Published in final edited form as:
Amino Acids. 2009 May ; 37(1): 153–168. doi:10.1007/s00726-008-0210-y.

Arginine metabolism and nutrition in growth, health and disease

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Abstract

L-Arginine (Arg) is synthesised from glutamine, glutamate, and proline via the intestinal-renal axis in humans and most other mammals (including pigs, sheep and rats). Arg degradation occurs via multiple pathways that are initiated by arginase, nitric-oxide synthase, Arg:glycine amidinotransferase, and Arg decarboxylase. These pathways produce nitric oxide, polyamines, proline, glutamate, creatine, and agmatine with each having enormous biological importance. Arg is also required for the detoxification of ammonia, which is an extremely toxic substance for the central nervous system. There is compelling evidence that Arg regulates interorgan metabolism of energy substrates and the function of multiple organs. The results of both experimental and clinical studies indicate that Arg is a nutritionally essential amino acid (AA) for spermatogenesis, embryonic survival, fetal and neonatal growth, as well as maintenance of vascular tone and hemodynamics. Moreover, a growing body of evidence clearly indicates that dietary supplementation or intravenous administration of Arg is beneficial in improving reproductive, cardiovascular, pulmonary, renal, gastrointestinal, liver and immune functions, as well as facilitating wound healing, enhancing insulin

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sensitivity, and maintaining tissue integrity. Additionally, Arg or L-citrulline may provide novel and effective therapies for obesity, diabetes, and the metabolic syndrome. The effect of Arg in treating many developmental and health problems is unique among AAs, and offers great promise for improved health and wellbeing of humans and animals.

Keywords

Arginine; Disease; Health; Nutrition; Physiology

Introduction

L-Arginine (Arg) is a basic amino acid (AA) in physiological fluids. Its content is relatively high in seafood, watermelon juice, nuts, seeds, algae, meats, rice protein concentrate, and soy protein isolate (Hou et al. 2008; King et al. 2008; Wu et al. 2007d), but low in the milk of most mammals (including cows, humans, and pigs) (Davis et al. 1994; Wu and Knabe 1994). Results of the third National Health and Nutrition Examination Survey indicate that mean Arg intake for the US adult population is 4.4 g/day, with 25, 20 and 10% of people consuming <2.6 (suboptimal), 5–7.5, and >7.5 g/day, respectively (King et al. 2008). In addition, preterm infants, who represent 10–12% of newborns, exhibit Arg deficiency (Becker et al. 2000), resulting in hyperammonemia and multiorgan dysfunction (Wu et al. 2004b). Moreover, Arg supplied in current diets is inadequate for maximal growth of milk-fed piglets (Wu et al. 2004c) or maximal reproductive performance of swine (Mateo et al. 2007). Thus, Arg nutrition remains a significant concern in both human and animal health, as well as livestock production.

Substantial amounts of orally administered Arg do not enter the systemic circulation in adults (humans, pigs, and rats), because 40% of dietary Arg is degraded by the small intestine in first pass metabolism (Castillo et al. 1993; Wu et al. 2007a). In contrast, there is little arginase activity in absorptive epithelial cells (enterocytes) of the neonatal small intestine and nearly all of the absorbed dietary Arg that is not utilized locally for protein synthesis can enter the portal vein of suckling infants (Wu et al. 1996b). Because Arg is the nitrogenous precursor for the synthesis of nitric oxide (NO; a key signaling molecule in virtually every cell type) by NO synthase (NOS) and regulates vital metabolic pathways (Jobgen et al. 2006), there is growing interest in Arg nutrition and physiology beyond protein synthesis (Morris 2007; Rhoads et al. 2008; Yao et al. 2008). Therefore, this article will review the versatile roles of Arg in multiorgan functions, as well as the prevention and treatment of major problems related to developmental biology and nutrient metabolism.

Arginine metabolism

Endogenous synthesis of Arg

Although it is often stated in biochemistry and nutrition textbooks that arginine is formed in the mammalian liver, there is no net synthesis of Arg via the hepatic urea cycle because the liver contains an exceedingly high arginase activity to hydrolyze Arg into urea plus ornithine (Wu and Morris 1998). Indeed, Arg concentrations in hepatocytes are very low (0.03–0.1 mM), compared with 0.5–10 mM for other AA (Li et al. 2008a, c). In adults, endogenous synthesis of Arg involves the intestinal-renal axis (Wu and Morris 1998). Namely, 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 Arg production (Fig. 1). In neonates, most of the citrulline synthesised in enterocytes is converted locally into Arg (Wu 1997; Wu and Knabe 1995). Interestingly, the uptake of citrulline by liver is negligible and this organ is not active in extracting Arg from the circulation (Wu et al. 2007c). Therefore, nearly 100 and 90% of the gut-derived citrulline and Arg, respectively, bypass the liver in pigs

(Wu et al. 2007c). Similar patterns of citrulline and Arg metabolism have recently been reported for humans (Ligthart-Melis et al. 2008; van de Poll et al. 2007).

Pyrroline-5-carboxylate (P5C) synthase, proline oxidase, and *N*-acetylglutamate (NAG) synthase are the three key regulatory enzymes of intestinal citrulline synthesis (Wu and Morris 1998). The enterocyte is the only mammalian cell type that expresses all three of these enzymes, indicating an essential role for the gut in whole-body homeostasis of citrulline and Arg (Flynn and Wu 1996). Thus, citrulline is a useful biomarker of intestinal failure in infants and adults (Crenn et al. 2008; Rhoads et al. 2005). NAG is an allosteric activator of both P5C synthase and carbamoylphosphate synthase-I; therefore it plays an important role in regulating citrulline production by enterocytes (Wu et al. 2004c). There are two isoforms of P5C synthase, with the short form expressed in the small intestine and the long-form in other cell types (Hu et al. 2008a, b). The short form of P5C synthase, but not the long form of the enzyme, is inhibited by ornithine (Hu et al. 2008a). Thus, in mammals, when dietary levels of arginine are high, intestinal synthesis of citrulline from glutamine and glutamate may be inhibited for sparing of glutamine and glutamate for other metabolic pathways.

Besides the kidney, citrulline is readily converted into Arg in nearly all cell types, including adipocytes, endothelial cells, enterocytes, macrophages, neurons, and myocytes (Wu and Morris 1998). Studies with macrophages (Wu and Brosnan 1992) and endothelial cells (Wu and Meininger 1993) demonstrated that citrulline is transported into cells by the N system which is selective for AAs with a side-chain amide group (e.g., glutamine and asparagine). Inside cells, conversion of citrulline into Arg via argininosuccinate synthase and lyase is the only pathway for citrulline utilization (Wu and Morris 1998). Animals (Wu et al. 2004c) and humans (Moinard et al. 2008) with functional kidneys have high rates of Arg synthesis from endogenous and exogenous citrulline.

