste of the L-arginine base and would not consume any unencapsulated crystalline L-arginine or its solution. Thus, when L-arginine-HCl (with a slight characteristic taste) is orally administered to humans, it is usually dissolved in a lemon-based solution to induce palatability (McNeal et al. 2018).

Table 12.2 Tastes of amino acids in humans

Taste	Amino acids
Sweet	L-Alanine[a], γ-aminobutyrate, L-citrulline[a], glycine, L-4-hydroxyproline[a], L-proline[a], L-serine[a], L-threonine[a];
	D-Alanine; D-asparagine, D-glutamine, D-histidine, D-isoleucine, D-leucine, D-methionine, D-phenylalanine, D-serine, D-threonine, D-tryptophan, D-tyrosine, D-valine
Sour	L-Aspartate (with a slight Umami flavor), L-cysteine-HCl, L-glutamic acid-HCl;
	D-Aspartate[b], D-aspartic acid, D-cysteine, D-glutamic acid, D-histidine-HCl[b]
Bitter	L-Arginine (a characteristically unpleasant taste), L-glutamate monoammonium, L-isoleucine[c], L-phenylalanine[c], L-lysine[c], L-histidine-HCl[c] (slightly bitter after an initial sour taste), L-valine[a] (bitter after an initial slightly sweet taste);
	D-Arginine, D-cysteine, D-4-hydroxyproline, D-lysine, D-lysine-HCl, D-proline
Umami[d]	L-Glutamate (e.g., L-monosodium glutamate and monopotassium glutamate), glutamic acid
Flat[e]	β-Alanine, L-cystine, L-methionine, L-tyrosine, D-Cystine;
	L-Asparagine (flat to slightly sweet), L-glutamine (flat to slightly sweet and meaty), D-glutamate (almost tasteless), taurine (flat to very slightly sour);
Flat[e] to SB	L-Cysteine, L-histidine, L-leucine, L-ornithine, L-tryptophan
SCT	L-Arginine-HCl, L-lysine, L-ornithine-HCl

[a]Slightly sweet
[b]Complex with sour and slightly bitter tastes
[c]Slightly bitter
[d]Meaty, broth-like, or savory taste
[e]Flat (lacking tastiness or flavor; tasteless) to slightly bitter
SB slightly bitter, *SCT* slight characteristic tastes. Each of these three amino acids have different characteristic tastes

12.4.2 Taste Receptors in the Plasma Membrane of Taste Receptor Cells

Taste cells express different taste receptors (transmembrane proteins) for different tastants. The gene *Tas2R* encodes for bitter receptor proteins, whereas the gene *Tas1R* encodes for sweet and umami receptor proteins (Jaggupilli et al. 2016); The latter have three subunits, Tas1R1, Tas1R2, and Tas1R3. Taste receptors for bitter, sweet, and umami tastants are all transmembrane G protein–coupled receptors that are composed of a GTP-binding α subunit and βγ-subunit (Banik et al. 2019). The G-protein's α-subunit (α-gustducin) is expressed in taste buds of all taste papillae (circumvallate, foliate and fungiform) (Lee and Owyang 2017). As noted previously, sour and salty taste receptors are H^+ and Na^+ channels, respectively.

Bitter receptors are Tas2Rs (or T2Rs) belong to the taste receptor family 2 proteins and are composed of about 30 members (Matsunami et al. 2000). They were originally identified in type II taste receptor cells in the taste bud of the tongue

(Chandrashekar et al. 2000, 2006; Shi et al. 2003), where they initiate bitter taste perception. Subsequent work has shown that T2Rs are expressed widely in many organs and cell types, including the intestinal, respiratory, genitourinary, central nervous, and immune systems, to mediate both tasting and nontasting responses of the organisms to bitter tastants (Jaggupilli et al. 2016).

