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REVIEW ARTICLE

Amino acids: metabolism, functions, and nutrition

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Abstract Recent years have witnessed the discovery that amino acids (AA) are not only cell signaling molecules but are also regulators of gene expression and the protein phosphorylation cascade. Additionally, AA are key precursors for syntheses of hormones and low-molecular weight nitrogenous substances with each having enormous biological importance. Physiological concentrations of AA and their metabolites (e.g., nitric oxide, polyamines, glutathione, taurine, thyroid hormones, and serotonin) are required for the functions. However, elevated levels of AA and their products (e.g., ammonia, homocysteine, and asymmetric dimethylarginine) are pathogenic factors for neurological disorders, oxidative stress, and cardiovascular disease. Thus, an optimal balance among AA in the diet and circulation is crucial for whole body homeostasis. There is growing recognition that besides their role as building blocks of proteins and polypeptides, some AA regulate key metabolic pathways that are necessary for maintenance, growth, reproduction, and immunity. They are called functional AA, which include arginine, cysteine, glutamine, leucine, proline, and tryptophan. Dietary supplementation with one or a mixture of these AA may be beneficial for (1) ameliorating health problems at various stages of the life cycle (e.g., fetal growth restriction, neonatal morbidity and mortality, weaning-associated intestinal dysfunction and wasting syndrome, obesity, diabetes, cardiovascular disease, the metabolic syndrome, and infertility); (2) optimizing efficiency of metabolic transformations to enhance muscle growth, milk production, egg and meat quality and athletic performance, while preventing excess fat deposition and reducing adiposity. Thus, AA have important functions in both nutrition and health.

Keywords Amino acids · Health · Metabolism · Nutrition

Abbreviations

AA	Amino acids
BCAA	Branched-chain amino acids
EAA	Nutritionally essential amino acids
eIF	Eukaryotic translation initiation factor
mTOR	Mammalian target of rapamycin
NEAA	Nutritionally non-essential amino acids
NO	Nitric oxide
PDV	Portal-drained viscera

Introduction

Amino acids (AA) are defined as organic substances containing both amino and acid groups. Except for glycine, all AA have an asymmetric carbon and exhibit optical activity. The absolute configuration of AA (L- or D-isomers) is defined with reference to glyceraldehydes. Except for proline, all protein AA have a primary amino group and a carboxyl group linked to the α-carbon atom (hence α-AA). In β-AA (e.g., taurine and β-alanine), an amino group links to the β-carbon atom. Post-translationally modified AA occur in some proteins (Galli 2007). Because of variations in their side chains, AA have remarkably different biochemical properties and functions (Brosnan 2001; Suenaga et al. 2008; Wu et al. 2007a). AA are generally stable in aqueous solution at physiological pH, except for (1) glutamine which is slowly

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cyclized to pyroglutamate (<1% per day at 1 mM at 25°C) and (2) cysteine which undergoes rapid oxidation to cystine.

Except for glycine, all AA can have L- and D-isoforms. Most D-AA, except for D-arginine, D-cystine, D-histidine, D-lysine, and D-threonine, can be converted into L-AA in animals via widespread D-AA oxidases and transaminases (Baker 2008; Fang et al. 2009). The efficiency of D-AA utilization, on a molar basis of the L-isomer, may be 20–100%, depending on substrates and species (Baker 2008).

Among more than 300 AA in nature, only 20 of them (α-AA) serve as building blocks of protein. However, non-protein α-AA (e.g., ornithine, citrulline, and homocysteine) and non-α AA (e.g., taurine and β-alanine) also play important roles in cell metabolism (Curis et al. 2007; Hu et al. 2008b; Manna et al. 2009; Perta-Kajan et al. 2007). Because of its large mass (representing 40–45% of body weight), skeletal muscle is the largest reservoir of both peptide-bound and free AA in the body (Davis and Fiorotto 2009). Over the past 20 years, much effort has been directed toward defining optimal requirements of AA by livestock species [including pigs (Wu et al. 2007a) and ruminants (Firkins et al. 2006)], birds (Baker 2008), fish (Li et al. 2008), and humans (Elango et al. 2009) under various nutritional, developmental, environmental, and pathological conditions. Additionally, results of recent studies indicate that the small intestine is a major site for extensive catabolism of AA in humans and animals, therefore modulating the entry of dietary AA into the portal circulation and the pattern of AA in plasma (Riedijk et al. 2007; Stoll et al. 1998; Wu 1998). Further, there is growing interest in regulatory functions of L- and D-AA in nutrition and physiology (Kim and Wu 2008; Tujioka et al. 2007; Wang et al. 2008b), as well as the underlying cellular and molecular mechanisms (Grillo and Colombatto 2007; Jobgen et al. 2006; Katane et al. 2008; Scolari and Acosta 2007; Wang et al. 2008c).

Although each AA has its own unique catabolic pathway(s), the catabolism of many AA exhibit a number of common characteristics in organisms (Table 1). Important metabolites of AA include ammonia, CO_2, long-chain and short-chain fatty acids, glucose, H_2S, ketone bodies, nitric oxide (NO), urea, uric acid, polyamines, and other nitrogenous substances with enormous biological importance (Blachier et al. 2007; Montanez et al. 2008; Morris 2007; Rider et al. 2007; Sugita et al. 2007) (Table 2). Complete oxidation of AA carbons occurs only if their carbons are ultimately converted to acetyl-CoA, which is oxidized to CO_2 and H_2O via the Krebs cycle and mitochondrial electron transport system. On a molar basis, oxidation of AA is less efficient for ATP production, compared with fat and glucose (Table 3). Thus, the efficiency of energy transfer from L-AA to ATP ranges from 29% for methionine to 59%

Table 1 Reactions initiating AA catabolism in animals

Reactions	Examples	
Transamination	Leucine + α-ketoglutarate ↔ α-ketoisocaproate + glutamate	(1)
Deamidation	Glutamine + H_2O → glutamate + NH_4^+	(2)
Oxidative deamination	Glutamate + NAD^+ ↔ α-ketoglutarate + NH_3 + NADH + H^+	(3)
Decarboxylation	Ornithine → putrescine + CO_2	(4)
Hydroxylation	Arginine + O_2 + BH4 + NADPH + H^+ → NO + BH4 + citrulline + $NADP^+$	(5)
Reduction	Lysine + α-ketoglutarate + NADPH + H^+ → saccharopine + $NADP^+$	(6)
Dehydrogenation	Threonine + NAD^+ → 2-amino-3-ketobutyrate + NADH + H^+	(7)
Hydrolysis	Arginine + H_2O → ornithine + urea	(8)
Dioxygenation	Cysteine + O_2 → cysteinesulfinate	(9)
One-carbon unit transfer	Glycine + MTHF ↔ serine + THF	(10)
Condensation	Methionine + Mg-ATP → *S*-adenosylmethionine + Mg-PPi + Pi	(11)
Oxidation	Proline + ½O_2 → pyrroline-5-carboxylate + H_2O	(12)
Amidotransferation	Glutamine + F6P ↔ glucosamine-6-phosphate + glutamate	(13)
Deaminated oxidation	D-Amino acid + O_2 + H_2O ↔ α-ketoacid + H_2O_2 + NH_3	(14)
Dehydration	Serine → aminoacrylate + H_2O	(15)
Cleavage	Glycine + NAD^+ + THF ↔ MTHF + CO_2 + NH_3 + NADH + H^+	(16)

Enzymes that catalyze the indicated reactions are: (1) BCAA transaminase; (2) phosphate-activated glutaminase; (3) glutamate dehydrogenase; (4) ornithine decarboxylase; (5) NO synthase; (6) lysine:α-ketoglutarate reductase; (7) threonine dehydrogenase; (8) arginase; (9) cysteine dioxygenase; (10) hydroxymethyltransferase; (11) *S*-adenosylmethionine synthase; (12) proline oxidase; (13) glutamine:fructose-6-phosphate transaminase; (14) D-amino acid oxidase; (15) serine dehydratase; (16) glycine synthase (glycine cleavage system). *F6P* fructose-6-phosphate, *MTHF* N^5-N^{10}-methylene-THF, *THF* tetrahydrofolate. BH4, tetrahydrobiopterin (required for hydroxylation of arginine, phenylalanine, tyrosine, and tryptophan)