Chickens, cats, and ferrets cannot produce citrulline from glutamine and glutamate due to a lack of P5C synthase in enterocytes (Wu and Morris 1998). Moreover, endogenous synthesis of citrulline via P5C synthase is limited in fish (Li et al. 2008b). Also, it is unlikely that there is significant net conversion of proline into citrulline in birds, carnivores, or aquatic animals (Flynn et al. 2002; Li et al. 2008b). Therefore, Arg is a nutritionally essential AA for these species.

Arginine degradation

Arg turns over rapidly in mammals with half lives in the circulation of 1.06, 0.75, and 0.65 h for adult, pregnant, and neonatal pigs, respectively (Wu et al. 2007a). Arg transport by cells involves the system y^+ (a high-affinity, Na^+-independent transporter) and Na-dependent transporters (e.g., $b^{0,+}$, $B^{0,+}$, and y^+L) in a cell-specific manner (Grillo et al. 2008). Once inside cells, there are multiple pathways for Arg degradation to produce NO, ornithine, urea, polyamines, proline, glutamate, creatine, and/or agmatine (Wu and Morris 1998). These pathways are initiated by arginases, three isoforms of NOS, Arg:glycine amidinotransferase, and Arg decarboxylase (Fig. 1). Quantitatively, <1 and 2% of metabolized Arg are utilized for polyamine synthesis and constitutive NO production, respectively, in mammalian cells (O'Quinn et al. 2002; Li et al. 2001).

In mammals, the arginase pathway is quantitatively most important for Arg catabolism. Type-I arginase is expressed abundantly in hepatocytes (Morris 2007) and to a limited extent, in extrahepatic cells, including enterocytes of postweaning mammals, endothelial cells, mammary epithelial cells, macrophages, and red blood cells (only in primates) (Li et al. 2002; O'Quinn et al. 2002; Wu et al. 1996b). In contrast, type-II arginase is widely expressed at relatively low levels in virtually all mitochondria-containing extrahepatic cells (including neuronal, renal, vascular, and muscle cells) and plays an important role in regulating the

synthesis of NO, proline and polyamines (Li et al. 2001; Odenlund et al. 2008; Orlando et al. 2008). Arginases I and II are encoded by two different genes and differ in their biochemical and immunological properties (Morris 2007). Some studies indicate that arginase activity is a limiting factor for polyamine synthesis and proliferation in endothelial cells (Li et al. 2002), macrophage cell lines (Kepka-Lenhart et al. 2000), and smooth muscle cells (Wei et al. 2001). Arginase, which can be released from cells and tissues, is present in extracellular fluid (e.g., plasma, wounds, intestinal lumen, and ovine allantoic fluid) to hydrolyze Arg to ornithine plus urea. Indeed, under conditions of inflammation and injury (particularly liver disease), a high activity of arginase in plasma results in a severe deficiency of Arg (Reid et al. 2007).

Arginase activity is absent from rapidly growing porcine placentae (Wu et al. 2005) and enterocytes of suckling piglets (Wu et al. 1996b). These metabolic strategies help maximize the supply of Arg from mother to fetus and from maternal milk to the systemic circulation of neonates. In both cell types, proline oxidase replaces arginase to provide ornithine for supporting the synthesis of polyamines that are required for high rates of protein synthesis and cell proliferation (Wu 1997; Wu et al. 2000a, b; Wu et al. 2008a). In contrast, a relatively high arginase activity is expressed in ovine placentae (Kwon et al. 2003b) to compensate for lower concentrations of proline in ovine maternal plasma (Kwon et al. 2003a). This species difference in placental arginase expression contributes to an unusual abundance of Arg (e.g., 4–6 mM at Day 40 of gestation) in porcine allantoic fluid (Wu et al. 1996a) but much lower concentrations of Arg (e.g., 0.82 mM at day 60 of gestation) in ovine allantoic fluid (Kwon et al. 2003a). Interestingly, citrulline is unusually abundant (e.g., 10 mM at day 60 of gestation) in ovine allantoic fluid as an effective precursor for Arg generation in the conceptus (Kwon et al. 2003a).

Large amounts of Arg (e.g., 0.17 g/day in a 2.5 kg piglet and 2.3 g/day in a 70 kg man) are utilized for the production of creatine via the interorgan cooperation of kidneys, pancreas, liver, and skeletal muscle (Wu and Morris 1998). Increasing evidence shows that creatine has anti-oxidative function (Fang et al. 2002), reduces inflammatory responses (Bassit et al. 2008), and improves glucose tolerance (Gualano et al. 2008) in humans. Because the methylation of guanidinoacetate to form creatine consumes more methyl groups than all other methylation reactions combined, creatine synthesis from Arg regulates availability of the methyl group donor for other methylation reactions, such as the synthesis of methionine from homocysteine (Brosnan and Brosnan 2007). Thus, Arg may indirectly affect one-carbon unit metabolism in the whole body.

There are three isoforms of NOS: NOS1, NOS2, and NOS3 (Alderton et al. 2001). The NOS1 isoform was first discovered in neuronal tissues, the NOS2 isoform was originally found to be inducible under certain conditions in macrophages, and the NOS3 isoform was first identified in endothelial cells. Both NOS1 and NOS3 are Ca^{2+}-dependent and constitutively expressed, whereas NOS2 is Ca^{2+}-independent and expressed abundantly in response to immunological challenges. The NOS isoforms can be present in the plasma membrane caveolae, cytoplasm, nucleus, rough endoplasmic reticulum, and mitochondria, depending on isoform and cell type (Jobgen et al. 2006; Montanez et al. 2008). The NOS isoforms are encoded by three different genes, have 51–57% homology in nucleotide sequences, and require Arg, O_2, (6R)-5,6,7,8-tetrahydrobiopterin (BH4), NADPH, calmodulin, FMN, and FAD for NO synthesis. Arginine, BH4, and heme promote and stabilize the active dimeric form of all isoforms of the NOS (Alderton et al. 2001).

Arginase and NOS compete for Arg. Therefore, relative changes in their enzymatic activities serve as major determinants of NO and polyamine production in many cell types (Durante et al. 2007; Li et al. 2001; Wei et al. 2001). A marked elevation in arginase activity, which reduces NO synthesis, provides a mechanism responsible for the survival of immunologically

challenged parasites (Gaur et al. 2007) and bacteria (Gobert et al. 2001). The K_m values of NOS for Arg are 3–20 μM (≤10% of intracellular Arg concentrations), depending on isoforms. However, increasing extracellular concentrations of Arg from 0.05 to 10 mM dose-dependently increases NO production by endothelial cells and activated macrophages (Wu and Meininger 2002). It is now clear that Arg promotes the translation of NOS2 mRNA in cytokine-stimulated astrocytes (Lee et al. 2003) and BH4 synthesis in endothelial cells (Shi et al. 2004), therefore enhancing NO production by inducible and constitutive NOS, respectively. Thus, there is a complex compartmentalization of Arg degradation at cellular, tissue, and whole-body levels, and dietary Arg supplementation may be a necessary strategy to maintain Arg homeostasis for good health and body functions under many physiological and pathological conditions.