Sweet taste receptors are heterodimers of taste 1 receptor member 2 (Tas1R2 or T1R2) and taste 1 receptor member 3 (Tas1R3 or T1R3; Li et al. 2002), and are present in many organs, including the gastrointestinal tract as well as the hypothalamus (Lee and Owyang 2017). Note that two T1R3 molecules can form a homodimeric low-affinity sweet taste receptor (T1R3-T1R3) (Zhao et al. 2003). Sweet taste receptors can be activated by such ligands as sugars (fructose, glucose, maltose and sucrose), artificial sweeteners [e.g., aspartame (L-aspartyl-L-phenylalanine), cyclamate and saccharin], as well as sweet-tasting AAs and proteins (e.g., brazzein, monellin and thaumatin).

Three known umami (meaty, broth-like, or savory taste) substances are glutamate, 5′-inosine monophosphate, and 5′-guanosine monophosphate (Kurihara 2009). Umami receptors (Tas1R1 + Tas1R3, mGluR4, and mGluR1) have been identified over the past 20 years. Tas1Rs include Tas1R1 and Tas1R3 and are the family C of transmembrane G protein–coupled receptors, as noted previously. These receptors have three regions: the large extracellular region, the seven-spanning transmembrane region, and the cytoplasmic region (Muto et al. 2007). A synergism exists between glutamate and 5′-nucleotides to enhance the response of taste cell receptors to glutamate (Kurihara 2009; San Gabriel et al. 2005, 2009). In humans and rats, the response of the taste cell receptors to a mixture of L-glutamate plus 5′-inosinate is approximately 8 and 1.7 times, respectively, greater than that to glutamate alone (Kurihara 2009). Mammalian milk contains not only abundant L-glutamate but also 5′-inosinate (Rezaei et al. 2016; Wu and Knabe 1994), and these nutrients are also present in the lumen of the small intestine as shown for AAs in Table 12.3. This explains why human infants and livestock neonates enjoy the consumption of their mothers' milk.

Sour taste is initiated by protons (H^+ ions) acting on specific receptors [e.g., hyperpolarization-activated and cyclic-nucleotide-gated (HCN) channels, HCN1 and HCN4] in a subset of taste cells on the tongue and palate epithelium (Stevens et al. 2001). These cells do not express gustducin, which is the G-protein participating in the sensing of bitter and sweet taste. Extracellular H^+ ions enter sour taste cells through HCN1 and HCN4, resulting in changes in membrane potential and intracellular acidification that play an important role in the transduction of sour taste to the central nervous system (Liman et al. 2014). A second element of the sensory transduction in sour taste cells involves an acid-sensitive K^+ channel ($K_{IR}2.1$), which is inhibited by intracellular acidification (Ye et al. 2015). The presence of $K_{IR}2.1$ allows for the amplification of the sensory response. This may help to explain why weak acids (e.g., acetic acid) taste sourer than strong acids (e.g., HCl and H_2SO_4).

The sodium taste receptor [also known as the epithelial sodium channel (ENaC)] has been shown to mediate the sense of salty taste (Chandrashekar et al. 2010). Passive influx of Na^+ ions through the ion channel via a Na^+ concentration gradient depolarizes taste receptor cells, leading to changes in their membrane potential and excitement. In mice, the taste for low concentrations of sodium ions (NaCl, typically conferring good appetite) is highly selective and is blocked by an ion-channel inhibitor called amiloride. However, detection of high concentrations of sodium ions (generally eliciting robust behavioral aversion) is not selective for sodium ions and is not blocked by amiloride, and can be substituted by other salts (Ye et al. 2015).

12.4.3 Mechanisms of Actions Bitter, Sweet, or Umami Tastes

Transduction of bitter, sweet, or umami tastes involves G protein-coupled receptors, as noted previously. The binding of a ligand (e.g., a bitter, sweet, or umami tastant) at its taste receptors (e.g., Tas2Rs, Tas1R2 + Tas1R3, or Tas1R1 + Tas1R3, respectively) leads to the dissociation of a heterotrimeric G-protein into an α

Table 12.3 Concentrations of free amino acids and ammonia (mM) in the jejunal luminal fluid of sow-reared piglets