Table 2 Major metabolites and functions of AA in nutrition and metabolism

AA	Products	Major functions
AA	Directly	Protein synthesis; osmolytes; regulation of hormone secretion, gene expression and cell signaling
Alanine	Directly	Inhibition of pyruvate kinase and hepatic autophagy; gluconeogenesis; transamination; glucose–alanine cycle
β-Alanine	Directly	A component of coenzyme A and pantothenic acid
	Dipeptides	Carnosine (β-alanyl-L-histidine), carcinine (β-alanyl-histamine), anserine (β-alanyl-1-methyl-L-histidine), and balenine (β-alanyl-3-methyl-histidine) with antioxidative function
Arginine	Directly	Activation of mTOR signaling; antioxidant; regulation of hormone secretion; allosteric activation of NAG synthase; ammonia detoxification; regulation of gene expression; immune function; activation of BH_4 synthesis; N reservoir; methylation of proteins; deimination (formation of citrulline) of proteins[a]
	NO	Signaling molecule; regulator of nutrient metabolism, vascular tone, hemodynamics, angiogenesis, spermatogenesis, embryogenesis, fertility, immune function, hormone secretion, wound healing, neurotransmission, tumor growth, mitochondrial biogenesis, and function
	Agmatine	Inhibition of NOS, ODC, and monoamine oxidase; ligand for α_2-adrenergic and imidazoline receptors
	Ornithine	Ammonia detoxification; syntheses of proline, glutamate, and polyamines; mitochondrial integrity; wound healing
	Methylarginines	Competitive inhibition of NOS
Asparagine	Directly	Cell metabolism and physiology; regulation of gene expression and immune function; ammonia detoxification; function of the nervous system
	Acrylamide[b]	Oxidant; cytotoxicity; gene mutation; food quality
Aspartate	Directly	Purine, pyrimidine, asparagine, and arginine synthesis; transamination; urea cycle; activation of NMDA receptors; synthesis of inositol and β-alanine
Citrulline	Directly	Antioxidant; arginine synthesis; osmoregulation; ammonia detoxification; N reservoir
Cysteine	Directly	Disulfide linkage in protein; transport of sulfur
	Taurine	Antioxidant; regulation of cellular redox state; osmolyte
	H_2S	A signaling molecule
Glutamate	Directly	Glutamine, citrulline, and arginine synthesis; bridging the urea cycle with the Krebs cycle; transamination; ammonia assimilation; flavor enhancer; activation of NMDA receptors; NAG synthesis
	GABA	Excitatory neurotransmitter; inhibition of T-cell response and inflammation
Glutamine	Directly	Regulation of protein turnover through cellular mTOR signaling, gene expression, and immune function; a major fuel for rapidly proliferating cells; inhibition of apoptosis; syntheses of purine, pyrimidine, ornithine, citrulline, arginine, proline, and asparagines; N reservoir; synthesis of NAD(P)
	Glu and Asp	Excitatory neurotransmitters; components of the malate shuttle; cell Metabolism; ammonia detoxification; major fuels for enterocytes
	Glucosamine-6-P	Synthesis of aminosugars and glycoproteins; inhibition of NO synthesis
	Ammonia	Renal regulation of acid–base balance; synthesis of glutamate and CP
Glycine	Directly	Calcium influx through a glycine-gated channel in the cell membrane; purine and serine synthesis; synthesis of porphyrins; inhibitory neurotransmitter in CNS; co-agonist with glutamate for NMDA receptors
	Heme	Hemoproteins (e.g., hemoglobin, myoglobin, catalase, and cytochrome *c*); production of CO (a signaling molecule)
Histidine	Directly	Protein methylation; hemoglobin structure and function; antioxidative dipeptides; one-carbon unit metabolism
	Histamine	Allergic reaction; vasodilator; central acetylcholine secretion; regulation of gut function
	Urocanic acid	Modulation of the immune response in skin
Isoleucine	Directly	Synthesis of glutamine and alanine; balance among BCAA
Leucine	Directly	Regulation of protein turnover through cellular mTOR signaling and gene expression; activator of glutamate dehydrogenase; BCAA balance; flavor enhancer
	Gln and Ala	Interorgan metabolism of nitrogen and carbon
	HMB	Regulation of immune responses
Lysine	Directly	Regulation of NO synthesis; antiviral activity (treatment of Herpes simplex); Protein methylation (e.g., trimethyllysine in calmodulin), acetylation, ubiquitination, and *O*-linked glycosylation
	OH-lysine	Structure and function of collagen
Methionine	Homocysteine	Oxidant; independent risk factor for CVD; inhibition of NO synthesis
	Betaine	Methylation of homocysteine to methionine; one-carbon unit metabolism
	Choline	Synthesis of betaine, acetylcholine, phosphatidylcholine, and sarcosine
	Cysteine	Cellular metabolism and nutrition
	DCSAM	Methylation of proteins and DNA; polyamine synthesis; gene expression
	Taurine	Antioxidant; osmoregulation; organ development; vascular, muscular, cardiac, and retinal functions; anti-inflammation
	Phospholipids	Synthesis of lecithin and phosphatidylcholine cell signaling
Phenylalanine	Directly	Activation of BH_4 (a cofactor for NOS) synthesis; synthesis of tyrosine; neurological development and function

Table 2 continued

AA	Products	Major functions
Proline	Directly	Collagen structure and function; neurological function; osmoprotectant
	H_2O_2	Killing pathogens; intestinal integrity; a signaling molecule; immunity
	P5C	Cellular redox state; DNA synthesis; lymphocyte proliferation; ornithine, citrulline, arginine and polyamine synthesis; gene expression; stress response
	OH-proline	Structure and function of collagen
Sarcosine	Directly	An intermediate in the synthesis of glycine from choline; possible treatment of certain mental disorders; a source of formaldehyde and H_2O_2; inhibition of glycine transport
Serine	Directly	One-carbon unit metabolism; syntheses of cysteine, purine, pyrimidine, ceramide and phosphatidylserine; synthesis of tryptophan in bacteria; gluconeogenesis (particularly in ruminants); protein phosphorylation
	Glycine	Antioxidant; one-carbon unit metabolism; neurotransmitter
	D-Serine[c]	Activation of NMDA receptors in brain
Theanine	Directly	An amino acid (glutamine analog) in tea leaves; antioxidant; increasing levels of GABA, dopamine, and serotonin in brain; neuroprotective effect
Threonine	Directly	Synthesis of the mucin protein that is required for maintaining intestinal integrity and function; immune function; protein phosphorylation and *O*-linked glycosylation; glycine synthesis
Tryptophan	Serotonin	Neurotransmitter; inhibiting production of inflammatory cytokines and superoxide
	NAS	Inhibitor of BH4 synthesis; antioxidant; inhibition of the production of inflammatory cytokines and superoxide
	Melatonin	Antioxidant; inhibition of the production of inflammatory cytokines and superoxide
	ANS	Inhibiting production of proinflammatory T-helper-1 cytokines; preventing autoimmune neuroinflammation; enhancing immune function
	Niacin	A component of NAD and NADP, coenzymes for many oxidoreductases
Tyrosine	Directly	Protein phosphorylation, nitrosation, and sulfation
	Dopamine	Neurotransmitter; regulation of immune response
	EPN and NEPN	Neurotransmitters; cell metabolism
	Melanin	Antioxidant; inhibition of the production of inflammatory cytokines and superoxide
Valine	Directly	Synthesis of glutamine and alanine; balance among BCAA
Arg and Met	Polyamines	Gene expression; DNA and protein synthesis; ion channel function; apoptosis; signal transduction; antioxidants; cell function; cell proliferation and differentiation
Arg, Met, and Gly	Creatine	Antioxidant; antiviral; antitumor; energy metabolism in muscle and brain; neurological and muscular development and function
Cys, Glu, and Gly	Glutathione	Free radical scavenger; antioxidant; cell metabolism (e.g., formation of leukotrienes, mercapturate, glutathionylspermidine, glutathione–NO adduct and glutathionylproteins); signal transduction; gene expression; apoptosis; cellular redox; immune response
Gln, Asp, Gly, and Ser	Nucleic acids	Coding for genetic information; gene expression; cell cycle and function; protein and uric acid synthesis; lymphocyte proliferation
	Uric acid	Antioxidant; the major end product of amino acid oxidation in avian species
Lys, Met, and Ser	Carnitine	Transport of long-chain fatty acids into mitochondria for oxidation; storage of energy as acetylcarnitine; antioxidant