Regulation of Arg metabolism

Arg metabolism is regulated by multiple factors that include dietary components (e.g., lysine, manganese, n-3 fatty acids), hormones (e.g., glucocorticoids, growth hormone, and leptin), cytokines, endotoxins, and endogenously generated substances (e.g., creatine, lactate, ornithine, P5C, and methylarginines) (Bush et al. 2002; Dekaney et al. 2008; Phang et al. 2008; Wu and Meininger 2002; Wei et al. 2008). Lysine competes with Arg for entry into cells and also inhibits arginase activity (Wu and Morris 1998). Therefore, the dietary Arg:lysine ratio is a critical factor influencing the effect of Arg supplementation. Under normal feeding conditions, the total amount of Arg in the diet should not be 150% greater than that of lysine (namely, Arg/lysine <2.5).

Glucocorticoids play a major role in upregulating Arg metabolism via the arginase pathway in many cell types, particularly hepatocytes and enterocytes (Flynn et al. 2008). In contrast, these hormones inhibit NO generation by suppressing NOS expression and BH4 synthesis (Shi et al. 2004). During weaning, the glucocorticoid surge induces expression of intestinal P5C synthase and arginase, resulting in enhanced citrulline production, Arg hydrolysis, and polyamine synthesis (Flynn and Wu 1997, Flynn et al. 1999; Wu et al. 2000c). Consequently, there is a metabolically significant urea cycle for ammonia detoxification in enterocytes of postweaning pigs (Wu 1995). Interestingly, a high level of circulating cortisol in the porcine fetus during late gestation and in the newborn does not induce arginase expression in their small intestines (Wu et al. 2004b). Thus, intestinal arginase expression is unresponsive to cortisol during the fetal and early neonatal periods, possibly due to limited expression of glucocorticoid receptors or inactive signal transduction in enterocytes (Flynn et al. 2008).

Cytokines (e.g., interleukin 4 and interferon-γ), other inflammatory stimuli (e.g., lipopolysaccharide), and cAMP can greatly stimulate expression of arginase I, arginase II, and ornithine decarboxylase in many cell types (Morris 2007; Wu et al. 1999). Inflammatory cytokines and endotoxins also strongly induce the expression of NOS2 and GTP cyclohydrolase I (the first and rate-controlling enzyme in de novo BH4 synthesis) in almost all cell types (Shi et al. 2004; Wu and Meininger 2002). Therefore, these substances upregulate Arg metabolism for the synthesis of urea, ornithine, proline, polyamines and NO in a cell-specific manner. For example, in response to intraperitoneal administration of lipopolysaccharide, whole-body NO production increases by 10- to 20-fold within 24 h (Wu et al. 1999). In contrast, lactate decreases intestinal citrulline synthesis by inhibiting proline oxidase via a noncompetitive mechanism (Dillon et al. 1999). Therefore, concentrations of Arg in plasma are reduced markedly in response to infection or inflammation (Li et al. 2007). Impaired synthesis from proline in enterocytes results in a deficiency of citrulline and arginine in neonates or adults with hyperlactacidemia (Dillon et al. 1999).

N^G-monomethy-$_L$-arginine (NMMA) and asymmetric dimethylarginine (ADMA) are competitive inhibitors of all NOS isoforms (Ki = 1.0–1.6 μM) (Alderton et al. 2001). However, concentrations of NMMA and ADMA are very low in plasma of healthy subjects (0.5–1 μM)

compared with those of arginine (100–250 μM) depending on nutritional state and developmental stages) (Marliss et al. 2006; Sotgia et al. 2008). Kohli et al. (2004) reported that 1 μM NMMA or ADMA did not affect NO synthesis in endothelial cells. Although much higher concentrations of NMMA and ADMA (e.g., 5–10 μM) can inhibit NO synthesis by these cells (Cardounel et al. 2007), the physiological significance of endogenous methylarginines in the regulation of NO production remains to be defined.

Arginine and reproduction

Fertility

On the basis of nitrogen balance, Arg was traditionally not considered as a nutritionally essential AA for healthy adult humans (Flynn et al. 2002) or livestock species (Wu et al. 2007c). However, this notion is not supported by studies on fertility in both males and females. Seminal fluid is particularly abundant in polyamines (putrescine, spermidine and spermine), polycationic products of Arg degradation, that are essential for cell growth and differentiation. For example, concentrations of polyamines are relatively high in porcine seminal fluid (~90 μM), in comparison with plasma (3–5 μM) (Fig. 2). Dietary supplementation with 1% Arg-HCl to sexually active boars for 30 days had no effect on the volume of ejaculated semen (Wu et al. 2007b), but enhanced concentrations of Arg, proline, ornithine, and polyamines in seminal fluid by 43, 41, 56, and 63%, respectively, compared with the control group (Fig. 2). In addition, dietary Arg supplementation to boars increased sperm counts by 18% and sperm motility by 8% (Wu et al. 2007b). Notably, Holt and Albanese (1944) reported that feeding an Arg-deficient diet to adult men for 9 days decreased both the number and motility of sperm cells by 90%. This striking observation underlines a critical role for Arg in spermatogenesis and argues that functional needs beyond tissue protein synthesis should be important criteria for the classification of AA as nutritionally essential or nonessential. Thus, enhancing Arg provision may improve fertility in males. For example, Tanimura (1967) reported that oral administration of Arg-HCl (0.5 g/day) to infertile men for 6–8 weeks markedly increased sperm counts and motility in most patients and resulted in successful pregnancies. The underlying mechanism(s) may include (1) enhanced synthesis of polyamines and Arg-rich basic proteins in sperm cells; and (2) a regulatory role for NO in sperm motility and capacitation (Balercia et al. 2004) as well as the sustenance of good-quality fertilized oocytes (Goud et al. 2008). Because men with high body mass indexes (BMI) are more likely to be infertile than their normal weight counterparts (Nguyen et al. 2007), it would be important to determine if dietary Arg supplementation will improve fertility in obese males.

Female fertility is also a significant problem in human medicine and animal agriculture. For example, a high BMI is associated with lower success rates in women for live births following the use of assisted reproductive technologies (Veleva et al. 2008). Also, studies of dairy cows and pigs indicate that up to 60% embryonic losses occur during early pregnancy, particularly under an adverse environment (e.g., hot and humid conditions) (Starbuck et al. 2004; Wu et al. 2006). Recognizing an important role for Arg in embryonic and conceptus survival and growth (Wu et al. 1996a, 2004a, b, c), we have developed Arg treatment protocols to enhance pregnancy outcome. For example, dietary supplementation with 1% Arg-HCl between days 30 and 114 of gestation increased fetal survival in gilts (Mateo et al. 2007). In addition, supplementing 1.3% Arg-HCl to the diet for female rats either throughout the entire pregnancy (21 days) or between days 1 and 7 of gestation increased embryonic survival and birth litter size by 30% (Zeng et al. 2008). It remains to be determined whether dietary Arg supplementation can ameliorate embryonic deaths in women and livestock species.