Amino acid	Day 2	Day 7	Day 14	Day 21
Ala	1.85 ± 0.24	2.09 ± 0.27	2.26 ± 0.31	2.21 ± 0.34
Arg	0.79 ± 0.09[b]	1.66 ± 0.20[a]	1.52 ± 0.23[a]	1.40 ± 0.25[a]
Asn	0.52 ± 0.08	0.61 ± 0.10	0.56 ± 0.07	0.50 ± 0.08
Asp	1.03 ± 0.16	1.27 ± 0.21	1.46 ± 0.19	1.35 ± 0.17
Citrulline	0.10 ± 0.02	0.09 ± 0.02	0.11 ± 0.02	0.12 ± 0.02
Gln	1.96 ± 0.21[b]	2.06 ± 0.25[b]	2.28 ± 0.32[b]	3.25 ± 0.34[a]
Glu	3.62 ± 0.52	3.04 ± 0.46	2.96 ± 0.30	2.88 ± 0.37
Gly	1.97 ± 0.24	2.05 ± 0.18	2.45 ± 0.14	2.24 ± 0.21
His	0.52 ± 0.07	0.55 ± 0.07	0.58 ± 0.08	0.53 ± 0.08
Ile	0.66 ± 0.07	0.70 ± 0.09	0.74 ± 0.08	0.76 ± 0.10
Leu	2.72 ± 0.29	2.94 ± 0.33	3.15 ± 0.36	3.23 ± 0.41
Lys	3.14 ± 0.32	2.98 ± 0.35	3.22 ± 0.38	3.06 ± 0.43
Met	0.61 ± 0.08	0.64 ± 0.07	0.67 ± 0.09	0.70 ± 0.08
Ornithine	0.40 ± 0.06	0.46 ± 0.05	0.53 ± 0.07	0.56 ± 0.08
Phe	0.88 ± 0.13	0.94 ± 0.15	0.96 ± 0.11	1.02 ± 0.17
Pro	3.72 ± 0.44	3.51 ± 0.48	3.39 ± 0.41	3.26 ± 0.47
Ser	1.27 ± 0.16	1.15 ± 0.18	1.34 ± 0.23	1.22 ± 0.19
Taurine	0.15 ± 0.02	0.18 ± 0.03	0.19 ± 0.03	0.21 ± 0.03
Thr	0.70 ± 0.09	0.76 ± 0.13	0.88 ± 0.15	0.82 ± 0.13
Trp	0.20 ± 0.03	0.22 ± 0.03	0.24 ± 0.03	0.21 ± 0.02
Tyr	1.06 ± 0.15	1.28 ± 0.21	1.40 ± 0.19	1.23 ± 0.22
Val	0.73 ± 0.09	0.78 ± 0.12	0.82 ± 0.10	0.85 ± 0.12
Ammonia[c]	0.28 ± 0.01	0.20 ± 0.02	0.22 ± 0.01	0.21 ± 0.01

Data are means ± SEM, n = 6. Milk was obtained from sows on Days 2, 7, 14 and 21 of lactation, as previously described (Wu and Knabe 1994). Jejunal luminal fluid samples were obtained from sow-reared piglets at 1 h after suckling

[a-b]Within a row, means not sharing the same superscript letters differ ($P < 0.05$), as analyzed by one-way ANOVA and the Student-Newmans-Keul multiple comparison test (Assaad et al. 2014)