ANS anthranilic acid, *BCAA* branched-chain AA, BH_4 tetrahydrobiopterin, *CNS* central nervous system, *CP* carbamoylphosphate, *CVD* cardiovascular disease, *DCSAM* decarboxylated *S*-adenosylmethionine, *EPN* epinephrine, *GABA* γ-aminobutyrate, *HMB* β-hydroxy-β-methylbutyrate, *NAG* *N*-acetylglutamate, *NAS* *N*-acetylserotonin, *NEPN* norepinephrine, *NOS* NO synthase, *ODC* ornithine decarboxylase, *P5C* pyrroline-5-carboxylate, *Tau-Cl* taurine chloramine

[a] Including myelin basic protein, filaggrin, and histone proteins

[b] Formed when asparagine reacts with reducing sugars or reactive carbonyls at high temperature

[c] Synthesized from L-serine by serine racemase

for isoleucine. However, glutamine is a preferred major fuel for rapidly dividing cells, including enterocytes, lymphocytes, macrophages, and tumors (Curthoys and Watford 1995; Rhoads et al. 1997). The major objective of this article is to provide insights into new developments in AA nutrition research, as well as their implications for both nutrition and health.

Definitions of essential, non-essential, and functional AA

On the basis of needs from the diet for nitrogen balance or growth, AA were traditionally classified as nutritionally essential (indispensable) or non-essential (dispensable) for humans and animals (Table 4). Essential AA (EAA) are

Table 3 Energetic efficiency of oxidation of amino acids, protein, and other substrates in animals

Nutrients	Combustion energy[a]		Net atp production		Efficiency of energy transfer to ATP[b] (%)
	kJ per		mol per		
	mol AA	g AA	mol AA	g AA	
Alanine	1,577	17.7	16	0.180	52.4
Arginine	3,739	21.5	29	0.167	40.0
Asparagine	1,928	14.6	14	0.106	37.5
Aspartate	1,601	12.0	16	0.120	51.6
Cysteine	2,249	18.6	13	0.107	29.8
Glutamate	2,244	15.3	25	0.170	57.5
Glutamine	2,570	17.6	23	0.157	46.2
Glycine[c]	973	13.0	13	0.173	68.9
Histidine	3,213	20.7	21	0.135	33.7
Isoleucine	3,581	27.3	41	0.313	59.1
Leucine	3,582	27.3	40	0.305	57.6
Lysine	3,683	25.2	35	0.239	49.0
Methionine	3,245	23.0	18	0.121	28.6
Ornithine	3,030	22.9	29	0.219	49.4
Phenylalanine	4,647	28.1	39	0.236	43.3
Proline	2,730	23.7	30	0.261	56.7
Serine	1,444	13.7	13	0.124	46.5
Threonine	2,053	17.2	21	0.176	52.8
Tryptophan	5,628	27.6	38	0.186	34.8
Tyrosine	4,429	24.4	42	0.232	48.9
Valine	2,922	25.0	30	0.256	53.0
Protein[d]	2,486	22.6	24	0.218	49.8
Glucose	2,803	15.6	38	0.211	70.0
Starch[e]	2,779	17.2	38	0.235	70.6
Palmitate	9,791	38.2	129	0.504	68.0
Fat[f]	31,676	39.3	409	0.507	66.6

[a] Adapted from Cox (1970)

[b] Calculated on the basis of 51.6 kJ/mol for one high-energy bond in ATP (moles of net ATP production/mol substrate × 51.6 kJ/mol ÷ combustion energy of kJ/mol substrate × 100)

[c] When 1 mol glycine is catabolized by the glycine cleavage system, 1 mol ATP is produced. When 1 mol glycine is converted to serine and then oxidized, 13 mol ATP are produced

[d] Assuming that the average molecular weight of an AA residue in protein is 110

[e] The average molecular weight of glucose residue in starch is 162

[f] Tripalmitoylglycerol is used as an example

defined as either those AA whose carbon skeletons cannot be synthesized or those that are inadequately synthesized de novo by the body relative to needs and which must be provided from the diet to meet optimal requirements. Conditionally essential AA are those that normally can be synthesized in adequate amounts by the organism, but which must be provided from the diet to meet optimal needs under conditions where rates of utilization are greater than rates of synthesis. However, functional needs (e.g., reproduction and disease prevention) should also be a criterion for classification of essential or conditionally essential AA. Non-essential AA (NEAA) are those AA which can be synthesized de novo in adequate amounts by the body to meet optimal requirements. It should be recognized that all of the 20 protein AA and their metabolites are required for normal cell physiology and function (El Idrissi 2008; Lupi et al. 2008; Novelli and Tasker 2008; Phang et al. 2008). Abnormal metabolism of an AA disturbs whole body homeostasis, impairs growth and development, and may even cause death (Orlando et al.

Table 4 EAA and NEAA in mammals, fish and poultry

Mammals and fish		Poultry	
EAA	NEAA	EAA	NEAA
Arginine[a]	Alanine	Arginine	Alanine
Histidine	Asparagine	Glycine	Asparagine
Isoleucine	Aspartate	Histidine	Aspartate
Leucine	Cysteine[2]	Isoleucine	Cysteine[b]
Lysine	Glutamate	Leucine	Glutamate
Methionine	Glutamine[b]	Lysine	Glutamine[b]
Phenylalanine	Glycine	Methionine	Serine
Threonine	Proline[c]	Phenylalanine	Taurine
Tryptophan	Serine	Proline	Tyrosine
Valine	Taurine[d]	Threonine	
	Tyrosine	Tryptophan	
		Valine	

[a] Arginine is an EAA for young mammals. Although it may not be required in the diet to maintain nitrogen balance in the adults of most species (including humans, pigs, and rats), dietary deficiency of arginine can result in metabolic, neurological or reproductive dysfunction. Thus, on the basis of functional needs, arginine is considered an EAA for vascular homeostasis, spermatogenesis, and fetal growth

[b] Conditionally essential AA in neonates and under stress conditions

[c] EAA for young pigs and some fish

[d] EAA for carnivores (e.g., cats), neonates, and some fish

2008; Willis et al. 2008; Wu et al. 2004c). Growing evidence shows that besides their role as building blocks of proteins and polypeptides, some AA are important regulators of key metabolic pathways that are necessary for maintenance, growth, reproduction, and immunity in organisms, therefore maximizing efficiency of food utilization, enhancing protein accretion, reducing adiposity, and improving health (Suenaga et al. 2008; Wu et al. 2007a, b, c). They are called functional AA, which include arginine, cysteine, glutamine, leucine, proline, and tryptophan.