Fetal growth and development

Polyamines and NO are essential to placental growth and angiogenesis (the growth of new vessels from the existing vasculature) and, therefore, for increasing uterine and placental-fetal blood flow (Wu et al. 2008b). Feeding Arg-free diets to pregnant rats or inhibiting NO synthesis resulted in increased fetal resorptions, intrauterine growth retardation (IUGR), increased perinatal mortality, and decreased number of live fetuses (Greenberg et al. 1997). Thus, endogenous synthesis of Arg is insufficient for pregnant dams and must be provided from diets to support fetal survival and growth. Accordingly, dietary Arg supplementation (0.2 or 2% Arg in drinking water) prevented hypoxia-induced fetal growth retardation in rats (Vosatka et al. 1998). Similarly, dietary supplementation with Arg to gilts or rats fed conventional diets during pregnancy increased the live-born litter weight by 24 and 30%, respectively (Mateo et al. 2007; Zeng et al. 2008). Similarly, intravenous administration of Arg-HCl (3 × 27 mg/kg body weight per day) enhanced fetal growth in ovine models of both undernutrition-induced and naturally-occurring IUGR (Lassala 2008). Finally, during late (week 33) gestation, daily intravenous infusion of Arg (20 g/day) for 7 days to women with unknown causes of IUGR increased birth weight at term (week 39) by 6.4% (Xiao and Li 2005). These findings provide a strong experimental basis for the use of Arg to prevent and treat IUGR in animals and humans.

Preeclampsia and preterm labor

There are both clinical and biochemical evidence for endothelial dysfunction in preeclamptic women (Roberts 1999). Additionally, plasma levels of Arg and placental NOS3 abundance are reduced in preeclamptic compared to healthy pregnant women (Kim et al. 2006). In a rat model of preeclampsia induced by chronic inhibition of NO synthesis, intravenous administration of Arg (0.16 g/kg body wt/day) from gestational day 10 through term reversed the disease-associated lesions (hypertension, IUGR, proteinuria, and renal glomerulus injury) (Helmbrecht et al. 1996). Beginning at 29 weeks of gestation, oral administration of Arg (3 g daily for 4 weeks) to women with preeclampsia increased NO synthesis, reduced blood pressure, prolonged pregnancy, improved fetal wellbeing, and enhanced fetal growth (Rytlewski et al. 2006). Also, administration of Arg (20 g/day intravenously for 5 days followed by 4 g/day orally for 2 weeks) to women with gestational hypertension prolonged pregnancy, reduced blood pressure, and decreased the frequency of babies with low birth weights (Facchinetti et al. 2007).

Preterm births account for 5–10% of all births and are a major cause of neonatal morbidity and mortality (Wu et al. 2004b). Thus, prevention of preterm labor has a profound impact on improving neonatal survival and reducing the high costs of intensive care. Some evidence from human and animal studies indicates that NO inhibits uterine contractility and may play an important role in maintaining uterine quiescence during pregnancy (Buhimschi et al. 1998). Thus, inhibition of NO synthesis resulted in preterm delivery in mice, which was reversed by infusion of sodium nitroprusside (an NO donor) (Tiboni and Giampietro 2000). Importantly, intravenous infusion of Arg (30 g over 30 min) into women with the preterm onset of uterine contractions reduced spontaneous uterine contractility (Facchinetti et al. 1996). Similarly, oral administration of Arg (3 g/day for 7 days) to women with threatened preterm labor beneficially increased pulsatility indexes of middle cerebral arteries and altered fetal-placental blood flow distribution (Rytlewski et al. 2008). These findings suggest that Arg administration may be a novel means to prevent preterm birth.

Arginine and neonatal growth

Neonates supported by total parenteral nutrition

Preterm infants are primarily supported by total parenteral nutrition (TPN) in the first weeks of life due to their inability to tolerate oral feeding. Past and current commercial TPN solutions

do not contain glutamine, although preterm infants have underdeveloped Arg-synthetic pathways and reduced intestinal mass for citrulline (and hence Arg) production (Wu et al. 2004b). As an allosteric activator of NAG synthase and a substrate of ornithine, Arg is required to maintain the urea cycle in an active state (Fig. 3). Consequently, life-threatening hyperammonemia can occur in preterm infants because of Arg deficiency (≤32 μM in plasma) (Heird et al. 1972). Importantly, increasing Arg provision may prevent hyperammonemia (Heird et al. 1972) and necrotizing enterocolitis (Amin et al. 2002) in preterm infants, as well as persistent pulmonary hypertension of neonates (McCaffrey et al. 1995).

Because the small intestine prefers to utilize enteral AA for citrulline synthesis (Wu and Morris 1998), dietary Arg requirements estimated on the basis of enteral feeding cannot be applied to TPN feeding. Brunton et al. (1999) reported that intravenous infusion of neonatal pigs with an AA solution (containing proline but no glutamine, ornithine, citrulline or Arg) elicited Arg deficiency, which resulted in hyperammonemia and death. However, enteral feeding of the same AA solution to neonatal pigs ameliorated Arg deficiency (Brunton et al. 1999), which further supports an important role for the small intestine in endogenous synthesis of Arg from proline (Wu 1997).

Neonates fed enteral diets

There are only a few reports of studies regarding Arg nutrition in enterally fed infants. On the basis of a nitrogen balance study involving only two term infants (1.5–3 week-old) and one preterm infant (3.5 month-old), Snyderman et al. (1959) concluded that Arg was not an essential AA for neonates. However, these authors did not measure either metabolic indicators of an Arg deficiency (e.g., plasma concentrations of Arg and ammonia, as well as urinary excretion of orotic acid and ammonia) or physiological parameters (e.g., cardiovascular, pulmonary, intestinal, muscular, immunological, and neurological functions). Feeding Arg-deficient diets to infants likely results in low Arg concentrations in their plasma (Wu et al. 2004b), which may affect all of the above metabolic and physiological parameters. Clearly, much research is needed to define Arg requirements of infants.