[c]NH_4^+ plus NH_3

subunit (α-gustducin) and βγ subunits (Fig. 12.1). The α-gustducin activates phospholipase C β2 (a membrane-associated enzyme), which stimulates the release of inositol-1,4,5-triphosphate (IP3) and diacylglycerol from membrane phospholipids (i.e., phosphatidylinositol 4,5-bisphosphate). The IP3 increases the intracellular concentration of Ca^{2+} through (1) activating Ca^{2+} channels for Ca^{2+} influx and (2) inducing the release of Ca^{2+} from the endoplasmic reticulum. This, in turn, activates the transient receptor potential cation channel M5 (TRPM5), leading to Na^+ influx and the depolarization of the plasma membrane, followed by the release of ATP from taste cells in the tongue or the production of NO and serotonin by taste cells in the gastrointestinal tract. In the tongue, ATP activates adjacent sensory gustatory (afferent) neurons that send signals (electrical impulses) to brain. In the gastrointestinal tract, the depolarization of the plasma membrane of taste cells enhances the direct release of serotonin from the cells and the release of serotonin from NO-stimulated enteroendocrine cells; serotonin acts on vagal nerve endings, which transmit signals to the forebrain. Thus, in chemosensory transduction, different taste receptors share the use of phospholipase phospholipase C β2 and the TRPM5 ion channel. The physiological responses help humans and other animals to avoid foods with strong bitter or sour tastes and protect the organisms against poisons.

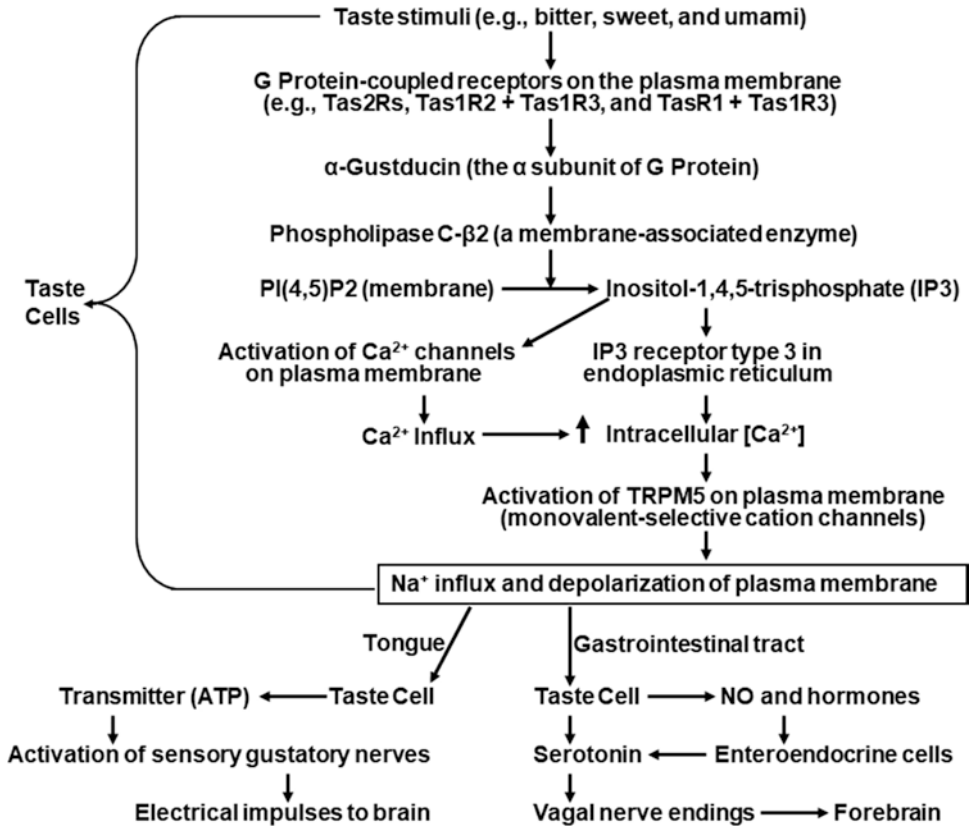

Fig. 12.1 Taste signaling mechanisms in the digestive tract (including the tongue) of humans and other animals. Extracellular taste stimuli induce a series of chemosensing cascade reactions that are common in both the tongue and the gastrointestinal tract. *NO* nitric oxide, *PI(4,5)P2* phosphatidylinositol 4,5-bisphosphate, *TRPM5* transient receptor potential melastatin 5 (selective cation channel), *Tas1R1* taste receptor type 1, member 1, *Tas1R2* taste receptor type 1, member 2, *Tas1R3* taste receptor type 1, member 3, *Tas2R* taste receptor type 2 (Tas2R)