Dynamic changes of AA in physiological fluids

Concentrations of AA in plasma are maintained relatively constant in the post-absorptive state of healthy adults. However, circulating levels of most AA undergo marked changes during the neonatal period, under catabolic conditions and in disease (Field et al. 2002; Flynn et al. 2000; Manso Filho et al. 2009). Additionally, results of recent studies indicate dynamic changes of free AA in milk (Haynes et al. 2009), skeletal muscle of lactating mammals (Clowes et al. 2005), and fetal fluids during pregnancy (Gao et al. 2009a; Kwon et al. 2003a). For example, concentrations of free glutamine in sow's milk increase from 0.1 to 4 mM between days 1 and 21 of lactation (Wu and Knabe 1994) and those in ovine allantoic fluid increase from 0.1 to 25 mM between days 30 and 60 of gestation (Kwon et al. 2003a). In contrast, intramuscular glutamine levels decrease by >50% in lactating sows (Clowes et al. 2005) and mares (Manso Filho et al. 2009), compared with their nonlactating counterparts; therefore, restoring intramuscular glutamine may provide a novel strategy to enhance milk production by mammals. Strikingly, arginine, ornithine, and citrulline are unusually abundant in porcine allantoic fluid (e.g., 4–6 mM arginine on day 40) and ovine allantoic fluid (e.g., 10 mM citrulline on day 60) during early to mid-gestation, compared with their plasma levels (e.g., 0.1–0.2 mM arginine and citrulline) (Wu et al. 1996b; Kwon et al. 2003a). These three AA plus glutamine represent approximately 70% of total α-AA nitrogen in the fetal fluids. The great increase (up to 80-fold) in their concentrations in allantoic fluid occurs during the most rapid period of placental growth. More recently, Gao et al. (2009a) reported that total recoverable amounts of glutamine, leucine, and isoleucine in ovine uterine flushings increased by 20-, 3-, and 14-fold, respectively, between days 10 and 15 of pregnancy, whereas those of arginine, histidine, ornithine, and lysine increased 8-, 22-, 5-, and 28-fold, respectively, between days 10 and 16. Such dynamic changes of AA in physiological fluids support the view that these nutrients play a crucial role in growth and development of the fetus and neonate.

Interorgan metabolism of AA and extensive catabolism of AA in the gut

Several NEAA (including glutamine, glutamate, and aspartate) are extensively oxidized by absorptive epithelial cells (enterocytes) of the mammalian small intestine, such that nearly all of them in a conventional diet do not enter the portal vein (Stoll et al. 1998; Wu 1998). Nitrogenous products include ornithine, citrulline, arginine, and alanine. The small intestine utilizes glutamine from both the arterial circulation and intestinal lumen, but takes up glutamate and aspartate only from the intestinal lumen. The circulating glutamine is synthesized from branched-chain AA (BCAA) and α-ketoglutarate (derived primarily from glucose) in skeletal muscle, adipose tissue, heart, and placenta (Curthoys and Watford 1995; Self et al. 2004). Enterocytes also actively degrade proline via the proline oxidase pathway to produce ornithine, citrulline, and arginine (Wu 1997). In adult mammals, the citrulline released from the small intestine is converted into arginine primarily in kidneys and, to a lesser extent, in other cell types (including endothelial cells, leukocytes, and smooth muscle cells) (Fig. 1). However, in neonates, most of the gut-derived citrulline is utilized locally for arginine synthesis (Wu and Morris 1998). Of particular note, enterocytes of post-weaning mammals have a high

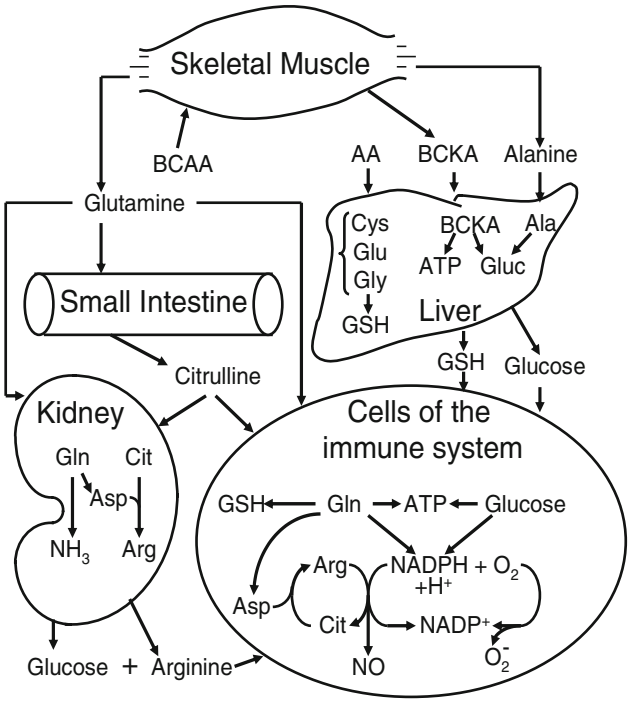

Fig. 1 Interorgan metabolism of branched-chain amino acids, glutamine and arginine and its role in immune function. Skeletal muscle takes up BCAA from the arterial blood, synthesizes both alanine and glutamine from BCAA and α-ketoglutarate, and releases these two amino acids into the circulation. The small intestine utilizes glutamine to synthesize citrulline, which is converted into arginine in kidneys, cells of the immune system, and other cell types. The liver is the primary organ for the synthesis of glutathione from glutamate, glycine, and cysteine and of glucose from alanine for use by extrahepatic cells (including immunocytes) and tissues. *Arg* arginine, *Asp* aspartate, *Cit* citrulline, *BCKA* branched-chain α-ketoacids, *Gluc* glucose, *GSH* glutathione. Reprinted from British Journal of Nutrition Li et al. (2007) with permission from The Nutrition Society

ability to catabolize arginine (Wu et al. 1996c) via both cytosolic type I and mitochondrial type II arginase (Davis and Wu 1998), which contributes to the extensive intestinal nitrogen recycling (Fuller and Redes 1998). As a mechanism for sparing proline and arginine carbons, their oxidation to CO_2 is limited in mucosal cells of the gut due to a low activity of pyrroline-5-carboxylate dehydrogenase (Wu 1997).

An exciting new aspect of AA nutrition is the finding that 30–50% of EAA in the diet may be catabolized by the small intestine in first-pass metabolism (Stoll et al. 1998; Wu 1998). For example, in milk protein-fed piglets, 40% of leucine, 30% of isoleucine, and 40% of valine in the diet were extracted by the portal-drained viscera (PDV) in first-pass, with <20% of the extracted BCAA being utilized for intestinal mucosal protein synthesis (Stoll et al. 1998). Similarly, large amounts of BCAA were catabolized by the sheep gastrointestinal tract (El-Kadi et al. 2006). This is consistent with a high activity of BCAA transaminase in intestinal mucosal cells (Chen et al. 2007, 2009). Accordingly, BCAA are actively transaminated in enterocytes to yield branched-chain α-ketoacids at rates comparable to those in skeletal muscle of young rats and chickens (Wu and Thompson 1987). The concept of intestinal AA metabolism has important implications for understanding efficiency of AA utilization and defining protein/AA requirements by humans and animals. The ammonia generated from intestinal AA catabolism either enters the portal vein or is utilized locally for urea synthesis (Wu 1995). The presence of a functional urea cycle in enterocytes serves as the first line of defense against ammonia toxicity in mammals.

Methionine, phenylalanine, lysine, threonine, and histidine were traditionally considered not to be catabolized by the intestinal mucosa (Wu 1998). However, Stoll et al. (1998) demonstrated that 50% of lysine and methionine, 45% of phenylalanine, and 60% of threonine in the diet were extracted in first-pass metabolism by the PDV of milk protein-fed pigs, with 30% of the extracted AA being catabolized by the small intestine. In addition, van Goudoever et al. (2000) found that intestinal oxidation of enteral lysine contributed one-third of total body lysine oxidation in growing pigs fed a high-protein diet. Subsequently, Riedijk et al. (2007) discovered extensive transmethylation and transsulfuration of methionine in the piglet gastrointestinal tract. Collectively, these in vivo findings suggest extensive oxidation of EAA in the gut.

Using the viable technique of enterocyte incubation, Chen et al. (2007) reported that there was no production of CO_2 or tricarboxylic-acid-cycle intermediates from carbon-1 or all carbons of lysine, histidine, threonine, and tryptophan in enterocytes of post-weaning pigs. Likewise, oxidation of methionine and phenylalanine in enterocytes was quantitatively negligible (Chen et al. 2007). Consistent with the metabolic data, there were no detectable activities of saccharopine dehydrogenase, threonine dehydrogenase, threonine hydratase, histidine decarboxylase, or phenylalanine hydroxylase in pig enterocytes (Chen et al. 2009). These results provide direct evidence for the lack of quantitatively significant catabolism of histidine, lysine, methionine, phenylalanine, threonine, and tryptophan in intestinal mucosal cells. The reported extensive catabolism of these EAA by the pig small intestine may result from the action of lumenal microbes (Fuller and Redes 1998). This may help explain why dietary supplementation with antibiotics or prebiotics improves efficiency of utilization of dietary AA for protein deposition and growth performance in pigs (Deng et al. 2007; Kong et al. 2008).