The relative contributions of milk versus endogenous synthesis to meet Arg requirements of the suckling neonate can be estimated on the basis of Arg intake and Arg accretion plus catabolism in the body. Published studies with the neonatal pig indicate that endogenous synthesis must provide at least 60% of Arg for a 7-day-old suckling pig (Wu et al. 2004c). Accordingly, inhibition of intestinal synthesis of citrulline and Arg for 12 h decreased plasma concentrations of citrulline and Arg by 52 and 76%, respectively, in the 4-day-old suckling pig (Flynn and Wu 1996). Both metabolic and growth data indicate that Arg deficiency, due to a low availability of mitochondrial NAG in enterocytes, is a major factor limiting maximal growth of milk-fed piglets (Wu et al. 2004c). Thus, either dietary supplementation with Arg (Kim and Wu 2004; Tan et al. 2008a; Yao et al. 2008) or activation of endogenous Arg synthesis (Frank et al. 2007) is effective in increasing Arg availability and growth performance in milk-fed piglets. Additionally, dietary Arg supplementation promotes lactogenesis in sows and the growth of suckling piglets with normal- or low-birth weights (Mateo et al. 2008; Kim and Wu 2008). Interestingly, the effect of Arg provision was greater for low-birth-weight piglets than for their normal-birth-weight littermates (Kim and Wu 2008), suggesting a lower rate of endogenous Arg synthesis in IUGR piglets. Similarly, oral administration of Arg (3 × 55–70 mg/kg body weight per day) for 3–18 months prevented ammonia toxicity and enhanced growth of infants with an inborn deficiency of Arg synthesis (Nagasaka et al. 2006).

Arginine and chronic metabolic disease

Obesity and the metabolic syndrome

Obesity in humans and animals occurs because of a chronic imbalance between energy intake and expenditure. This disease is a major 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) (Zou and Shao 2008). Growing evidence indicates that Arg supplementation may be a novel therapy for obesity and the metabolic syndrome. First, dietary supplementation with Arg decreased plasma levels of glucose, homocysteine, fatty acids, and triglycerides, and improved insulin sensitivity in chemically induced diabetic rats (Kohli et al. 2004), genetically obese Zucker diabetic fatty rats (Fu et al. 2005; Wu et al. 2007d), and diet-induced obese rats (Jobgen 2007). Similar results have been reported for obese humans with type-II diabetes receiving oral (Lucotti et al. 2006) or intravenous (Wascher et al. 1997) administration of Arg. Second, citrulline or Arg supplementation retarded the progression of high fat diet-induced atherosclerosis in obese rabbits (Böger et al. 1997; Hayashi et al. 2005). Third, supplementing conventional diets for growing-finishing pigs with Arg reduced body-fat accretion, enhanced muscle gain, and improved the metabolic profile (He et al. 2008; Tan et al. 2008b). A distinct advantage of Arg over drugs (e.g., metformin and thiazolidinediones) is that dietary Arg supplementation reduces adiposity, while improving insulin sensitivity (Fu et al. 2005; Wu et al. 2007d). The possible underlying mechanisms for the effect of Arg may involve multiple NO-dependent pathways that favor the whole-body oxidation of fatty acids and glucose (Jobgen et al. 2006).

Endothelial dysfunction

An NO deficiency is a major factor contributing to endothelial dysfunction, which occurs in a variety of metabolic disorders, including diabetes, hypercholesterolemia, hypertension, tobacco smoking, and malaria (Wu and Meininger 2000). Several lines of evidence demonstrate that Arg administration is effective in reversing endothelial dysfunction under these conditions. First, oral administration of Arg (1.25% Arg in drinking water) to diabetic rats reversed the defective endothelium-dependent relaxation (Pieper 1997). Also, dietary supplementation with Arg or watermelon (rich in citrulline) increased circulating levels of Arg, endothelial BH4 availability and NO synthesis, and enhanced endothelium-dependent relaxation in Zucker diabetic fatty rats (Wu et al. 2007d). Second, the impaired endothelium-dependent relaxation in hypercholesterolemic humans could be improved by intravenous infusion of Arg (10 mg/kg body weight per min for 20 min) or oral administration of Arg (7 g × 3/day) (Clarkson et al. 1996; Creager et al. 1992). Third, acute supplementation of 15 g Arg ameliorated endothelial dysfunction and oxidative stress in young men fed a high fat diet (Lin et al. 2008). Fourth, hypertension in adult rats with intestinal resection (Wakabayashi et al. 1994) or patients with lysinuric protein intolerance (Kamada et al. 2001) could be prevented by parenteral administration of Arg. Fifth, intravenous infusion of Arg (30 g Arg-HCl over 45 min) normalized coronary vasomotion in long-term smokers (Campisi et al. 1999), and 3-day oral administration of Arg (7 g/day) prevented smoking-induced impairment of endothelial function in young adults (Siasos et al. 2008). Finally, intravenous infusion of Arg reversed endothelial dysfunction in patients with severe falciparum malaria, which is characterized by the cytoadherence of parasitized erythrocytes to the microvascular endothelium, hemolysis and impaired blood flow (Yeo et al. 2007). The mechanisms by which Arg administration may prevent cardiovascular dysfunction include: (1) restoring endothelial NO synthesis and decreasing superoxide production; (2) reducing vascular oxidative damage; and (3) inhibiting platelet adherence and aggregation, leukocyte adherence to the endothelium, and the proliferation of vascular smooth muscle cells (Wu and Meininger 2000, 2009).

Arginine and tissue injury and wound healing

Sickle cell disease vasculopathy

Sickle cell disease is associated with hemolysis or premature destruction of red blood cells (Wood et al. 2008). This, along with the injury to other cell types, results in elevated levels of arginase and reduced concentrations of Arg in plasma, impaired NO synthesis by endothelial cells (Morris et al. 2005; Romero et al. 2002), as well as microvascular vaso-occlusion, vasoculopathy, and multiorgan injury (Wood et al. 2008). Studies involving a transgenic-knockout mouse model with sickle cell disease showed that dietary Arg supplementation reduced red blood cell density and Gardos channel activity (Romero et al. 2002), while increasing systemic NO synthesis, reducing oxidative stress, and improving microvascular function (Kaul et al. 2008). Importantly, oral administration of L-citrulline (0.1 g/kg body weight per day) normalized circulating levels of Arg and total leukocyte counts, and improved wellbeing in patients with sickle cell disease (Waugh et al. 2001).

Renal injury

Proline, polyamines and NO play important roles in renal function, but high concentrations of these substances also contribute to renal injury and fibrosis (Satriano 2007). Of particular interest, proline is a major component of collagen, which makes up fibrotic extracellular matrix, whereas polyamines are required for proliferative responses in many renal diseases. At physiological concentrations, NO regulates glomerular and medullary hemodynamics, renin release, tubuloglomerular feedback response, and extracellular fluid volume (Baylis 2008). However, excessive production of NO can lead to increased formation of peroxynitrite anion, nitration of protein tyrosine, and production of hydroxyl radical, and may contribute to the pathogenesis of several common renal diseases, such as immune-mediated glomerulonephritis and postischemic renal failure (Baylis 2008). In these cases, excess dietary Arg may be detrimental. For example, Peters et al. (1999) reported that oral Arg administration (1% Arg in drinking water) for 1 week increased mesangial cell injury and subsequent tissue fibrosis in a rat model of glomerulonephritis. On the other hand, decreased NO synthesis by constitutive NOS may contribute to the pathogenesis of volume-dependent hypertension and of glomerular injury resulting from elevated intraglomerular pressure (Baylis 2008). Thus, most studies have shown a beneficial effect of supplemental Arg in preventing or slowing the progression of a number of experimental renal diseases characterized by systemic hypertension and increased intraglomerular pressure (e.g., Bellinghieri et al. 2006; Ito et al. 2005).