12.5 Metabolism and Functions of AAs in the Ear

The ear, which is the organ of hearing and in mammals also balance, consists of the outer ear (the pinna and the ear canal), the middle ear (including the tympanic cavity and the three ossicles), and the inner ear (the semicircular canals, the utricle and saccule, and the cochlea for hearing). A signaling cascade enables hearing and balance via a transmembrane channel-like protein (TMC-1) located in the inner ears that is present in mammals, birds, fish, amphibians, and reptiles (Pan et al. 2018). This protein forms a sound- and motion-activated pore that allows the conversion of sound or head movement into nerves that send signals to the brain. Severe damage to the inner ear (as inflicted by cochlear ablation) results in decreases in the concentrations of glutamate and aspartate in the ipsilateral ventral cochlear nucleus and the deep layer of the dorsal cochlear nucleus (Godfrey et al. 2015). Conversely, impaired AA metabolism, a deficiency of glutathione, and oxidative stress increases risk for sensorineural hearing loss (Capaccio et al. 2012). For example, accumulation of homocysteine in the cochlea because of a defect in its recycling into methionine due to mutations of the genes (including methionine synthase) or a deficiency of water-soluble vitamins contributes to the onset and progression of sensorineural hearing loss (Partearroyo et al. 2017). In addition, excessive production of NO from arginine by inducible NO synthase under

inflammatory conditions may result in cochlear damage, and the injury can be prevented by inhibiting inducible NO synthase (Watanabe et al. 2000). Furthermore, the cochleas of normal rats contain substantial amounts of spermidine and spermine but no detectable putrescine (Schweitzer et al. 1986). Interestingly, ornithine decarboxylase activity is not detectable in this tissue (Schweitzer et al. 1986), raising an important question of whether spermidine and spermine in the cochlea are derived from the blood circulation. At present, there is a paucity of information regarding the metabolism of AAs in the different parts of the ear. However, there are reports that the concentrations of aspartate and glutamate are higher in the endolymph than in the perilymph, whereas those of all other AAs are substantially lower in the endolymph than in the perilymph (Thalmann and Thalmann 1987). Thus, it is possible that cell-specific synthesis and catabolism of AAs exist in the ear.

12.6 Metabolism and Functions of AAs in the Skin

The skin is a large organ in the body to provide a physical barrier from the external environment (including water, toxic chemicals, and pathogens), regulate the body temperature, and permit the sensations of physical contact. The skin has several layers, including (1) the epidermis (the outermost layer of skin), (2) the dermis (beneath the epidermis, containing tough connective tissue, hair follicles, and sweat glands); and (3) the deeper subcutaneous tissue (hypodermis) that consists of fat and connective tissue (Slominski et al. 2012). The skin has an extensive neural network represented by somato-sensory and autonomic nerve fibers. Skin health is particularly important for aquatic animals, which are constantly in contact with water and toxic molecules in the living environment. In this volume, Solano (2020) has extensively reviewed the metabolism and functions of AAs in the skin, and here we only highlight key aspects related to skin health and growth. Epidermis melanocytes of the human skin contain all the enzyme [including phenylalanine hydroxylase and tyrosine hydroxylase (Schallreuter et al. 2004)] for converting: (1) phenylalanine into tyrosine and then the tyrosine molecule into the pigment melanin (Serre et al. 2018), and (2) tryptophan into melatonin and serotonin (Slominski et al. 2008, 2012). Human phenylalanine hydroxylase is activated by H_2O_2, which provides a mechanism for increasing the intracellular provision of L-tyrosine for melanogenesis in melanocytes (Schallreuter et al. 2004). The skin's color is determined by the amount of melanin and related metabolites in melanocytes. Melanin absorbs the harmful sunlight UV rays and protects the cutaneous DNA from damage (Fajuyigbe et al. 2018), whereas melatonin protects the skin from oxidative stress and regulates hair growth (Slominski et al. 2008). Similarly, urocanic acid (a metabolite of histidine in melanocytes) confers a photoprotective effect in the skin. Furthermore, histamine is produced from histidine by both mast cells and other cells of epidermis and dermis to act locally through binding to H1-H4 receptors (Slominski et al. 2012).