Regulatory roles of AA

Gene expression

Regulation of gene expression by AA can occur in any step of the highly specific processes that involve the transfer of

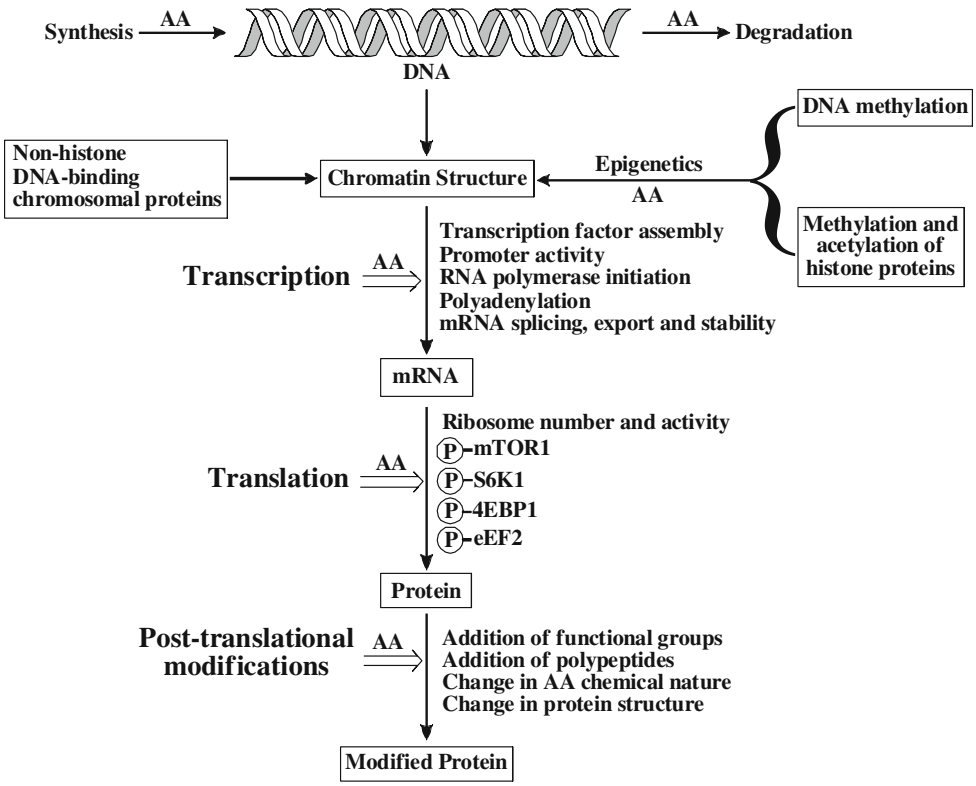

Fig. 2 Possible mechanisms responsible for AA regulation of gene expression in cells. AA may regulate gene expression in animal cells at the levels of transcription, translation, and post-translational protein modifications. Post-translational protein modifications include acetylation, ADP-ribosylation, biotinylation, γ-carboxylation, disulfide linkage, flavin attachment, glutamylation, glycation, glycosylation, glycylation, heme attachment, hydroxylation, methylation, myristoylation, nitrosylation, oxidation, phosphorylation, palmitoylation, proteolytic cleavage, racemization, selenoylation, sulfation, and ubiquitination

information encoded in a gene into its product (RNA and/or protein) (Fig. 2). These biochemical events are transcription, translation, and post-translational modifications. Gene transcription can also be regulated by epigenetics and genomic imprinting (Wu et al. 2006). Results of cell culture studies indicate that deficiency of an AA, either an EAA or an NEAA, results in increased availability of uncharged tRNA that binds and activates the general control non-derepressible protein 2 (GCN2) kinase (Kilberg et al. 2005; Palii et al. 2008). This kinase phosphorylates the eukaryotic translation initiation factor (eIF)-2α, leading to a decrease in global protein synthesis. However, under conditions of nutrient deprivation, some mRNA may undergo enhanced translation via mechanisms involving GCN4 and activating transcription factor 4. In contrast, excess of an AA may down- or up-regulate expression of genes depending on its side chains and target proteins (Flynn et al. 2008; Stipanuk et al. 2008), indicating the complexity of regulatory mechanisms for protein synthesis. For example, glutamine stimulates argininosuccinate synthetase gene expression in Caco-2 cells at the transcriptional level (Brasse-Lagnel et al. 2003) but reduces glutamine synthetase protein levels in mouse C2C12 skeletal muscle cells probably at the post-translational level (Huang et al. 2007). Moreover, either excess or deprivation of arginine modulates global gene expression in mammalian cells (Leong et al. 2006), whereas methionine deficiency stimulates osteopontin expression in hepatocytes through the hypomethylation of DNA and protein (Sahai et al. 2006). Consistent with these in vitro studies, microarray analysis indicates that dietary supplementation with glutamine or arginine increases expression of anti-oxidative genes and reduces expression of proinflammatory genes in the small intestine and adipose tissue (Fu et al. 2005; Jobgen et al. 2009b; Wang et al. 2008a). Additionally, dietary intake of methionine may affect expression of the fetal genome and pregnancy outcomes (Rees et al. 2006), but direct evidence is lacking.

Cell signaling via the mammalian target of rapamycin (mTOR; a highly conserved serine/threonine protein kinase), also known as FK506 binding protein 12-rapamycin associated protein 1, is another major mechanism for regulation of protein synthesis (Liao et al. 2008). The mTOR system consists of (1) rapamycin-sensitive complex 1 (mTOR1) [mTOR, raptor (regulatory associated protein of TOR), and G protein β-subunit-like protein)] which can be activated by AA; and (2) rapamycin-insensitive complex 2 (mTOR2) [mTOR, rictor (rapamycin-insensitive companion of TOR), mitogen-activated-protein kinase-associated protein 1, and G protein β-subunit-like protein)]. These two complexes are structurally and functionally distinct in cells. mTOR1 phosphorylates 4E-BP1 (eIF4E-binding protein-1) and ribosomal protein S6 kinase-1 (S6K1), resulting in initiation of protein synthesis and possibly inhibition of autophagy (a major mechanism for the entry of proteins into the lysosome for their hydrolysis) (Fig. 3). mTOR2 phosphorylates

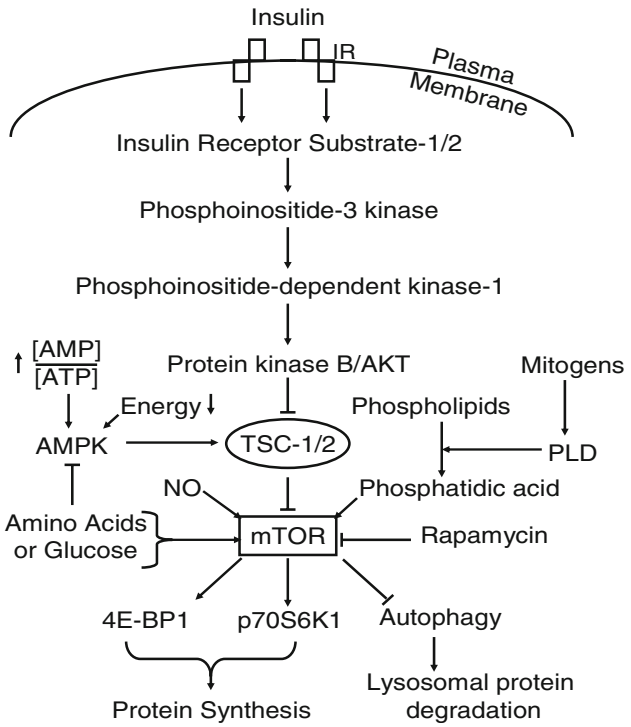

Fig. 3 Activation of protein synthesis by AA and growth factors through the mTOR signaling pathway. mTOR (a protein kinase) phosphorylates eIF4E-binding protein-1 (4E-BP1) and ribosomal protein S6 kinase-1 (S6K1), thereby stimulating protein synthesis and inhibiting autophagy (a key step in lysosomal proteolysis). mTOR is inhibited by TSC-1/2 (tuberous sclerosis complex-1/2) whose activity is enhanced by AMPK (AMP-activated protein kinase) but suppressed by protein kinase B (also known as AKT). Phosphorylation of AKT in response to insulin and other growth factors relieves an inhibitory effect of TSC-1/2 on mTOR. Additionally, certain nutrients (e.g., glutamine, arginine, leucine, and glucose) and phosphatidic acid produced by phospholipase D (PLD) stimulate mTOR phosphorylation and thus increase its activity. Oxidation of AA, glucose, and fatty acids increases cellular ratios of ATP:AMP, therefore reducing AMPK activity. Reprinted from Livestock Science Wu et al. (2007a) with permission from Elsevier