Gastrointestinal and liver injury

A new development in Arg research is the discovery of activation of mTOR, MAP kinase, and ribosomal $p70^{s6\,k}$ signaling by Arg in enterocytes that, in turn, stimulates protein synthesis, cell migration, and intestinal restitution (Rhoads et al. 2004, 2007, 2008). Either underproduction or overproduction of NO is injurious to the digestive tract (Wang et al. 2008). Arg depletion and putative reductions in the synthesis of NO, polyamines, and collagen may predispose an individual to delayed gastrointestinal recovery from injury, multiorgan system failure, and endotoxemia. Most studies have demonstrated beneficial effects of Arg on improving gastrointestinal function and gastric ulcer healing, accelerating intestinal mucosal regeneration, enhancing bacterial clearance, and reducing histological bowel necrosis (Wang et al. 2008). Moreover, hepatic ischemia/reperfusion injury in animal models could be attenuated by infusion of Arg, citrulline or an inhibitor of arginase (Jeyabalan et al. 2008; Nikolic et al. 2007; Reid et al. 2007). However, high doses of Arg could have deleterious effects in the setting of an inflamed intestine. For example, in neonatal pigs fed a basal diet that provided daily 0.53 g Arg/kg body weight, oral administration of additional Arg (1.65 g/kg body weight per day) stimulated a prostaglandin-dependent secretory diarrhea while not

exerting the expected benefit on epithelial defense or barrier function in the *Cryptosporidium parvum*-infected intestine (Gookin et al. 2008).

Cystic fibrosis

A genetic mutation of the chloride transport channel in the apical membrane of epithelial cells in the pulmonary alveoli results in a disease called cystic fibrosis, which is one of the most common genetic disorders among Caucasians (5% of population carries the defective gene). Because arginase is released from the injured lung, circulating levels of arginase are markedly elevated while levels of Arg are substantially reduced in patients with cystic fibrosis (Grasemann et al. 2006b). A single inhalation of nebulized Arg solution can enhance NO synthesis and improve pulmonary function in patients with cystic fibrosis (Grasemann et al. 2006a). A large clinical trial is necessary to confirm and extend this novel and important finding.

Wound healing

Successful wound healing is critical to the recovery of patients from tissue injury. A critical role for NO in the wound healing process was established by the finding that NOS2 gene transfer reversed impaired wound repair in *Nos2*-deficient mice (Yamasaki et al. 1998). Additionally, studies of patients with inherited prolidase deficiency (Lupi et al. 2008) or recessive dystrophic epidermolysis bullosa (Wessagowit et al. 2004) suggest that the Arg-proline pathway is crucial for wound healing. Many animal and human studies have concluded that Arg supplementation enhances hydroxyproline content and tensile strength in wound tissues via a proline-dependent mechanism (Witte and Barbul 2008). Notably, subcutaneous administration of Arg at the site of foot ulcers in diabetic patients increased wound healing (Arana et al. 2004), which might involve enhanced proline and polyamine syntheses, as well as NO-dependent increases in blood flow and nutrient supply. Important validation of the hypothesis that dietary Arg is essential to wound healing was provided by the Arg kinetic study of Yu et al. (2001). These authors demonstrated that, in burn patients, Arg degradation is markedly increased relative to *de novo* synthesis of Arg, leading to reduced concentration of Arg in plasma. Thus, exogenous Arg must be supplied for maintaining a positive nitrogen balance and accelerated wound healing in patients with tissue injury.

Arginine and immunity

Arg regulates NO synthesis by NOS2, production of antibodies by B-cells, as well as T-cell receptor expression and B-cell development (De Jonge et al. 2002). Thus, Arg plays an important role in both innate and acquired immunity. Inadequate intake of dietary Arg impairs NO synthesis by both constitutive and inducible NOS in mammals (Wu and Meininger 2002), indicating a role for adequate Arg nutrition in immune function. Available evidence shows that Arg is required for defense against viruses, bacteria, fungi, malignant cells, intracellular protozoa, and parasites in mammals, birds, terrestrial animals, lower vertebrates, and invertebrates (Li et al. 2007). For example, adequate provision of Arg is required for lymphocyte proliferation and development, and dietary Arg supplementation enhances immune responses in various models of immunological challenges (Li et al. 2007). Moreover, dietary supplementation with 1 or 2% Arg (approximately 1 or 2 times the Arg content of the regular diet) to tumor-bearing or septic rats increased thymic weight, the number of thymic lymphocytes, T-lymphocyte proliferation, the cytotoxicity of specific cells (T lymphocyte, macrophages, and NK cells), IL-2 production, IL-2 receptor expression on T lymphocytes, and the delayed type hypersensitivity response (Li et al. 2007). Further, dietary supplementation with 1% Arg-HCl enhanced the immune status of pregnant sows and neonatal pigs, thereby reducing morbidity and mortality in response to infectious pathogens (Han et al. 2008; Li et al. 2007; Tan et al. 2008a).

Arginine and cancers

Elevated levels of polyamines and NO promote and inhibit tumor growth, respectively (Mannick 2007; Shantz and Levin 2007). Thus, whether Arg suppresses or enhances tumor growth depends on the relative activities of NOS and arginase pathways, whose expression may vary with the stage of carcinogenesis. This may explain apparently conflicting findings in the literature that Arg both stimulated and inhibited the growth of tumors (Eremin 1997). The majority of in vivo studies have shown that dietary Arg supplementation from the time of tumor induction or inoculation resulted in protection against the growth of transplantable tumors and the tumorigenicity of carcinogens (Eremin 1997). For example, low-dose oral supplementation of Arg (50 mg/kg body wt/day) for 1 year decreased the total number of tumors and increased survival in mice, via NO-mediated cytotoxicity against tumor cells and blocking the formation of lipid peroxidation products (Lubec et al. 1996). Furthermore, Arg supplementation (1% Arg in drinking water) during the initiation stage of carcinogenesis decreased colorectal tumor production and crypt cell hyperproliferation, but Arg supplementation during the promotion stage stimulated colorectal tumor growth (Ma et al. 1999). New knowledge about the regulation of Arg metabolism in tumor tissues is key to designing sound therapeutic means to effectively prevent and treat cancers.