Collagen is the most abundant protein in the skin. Thus, adequate provision of glycine and proline is essential for skin health (Li and Wu 2018). These two AAs are generally deficient in plant-source foods (Hou et al. 2019) but are highly abundant in meat (Wu 2020b). Thus, regular consumption of meat (e.g., beef) is beneficial for delaying skin ageing and maintaining bone strength in humans. For a 70-kg healthy adult consuming 52.5 g protein/day, dietary intake provides only 14% of the daily requirement for glycine, and the endogenous synthesis of glycine from 4-hydroyproline in tissues (including the skin, Fig. 12.2) contributes to 59% of the daily requirement for glycine (Wu 2020b). Likewise, for piglets reared by sows, milk-borne and endogenous collagen-derived 4-hydroxyproline contribute to 14% and 31%, respectively, of glycine needed by the young pig (Wu et al. 2019). Nearly all ($\geq$ 95%) of the skin-derived 4-hydroxyproline is used for glycine synthesis in the body. This metabolic pathway allows for the conversion of (1) arginine and proline into glycine via the post-translational hydroxylation of collagen's proline residues in all animals, and (2) glutamine and

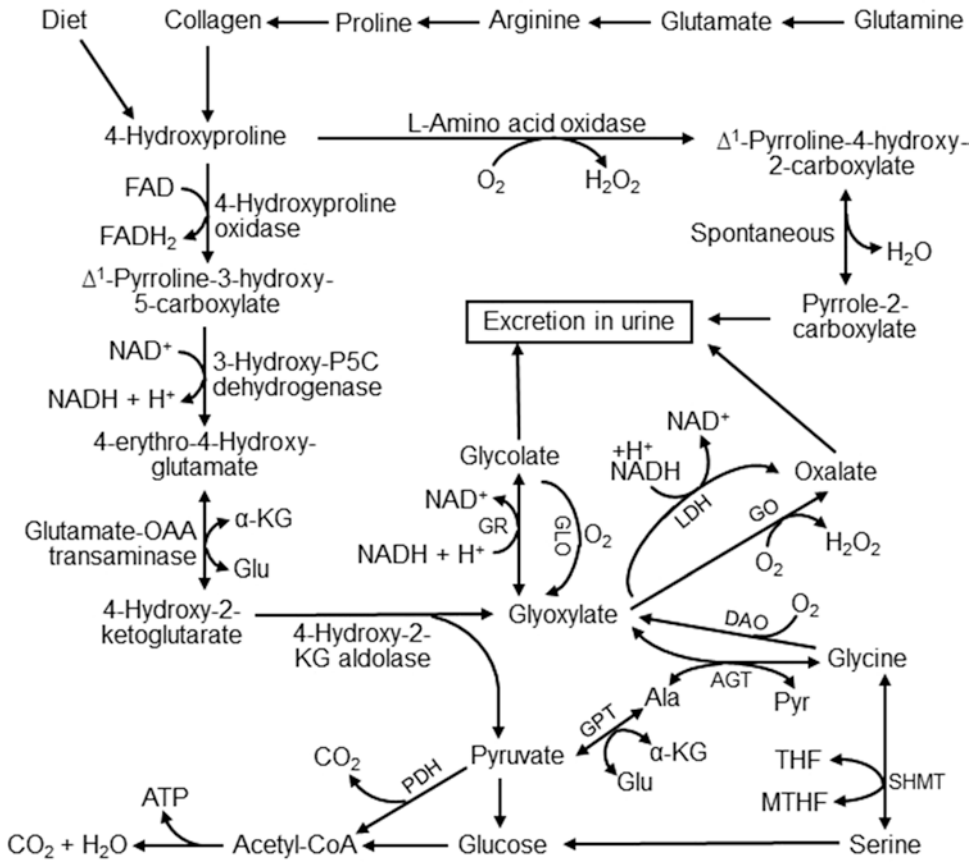

Fig. 12.2 Catabolism of 4-hydroxyproline in animal tissues. This metabolic pathway occurs in humans and other animals (including fish), with the skin, small intestine, liver, kidneys and skeletal muscle being the major sites for the conversion of 4-hydroxyproline into glycine. Through L-amino acid oxidase, 4-hydrocyproline is oxidized to pyrrole-2-carboxylate. *Ala* alanine, *AGT* alanine-glyoxylate aminotransferase, *DAO* D-amino acid oxidase, *GLO* glycolate oxidase, *Glu* glutamate, *GO* glyoxylate oxidase, *GPT* glutamate pyruvate transaminase, *GR* glyoxylate reductase, *α-KG* α-ketoglutarate, *LDH* lactate dehydrogenase, *MTHF* N^5-N^{10}-methylene tetrahydrofolate, *OAA* oxaloacetate, *PDH* pyruvate dehydrogenase, *Pyr* pyruvate, *SHMT* serine hydroxymethyl transferase, *THF* tetrahydrofolate. Adapted from Wu et al. (2019)

glutamate into glycine in humans and other mammals (including pigs, cattle and rats), as well as birds and fish.

Lysine is required for the proper structure of collagen and elastin proteins (Li and Wu 2018). Specifically, some lysine residues in these proteins must be hydroxylated by vitamin C-dependent lysyl hydroxylase and lysyl oxidase for their maturation and function. In humans, about 20% of the collagen's lysine residues are 5-hydroxylated (Veis and Anesey 1965), followed by the addition of carbohydrate moieties through the action of glycosyl transferases. Once the tropocollagen or proelastin is secreted to the extracellular matrix, some ε-amine groups of lysyl or 