protein kinase B/Akt and may function to regulate cell proliferation, differentiation, migration, and cytoskeletal reorganization (Sarbassov et al. 2005). Some AA (e.g., glutamine, arginine, and leucine) are known to stimulate the phosphorylation of mTOR1 in a cell-specific manner, thereby regulating intracellular protein turnover (Escobar et al. 2005, 2006; Meijer and Dubbelhuis 2004; Yao et al. 2008). It is unknown whether AA directly or indirectly phosphorylate mTOR. Future studies are warranted to address this important question. It is noteworthy that recent in vitro studies have shown that Rag GTPases bind raptor and mediate AA signaling to mTORC1 (Sancak et al. 2008).

Synthesis and secretion of hormones

Many low-molecular-weight hormones are synthesized from specific AA (Table 2). For example, tyrosine (or phenylalanine) is the precursor for the synthesis of epinephrine, norepinephrine, dopamine, and thyroid hormones. High concentrations of AA, which are often achieved by oral or intravenous administration of pharmacological doses that are 10–20 times intake from the diet, can also stimulate secretion of hormones from endocrine cells (Newsholme et al. 2005). Among them, arginine, glutamine, and leucine are the best characterized secretagogues. For example, pharmacologic doses of L-arginine (e.g., 0.1–0.3 g/kg body weight over 20 min) stimulate the secretion of insulin, growth hormone, prolactin, glucagon, progesterone, and placental lactogen from their respective endocrine organs (Wu et al. 2008b), whereas glutamine and leucine increase insulin release from pancreatic β-cells (Newsholme et al. 2005). Further, dietary supplementation with glutamine reduces the production of glucocorticoids (a stress hormone) in weanling pigs (Li et al. 2007). Elevated levels of these AA may partly mediate the effect of high-protein intake on circulating concentrations of hormones in animals. It should be recognized that the effects of dietary AA supplementation on hormone secretion depend on dose, nutritional status, and developmental stage.

Nutrient metabolism and oxidative defense

Available evidence shows that AA directly participate in cell signaling (Ou et al. 2007; Rhoads and Wu 2008), cell-specific metabolism of nutrients (Jobgen et al. 2006), oxidative stress (Galli 2007; Mannick 2007), and efficiency of utilization of dietary protein (Wang et al. 2008a). For example, arginine is an allosteric activator of *N*-acetylglutamate synthase, a mitochondrial enzyme that converts glutamate and acetyl-CoA into *N*-acetylglutamate (an allosteric activator of carbamoylphosphate synthase I) (Wu and Morris 1998). Thus, arginine and glutamate maintain the hepatic urea cycle in an active state for ammonia detoxification. Second, alanine inhibits pyruvate kinase, thereby regulating gluconeogenesis and glycolysis to ensure net glucose production by hepatocytes during periods of food deprivation (Meijer 2003). Third, glutamate and aspartate mediate the transfer of reducing equivalents across the mitochondrial membrane and thus regulate glycolysis and cellular redox state (Brosnan 2001). Fourth, arginine and phenylalanine increase GTP cyclohydrolase-I expression and activity, thereby increasing the availability of tetrahydrobiopterin for NO synthesis and the hydroxylation of aromatic AA (Shi et al. 2004). The arginine–NO pathway can also be modulated by a number of other AA, including taurine, lysine, glutamate, homocysteine, and asymmetric dimethylarginine to exert their physiological and pathological effects (Wu and Meininger 2002). While physiological levels of NO play an important role in

cellular signaling, excess NO produced by inducible NO synthase can result in oxidative injury and apoptosis (Jobgen et al. 2006). Fifth, arginine increases expression of key proteins and enzymes (e.g., AMP-activated protein kinase and peroxisome proliferator-activated receptor γ coactivator-1α) responsible for mitochondrial biogenesis and substrate oxidation, thereby reducing excess fat mass in obese animals (Fu et al. 2005). Likewise, glutamine regulates expression of genes in the small intestine that are related to oxidative defense, signal transduction, and protein turnover (Wang et al. 2008a), therefore preventing intestinal atrophy and enhancing growth in weanling pigs (Wu et al. 1996a). In addition, H_2S and CO, which are products of cysteine and heme degradation, respectively, may also play signaling roles in nutrient metabolism (e.g., stimulation of glucose and fatty acid oxidation) (Li et al. 2009). Sixth, methionine, glycine, serine, and histidine actively participate in one-carbon metabolism and, thus, the methylation of proteins and DNA, thereby regulating gene expression and the biological activity of proteins (Table 2). Seventh, glutathione, which is formed from cysteine, glutamate, and glycine, is the major antioxidant in cells and regulates the homeostasis of free radicals (Wu et al. 2004b). Finally, coordination of AA metabolism among liver, skeletal muscle, intestine, and immune cells maximizes glutamine availability for renal ammoniagenesis under acidotic conditions, while producing arginine, proline, and glutathione in response to physiological and nutritional needs (Fig. 1).

Intracellular protein turnover

The continuous synthesis and degradation of proteins in cells is collectively termed intracellular protein turnover, which determines protein balance in tissues. Protein turnover requires large amounts of ATP (e.g., 20–25% of whole body energy expenditure in adults). However, this costly metabolic cycle fulfills key obligatory functions, including protein homeostasis, cell turnover, removal of aged and damaged proteins, synthesis of heat-shock and immunological proteins, gluconeogenesis, wound healing, tissue repair, adaptation to nutritional and pathological alterations, and immune responses.

Leucine is an inhibitor of protein degradation in incubated skeletal muscle (Nakashima et al. 2007; Tischler et al. 1982) and the perfused liver (Meijer and Dubbelhuis 2004). In addition, leucine stimulates muscle protein synthesis under both in vitro and in vivo experimental conditions (Suryawan et al. 2008a, b; Tischler et al. 1982). The underlying mechanisms may involve activation of the mTOR signaling to enhance translation initiation and inhibit autophagy (a key step in lysosomal proteolysis) in liver and muscle (Fig. 3), as reported for intestinal epithelial cells (Rhoads et al. 2008). Interestingly, other two BCAA, isoleucine and valine, have no effect on mTOR phosphorylation or muscle protein turnover (Escobar et al. 2005, 2006; Tischler et al. 1982), indicating a structural specificity of leucine in cell signaling and function. Surprisingly, chronic dietary supplementation with leucine failed to promote muscle protein accretion or whole body growth in young rats (Lynch et al. 2002). Likewise, supplementing 1, 2, and 4% leucine to a corn- and soybean-based diet for 16 days did not affect feed intake or daily weight gain of young pigs weaned at 4 weeks of age (Edmonds and Baker 1987). It is possible that supplementation of leucine alone may result in BCAA imbalance in the diet and circulation. Therefore, simultaneous supplementation of all of the three BCAA may be necessary to realize the potential of leucine in increasing muscle growth.