Arginine and skeletal muscle function

Skeletal muscle represents 40–45% of the adult body weight and is crucial for whole-body homeostasis of protein and AAs (Jobgen et al. 2006). Recent studies have demonstrated that Arg plays a cell signaling role in this tissue. For example, Arg activates the mTOR cell signaling pathway in skeletal muscle to enhance protein synthesis and whole-body growth (Yao et al. 2008). There is also indirect evidence that Arg inhibits proteolysis in skeletal muscle (Frank et al. 2007). Thus, regulation of intracellular protein turnover by Arg favors muscle gain (Tan et al. 2008b), which may have important implications for health. In support of this view, oral administration of Arg enhanced exercise endurance and muscle force generation in humans (Fricke et al. 2008). Moreover, Arg administration reduced inflammation and maintained muscle integrity in a mouse model of Duchenne muscular dystrophy (Hnia et al. 2008), the most common muscle wasting disease. Because muscle wasting occurs in astronauts or patients on bed rest, it is important to determine whether this disorder can be prevented by dietary Arg supplementation.

Safety of supplemental arginine

Arg is stable under sterilization conditions (e.g., high temperature and high pressure) and is not toxic to cells (Flynn et al. 2002). Thus, its administration at an appropriate dose, chemical form, and means is safe for animals and humans. For example, neonatal pigs, growing-finishing pigs, pregnant pigs, and adult rats tolerated large amounts of chronic supplemental Arg-HCl (Ajinomoto Inc., Tokyo, Japan) (at least 0.62, 0.32, 0.21, and 2.14 g Arg/kg body weight per day, respectively) administered via enteral diets without any adverse effects (Wu et al. 2007a). Additionally, long-term intravenous infusion of Arg-HCl (Ajinomoto Inc.) to ewes at 81 mg/kg body weight per day between days 60 and 147 (term) of gestation was safe for both mother and fetus (Wu et al. 2007a). Furthermore, intravenous Arg infusion (up to 0.5 g Arg-HCl/kg body weight for infants, or 30 g Arg-HCl for adults over 30–60 min) or oral Arg (9 g Arg-HCl/day for adults) has no adverse effects on humans (Shao and Hathcock 2008). However, higher oral doses of Arg-HCl (>9 g/day) are occasionally associated with nausea, gastrointestinal discomfort, and diarrhea for some subjects (Grimble 2007), which may result from a rapid and excess production of NO by the gastrointestinal tract and from impaired intestinal absorption of other dietary basic AA (lysine and histidine). A solution to this potential problem may be the alternative use of L-citrulline, a precursor for arginine synthesis (Wu and

Meininger 2000). As a neutral AA, L-citrulline does not compete with basic AA for transport by cells, its conversion to Arg consumes one mole of ammonia in the form of aspartate, and its administration does not require equimolar HCl. Thus, enteral or parenteral L-citrulline may be particularly useful for patients with elevated ammonia concentrations, impaired Arg transport, enhanced intestinal Arg catabolism, or a relatively high activity of constitutively expressed arginase (e.g., ovine placentae). Because excessive production of NO is destructive to cells, it would not be advisable to administer Arg alone to animals or patients with severe infections, active inflammatory or autoimmune disorders, active malignancy (e.g., late stages of tumorigenesis), or pathological angiogenesis. Finally, as with other nutrients (e.g., glucose, fatty acids, vitamins and minerals), improper use of Arg (e.g., high dose and disturbance of acid-base balance) may yield an undesirable effect and, thus, should be avoided in dietary supplementation and clinical therapy. Therefore, it is important that arginine be taken in divided doses on each day of administration (1) to prevent gastrointestinal tract disorders due to abrupt production of large amounts of NO; (2) to increase the availability of circulating arginine over a longer period of time; and (3) to avoid a potential imbalance among AAs (Wu et al. 2007a).

Conclusion and perspectives

Arg is a component of dietary protein and body fluids. In animals and humans, this AA fulfills versatile physiological functions. Based on nitrogen balance (or growth) and functional needs beyond tissue protein synthesis, Arg is classified as a nutritionally essential AA for: (1) the conceptuses of mammals; (2) neonates; (3) birds, cats, ferrets, and fish; and (4) adults under certain conditions (e.g., intestinal resection or dysfunction, burns, or renal dysfunction associated with NO deficiency). Compelling evidence shows that Arg plays an important role in reproduction, fetal and postnatal development, wound healing, immune function, and tissue integrity, as well as prevention and treatment of endothelial dysfunction (Table 1). Additionally, Arg or L-citrulline may provide novel and effective therapies for obesity, diabetes, and the metabolic syndrome. The beneficial effect of Arg in treating many developmental and health problems is unique among AA, and Arg may become a useful “nutraceutical”. Appropriate use of Arg is safe for animals and humans in dietary supplementation and clinical therapy.

Acknowledgements

This work was supported, in part, by grants from National Institutes of Health (1R21 HD049449), National Research Initiative Competitive Grants (2006-35203-17283, 2008-35206-18762, 2008-35206-18764, and 2008-35203-19120) from the USDA Cooperative State Research, Education, and Extension Service, American Heart Association (0655109Y and 0755024Y), Texas AgriLife Research (H-8200), National Natural Science Foundation of China (30371038 and 30528006), and the Outstanding Overseas Chinese Scholars Fund of The Chinese Academy of Sciences (2005-1-4). We thank Drs. Nick Flynn and Nancy Turner for helpful comments on the paper.

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Abbreviations

AA
: Amino acid

Arg
: L-arginine

ADMA
: Asymmetric dimethylarginine

BH4
: (6R)-5,6,7,8-tetrahydrobiopterin

BMI
: Body mass index

IUGR
: Intrauterine growth retardation

NAG
: *N*-acetylglutamate

NMMA
: N^G -monomethy-L-arginine (NMMA)