5-hydroxylysyl residues are oxidized to aminoadipic semialdehyde by lysyl oxidases (a copper-containing amine oxidase in the skin). The aldehyde group of aminoadipic semialdehyde further reacts with some ε-NH_2 groups of unaltered lysyl residues to form covalent cross-linked bonds among tropocollagen units. This is essential to tensile structure and strength. Other cross-linking reactions may result in the formation of lysinonorleucine, merodesmosine, and desmosine or isodesmosine. Note that desmosine

is primarily responsible for the elasticity of elastin and is often used to indicate the content of the elastin (Stoilov et al. 2018).

12.7 Conclusion and Perspectives

AAs as well as their receptors and transporters play an important role in the growth, development, and functions of sense organs in humans and other animals (including fish). Knowledge of the smell and taste of AAs can guide the practice of their supplementation to diets for humans and other animals. Abnormal metabolism of AAs results in a deformity of tissue structures and the dysfunction of the organs. For AAs that are synthesized in the sense organs, the necessary biochemical pathways are cell-, tissue, and species-specific. This metabolic principle also applies to the catabolism of all AAs in the organs. To date, there is a large database for AA metabolism in the eye and skin under normal (e.g., developmental changes and physiological responses) and pathological (e.g., nutritional and metabolic diseases, nutrient deficiency, infections, and cancer) conditions. However, relatively little is known about the synthesis or catabolism of AAs in the ear, nose, and tongue. This should be an active area of biomedical research, particularly regarding healthy ageing, nasal and sinus cancers**,** the regulation of food intake, and oral cavity health). Because BCAA transaminase, glutamate-pyruvate transaminase, and glutamate-oxaloacetate transaminase are widely spread in animal tissues (Wu 2013), it is expected that BCAAs, glutamate, alanine, and aspartate are degraded in the ear, nose, and tongue as in the eyes and skin. The development and health of the sense organs are particularly vital for aquatic animals because they are constantly exposed to water, potentially harmful chemicals, and predators in their naturally living environment. Future research is warranted to study how prenatal and postnatal nutrition and environmental pollution affect the growth, development and health of the sense organs, as well as their expression of genes (including epigenetics) and proteins in humans and other animals. This knowledge can be applied to prevent and treat disorders in the eyes, ears, nose, tongue, and skin.

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