Intramuscular levels of glutamine exhibit a marked decline under various catabolic conditions (e.g., injury, sepsis, and lactation) associated with negative protein balance in skeletal muscle (Curthoys and Watford 1995; Clowes et al. 2005). These findings suggest a possible link between this AA and protein turnover. In support of this possibility, Rennie and co-workers demonstrated that infusion of glutamine into rat skeletal muscle increased protein synthesis (Maclennan et al. 1987) and inhibited protein breakdown (Maclennan et al. 1988). Subsequently, Wu and Thompson (1990) found that elevating extracellular concentrations of glutamine from 1 mM (physiological level in chick plasma) to 15 mM dose-dependently increased protein synthesis and decreased protein degradation in incubated chicken skeletal muscle. Although direct in vivo evidence is lacking, there is a positive relationship between intramuscular concentrations of glutamine and muscle protein synthesis in chickens (Watford and Wu 2005). Besides skeletal muscle, glutamine also stimulates protein synthesis and inhibits proteolysis in mucosal cells of the small intestine (Coeffier et al. 2003). The underlying mechanisms are unknown, but may involve mTOR signaling events, as reported for cardiac myocytes (Xia et al. 2003). Activation of the mTOR signaling pathway may be partly responsible for the beneficial effect of dietary L-glutamine supplementation on preventing intestinal atrophy and improving growth performance in early weaned pigs (Wang et al. 2008a; Wu et al. 1996a). Because leucine, isoleucine, and valine are substrates for glutamine synthesis in animal tissues, glutamine may play a role in mediating the anabolic effect of BCAA in animals. Such an effect is likely important for the lactating mammary gland, which produces more glutamine than it takes up from arterial blood (Kim and Wu 2008). Additionally, placental BCAA catabolism results in glutamine synthesis and its releases into the fetal circulation (Self et al. 2004), which is a major

source of the circulating glutamine in the fetus (Wu et al. 1995, 2006). It is unknown whether dietary supplementation with BCAA or glutamine may enhance placental and fetal growth in mammals.

There is emerging evidence that arginine increases protein synthesis in the pig small intestine under catabolic conditions, including viral infection and malnutrition (Corl et al. 2008). Recent studies have shown that arginine activates mTOR and other kinase-mediated signaling pathways in intestinal epithelial cells (Ban et al. 2004; Rhoads et al. 2006), thereby stimulating protein synthesis, enhancing cell migration, and facilitating the repair of the damaged intestinal epithelium. These findings may provide a mechanism for the beneficial effect of arginine in maintaining intestinal integrity and function in neonates (Wu et al. 2004c). Increasing concentrations of arginine in plasma of milk-fed piglets through either dietary arginine supplementation (Wu et al. 2004c) or metabolic activation of endogenous arginine synthesis by *N*-carbamoylglutamate (Frank et al. 2007) increased protein accretion in skeletal muscle and the whole body. This anabolic effect of arginine was associated with an increase in muscle mTOR activation and protein synthesis (Yao et al. 2008). It can be surmised that inadequate provision of arginine and glutamine from a low-protein (12.7% crude protein) diet may explain why dietary supplementation with deficient EAA (lysine, methionine, threonine, tryptophan, leucine, isoleucine, and valine) was ineffective in restoring protein synthesis or whole body growth in weanling piglets (Deng et al. 2008). Inclusion of crystalline AA in low-protein diets may substantially reduce nitrogen excretion from animals, therefore minimizing an impact of animal production on environmental pollution.

Immune function

Protein deficiency has long been known to impair immune function and increases the susceptibility of animals to disease. However, only in the past 20 years, have the underlying cellular and molecular mechanisms begun to unfold. A dietary deficiency of protein reduces the availability of most AA in plasma, particularly glutamine, arginine, tryptophan, methionine, and cysteine (Li et al. 2007). The roles of glutamine, arginine, methionine, and cysteine in enhancing the immune function have been well established (Li et al. 2007; Tan et al. 2008a; Van Brummelen and du Toit 2007). The underlying mechanisms may involve mTOR activation, NO and glutathione synthesis, H_2S signaling, and cellular redox state. Because the availability of cysteine is a major factor that limits the synthesis of glutathione (Wu et al. 2004b), dietary supplementation with *N*-acetyl-cysteine (a stable precursor of cysteine) is highly effective in enhancing immunity under various disease states (Grimble 2006). It is noteworthy that a large amount of NO synthesized from arginine by inducible NO synthase is cytotoxic to pathogenic microorganisms and viruses (Bronte and Zanovello 2005). Accordingly, dietary supplementation with arginine improves the immune status of humans and animals (Li et al. 2007; Tan et al. 2008a, b).

There has been growing interest in recent years in the role of tryptophan and proline in immune functions. Notably, concentrations of tryptophan progressively decline in plasma of pigs with chronic lung inflammation (Melchior et al. 2003). Catabolism of tryptophan via the indoleamine 2,3-dioxygenase (IDO) appears to be critical for functions of both macrophages and lymphocytes (Macchiarulo et al. 2008). Thus, anthranilic acid (a metabolite of tryptophan via the IDO pathway) inhibits production of proinflammatory T-helper-1 cytokines and prevents autoimmune neuroinflammation (Platten et al. 2005). Additionally, Ha et al. (2005) discovered that the lack of proline catabolism due to a deficiency of intestinal proline oxidase impairs gut immunity in Drosophila. A major mediator derived from proline oxidation is H_2O_2, which is cytotoxic to pathogenic bacteria and is also a signaling molecule (Shi et al. 2004). A high activity of proline oxidase in placentae (Wu et al. 2005, 2008a) and the small intestine of mammals (Wu 1997) may play a crucial role in protecting these organs from infections during the critical periods of fetal and neonatal development. Further, proline oxidase is present in milk and may play a role in protecting the neonatal intestine from infectious agents (Sun et al. 2002). This may explain, in part, why neonates fed a non-milk diet have a high risk of intestinal dysfunction in comparison with those nursed by their mothers (Wu et al. 1996c).

Reproduction

Arginine was not traditionally considered an EAA for healthy adult males on the basis of nitrogen balance. However, it has been known for over 50 years that feeding an arginine-deficient diet to adult men for 9 days decreases sperm counts by ~90% and increases the percentage of non-motile sperm approximately tenfold (see Wu et al. 2008b, for review). This striking observation underlines a critical role for arginine in spermatogenesis and argues that functional needs should be a criterion for the classification of arginine as an EAA for adult males of reproductive age. Interestingly, concentrations of polyamines (products of arginine catabolism) are relatively high in porcine seminal fluid (~90 μM), in comparison with 3–5 μM for plasma (Wu et al. 2008b). Dietary supplementation with 1% L-arginine–HCl to sexually active boars for 30 days had no effect on the volume of ejaculated semen but enhanced

concentrations of arginine, proline, ornithine, and polyamines in seminal fluid by 43, 41, 56, and 63%, respectively, compared with the control group (Wu et al. 2008b). Accordingly, dietary arginine supplementation increased sperm counts by 18% and sperm motility by 7.6%. The underlying mechanisms may involve augmented synthesis of both NO and polyamines that are essential to spermatogenesis and sperm viability. Dietary supplementation may provide a novel means to improve fertility in male breeding animals (including boar, bull, cock, ram, and stallion) particularly under stress conditions.

There are marked changes in NO synthesis and polyamine concentrations in the conceptus during early gestation when placental growth is most rapid (Kwon et al. 2003b, 2004). Additionally, recent studies from female rodents, pigs, and sheep suggest that leucine, glutamine, arginine, and proline may play an important role in embryonic, placental, and fetal development during pregnancy (Martin et al. 2003; Wu et al. 2004a, 2008a). Of particular interest, BCAA are extensively degraded in placentae to form glutamine (Self et al. 2004), whereas proline is a major AA for placental synthesis of polyamines (Wu et al. 2005, 2008a). Further, as noted above, concentrations of glutamine, arginine, and leucine increased markedly in uterine fluids between days 10 and 15 of gestation (Gao et al. 2009a) in association with increased expression of AA transporters in the conceptus (Gao et al. 2009b, c, d, e). The unusual abundance of these AA at sites critical for embryonic and fetal development raised an important question of whether they play a crucial role in embryogenesis, angiogenesis, implantation, as well as placental/fetal growth and development (Wu et al. 2004a, 2006). In support of this notion, dietary supplementation with 1.0% L-arginine–HCl (equivalent to 0.83% L-arginine) to gilts fed a 2-kg diet daily between day 30 of gestation and parturition increased the number and total litter weight of live-born piglets by 2 and 24%, respectively (Mateo et al. 2007). Additionally, Zeng et al. (2008) reported that supplementing L-arginine to the diet of rats during early or mid-gestation enhanced embryonic survival and litter size at term birth. These important discoveries provide a new means to improve pregnancy outcomes in both livestock species and humans.

Obesity, diabetes, and the metabolic syndrome

Obesity in humans is a major public health crisis worldwide and is a leading risk factor for insulin resistance, type II diabetes, atherosclerosis, stroke, hypertension, and some types of cancer (including colon and breast cancers) (Hill et al. 2008). Unfortunately, clinicians have few tools to fight the obesity epidemic, because current anti-obesity drugs are not highly effective and are fraught with side effects. Similarly, livestock species exhibit excessive amounts of subcutaneous adipose tissue at market weight, and there are few means of reducing white adipose tissue that are acceptable to consumers (Smith et al. 2008). Recent work has shown that dietary supplementation with arginine or watermelon (rich in citrulline) reduced plasma levels of glucose, homocysteine, and asymmetric dimethylarginine [risk factors for the metabolic syndrome (Marliss et al. 2006)], while improving endothelium-dependent relaxation (an indicator of cardiovascular function) in both type I and type II models of diabetes mellitus (Fu et al. 2005; Kohli et al. 2004). Excitingly, the arginine treatment reduced white adipose tissue but increased brown fat mass in Zucker diabetic fatty rats (type II diabetes) and diet-induced obese rats (Fu et al. 2005; Jobgen et al. 2009a; Wu et al. 2007c). Arginine and/or its metabolites (NO and polyamines) may enhance the proliferation, differentiation, and function of brown adipocytes. In addition, both skeletal muscle mass and whole body insulin sensitivity were enhanced in response to arginine supplementation via mechanisms involving increases in muscle mTOR and NO signaling (Wu and Meininger 2009). Similarly, long-term oral administration of arginine decreased fat mass in adult obese humans with type II diabetes (Lucotti et al. 2006). Moreover, Tan et al. (2008b) reported that supplementing arginine to a conventional corn- and soybean-based diet reduced fat accretion and promoted protein deposition in the whole body of growing-finishing pigs. Interestingly, elevated levels of BCAA in mice with BCAA transaminase knockout were associated with an increase in muscle protein synthesis and a decrease in fat mass and augmentation of whole-body energy expenditure (She et al. 2007). In contrast to leucine which inhibits NO production by the endothelium, high concentrations of arginine in plasma enhance NO availability and improve vascular insulin sensitivity (Wu and Meininger 2009). Thus, dietary supplementation with arginine may provide a novel means to treat obesity and the metabolic syndrome in mammals.

Efficacy and safety of crystalline AA supplementation

In contrast to protein, crystalline AA in the diet do not undergo digestion and are directly available for absorption by the small intestine. Therefore, they are absorbed into enterocytes and appear in the portal vein more rapidly than protein-bound AA. This may result in a transient imbalance among AA in the systemic circulation whose extent likely depends on both the quality and quantity of dietary protein. This raises a question about the bioequivalence of supplemental AA relative to proteins and peptides in diets. However, experimental evidence from studies with humans, pigs, chickens, and rats consistently indicate that

Table 5 Important roles for AA in nutrition

Nutrient absorption and metabolism (e.g., nutrient transport, protein turnover, fat synthesis and oxidation, glucose synthesis and oxidation, amino acid synthesis and oxidation, urea and uric synthesis for ammonia detoxification, and efficiency of food utilization)
Cellular signaling via mTOR, cAMP, and cGMP activation pathways, as well as the generation of NO, CO, and H_2S
Hormone synthesis and secretion (e.g., insulin, glucagon, growth hormone, glucocorticoids, prolactin, placental lactogen, and epinephrine)
Endothelial function, blood blow, and lymph circulation
Immune function and health (e.g., T-cell proliferation and B-cell maturation, antibody production by B-cells, killing of pathogens, obesity, diabetes, and metabolic syndrome)
Reproduction and lactation (e.g., spermatogenesis, male fertility, ovulation, ovarian steroidogenesis, embryo implantation, placental angiogenesis and growth, fetal growth and development, and lactogenesis)
Acid–base balance, neurotransmission, extracellular and intracellular osmolarity, antioxidative defense, and whole body homeostasis
Fetal and postnatal growth and development, as well as tissue regeneration and remodeling

crystalline AA have high-nutritional values when they are added to a diet deficient in those AA (Baker 2008; Elango et al. 2009; Mateo et al. 2008; Wu et al. 2004c, 2007b). Extensive research has also shown that supplementing appropriate amounts of an AA (usually <0.2–2.5% of the diet on a dry matter basis depending on AA and species) is generally safe for animals (Edmonds and Baker 1987; Kim and Wu 2004; Wang et al. 2007; Wu et al. 2008b). However, excess AA can result in severe adverse effects, including reduced food intake, abnormal behavior, impaired growth, and even death, owing to AA imbalance (disproportions of dietary AA) or antagonism (the mutually adverse and opposing action of AA). AA imbalances may occur among AA regardless of their structure and can be prevented by addition of one or more of the limiting AA to the diet. In contrast, AA antagonism commonly occurs among chemically or structurally related AA (e.g., lysine-arginine-ornithine, leucine-isoleucine-valine, and threonine-tryptophan) but can be overcome by addition of a structurally similar AA. AA imbalance or antagonism may result from (1) impairment of intestinal AA absorption and transport by extraintestinal cells; (2) disturbance of AA metabolism and homeostasis; (3) dysfunctional generation of signaling molecules (e.g., GABA, NO, CO, and H_2S); and (4) excess production of toxic substances (e.g., ammonia and homocysteine). Thus, like all other nutrients (e.g., glucose, fatty acids, minerals, and vitamins), excess amounts of supplemental AA or their metabolites can be toxic to organisms and should be avoided in dietary formulation and clinical therapy. Safety levels for AA supplementation can be established by well-controlled studies with animals and humans.

Conclusion and perspectives

Amino acids display remarkable metabolic and regulatory versatility (Table 5). They serve as essential precursors for the synthesis of a variety of molecules with enormous importance, and also regulate key metabolic pathways and processes that are vital to the health, growth, development, reproduction, and homeostasis of organisms. These findings exemplify the power of basic research on AA biochemistry and nutrition to discover new knowledge of animal biology and solve significant practical problems in medicine and animal agriculture. Dietary supplementation with one or a mixture of functional AA may be beneficial for (1) ameliorating health problems at various stages of the life cycle (e.g., fetal growth restriction, neonatal morbidity and mortality, weaning-associated intestinal dysfunction and wasting syndrome, obesity, diabetes, cardiovascular disease, the metabolic syndrome, infertility, and infection); (2) optimizing efficiency of metabolic transformations to enhance protein synthesis, muscle growth, milk production, egg and meat quality, and athletic performance, while preventing excess fat deposition and favoring reduction of adiposity in humans and animals.

Studies of AA nutrition have been largely based on traditional approaches (e.g., digestibility trials, nitrogen balance, assessments of growth and reproductive performance, isotope tracer techniques, as well as northern and western blots) (Dekaney et al. 2008; Mateo et al. 2007, 2008; Wang et al. 2008c). These techniques have played historically significant roles in the development of this field and remain relevant in current research. However, recent advances of high-throughput functional genomics, microarray, metabolomics, and proteomics (He et al. 2008; Hu et al. 2008a; Ptolemy et al. 2007; John et al. 2008; Wang et al. 2009; Yan and He 2008) have provided powerful discovery tools to study regulatory roles for AA in gene expression and protein function. It would be important to capitalize on these revolutionary methods in future endeavors so as to rapidly and extensively expand our knowledge of AA biochemistry and nutrition in mammals and other species.

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