NO
: Nitric oxide

NOS
: Nitric-oxide synthase

P5C
: Pyrroline-5-carboxylate

TPN
: Total parenteral nutrition

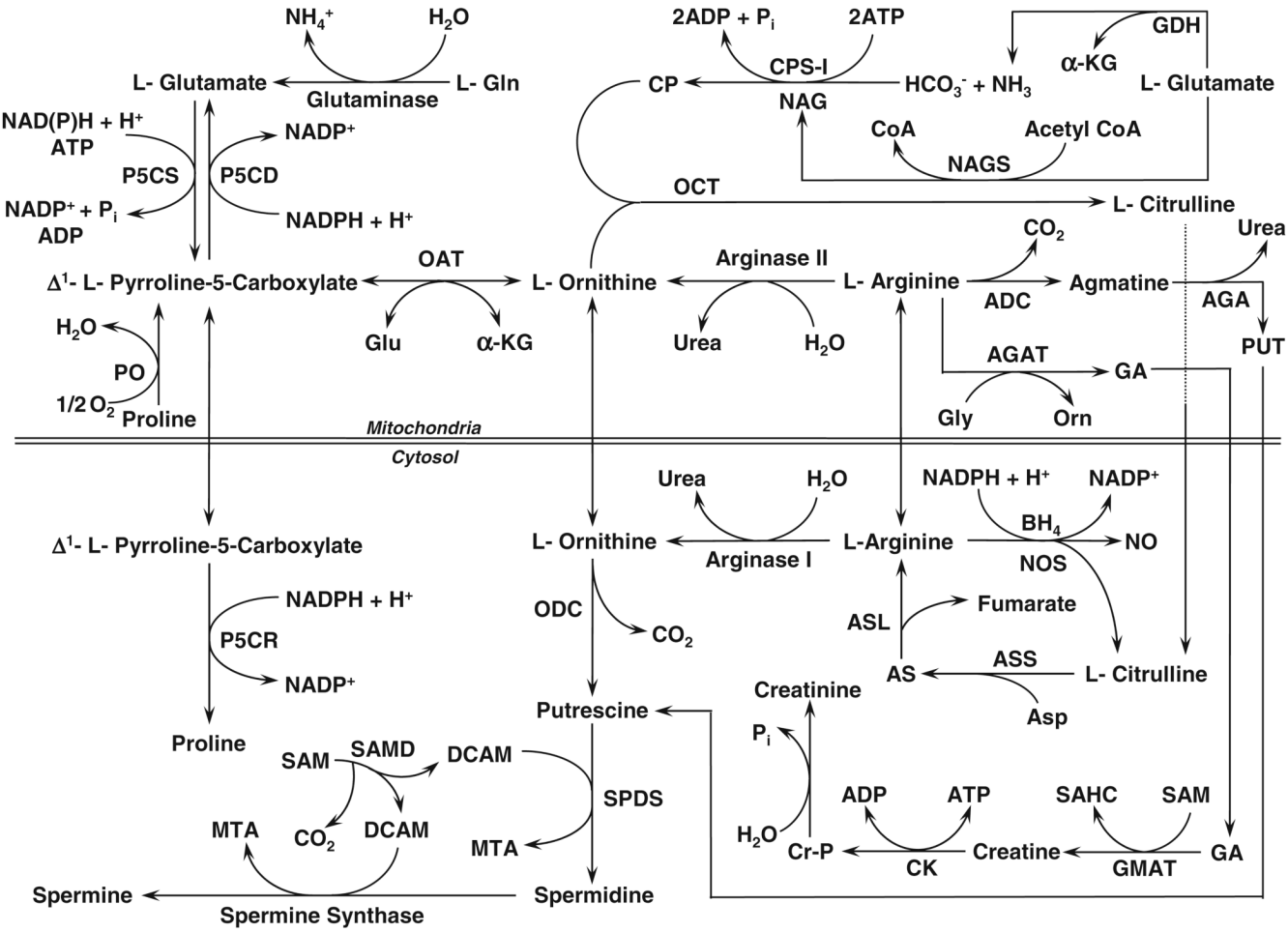

Fig. 1.

Arginine metabolism in mammals. *ADC* arginine decarboxylase, *AGA* agmatinase, *AGAT* arginine:glycine amidinotransferase, *ASL* argininosuccinate lyase, *ASS* argininosuccinate synthase, *AS* argininosuccinate, *Asp* aspartate, BH_4 (6R)-5,6,7,8-tetrahydro-L-biopterin, *CP* carbamoylphosphate, *CPS-I* carbamoylphosphate synthetase-I (ammonia), *DCAM* decarboxylated *S*-adenosylmethionine, *Glu* glutamate, *Gln* glutamine, *GDH* glutamate dehydrogenase, *GA* guanidinoacetate, *GMAT* guanidinoacetate *N*-methyltransferase, *CK* creatine kinase, *Cr–P* creatine-phosphate, *α-KG* *α*-ketoglutarate, *MTA* methylthioadenosine, *NAG* *N*-acetylglutamate, *NAGS* *N*-acetylglutamate synthase, *NO* nitric oxide, *NOS* nitric oxide synthase, *OAT* ornithine aminotransferase, *OCT* ornithine carbamoyltransferase, *ODC* ornithine decarboxylase, *PO* proline oxidase, *P5CD* pyrroline-5-carboxylate dehydrogenase, *P5CR* pyrroline-5-carboxylate reductase, *P5CS* pyrroline-5-carboxylate synthase, *PUT* putrescine, *SAM* *S*-adenosylmethionine, *SAMD* *S*-adenosylmethionine decarboxylase, *SAHC* *S*-adenosylhomocysteine, *SPDS* spermidine synthase. Reprinted from Biomedicine and Pharmacotherapy Fang et al. (2002) with permission from Elsevier

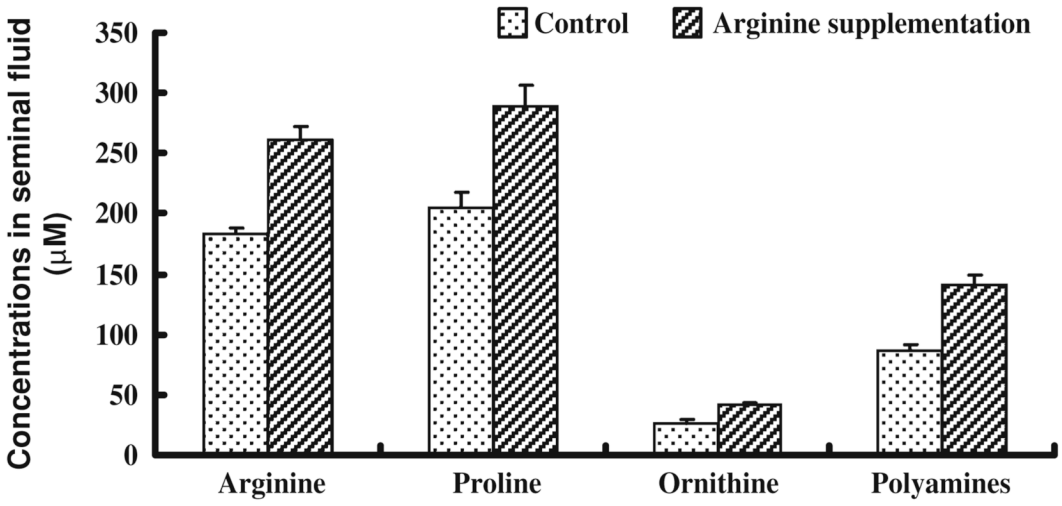

Fig. 2.

Concentrations of arginine, proline, ornithine, and polyamines (putrescine + spermidine + spermine) in seminal fluid of boars. Sexually active boars had free access to a corn and soybean meal-based diet supplemented with 1.0% L-arginine-HCl or 1.7% L-alanine (isonitrogenous control) for 30 days. At the end of arginine supplementation, semen samples were obtained for analysis of amino acids. Data are mean ± SEM, $n = 5$. Values of all the measured parameters are greater ($P < 0.01$) for arginine-supplemented boars compared with the control group. Adapted from Wu et al. (2007b)

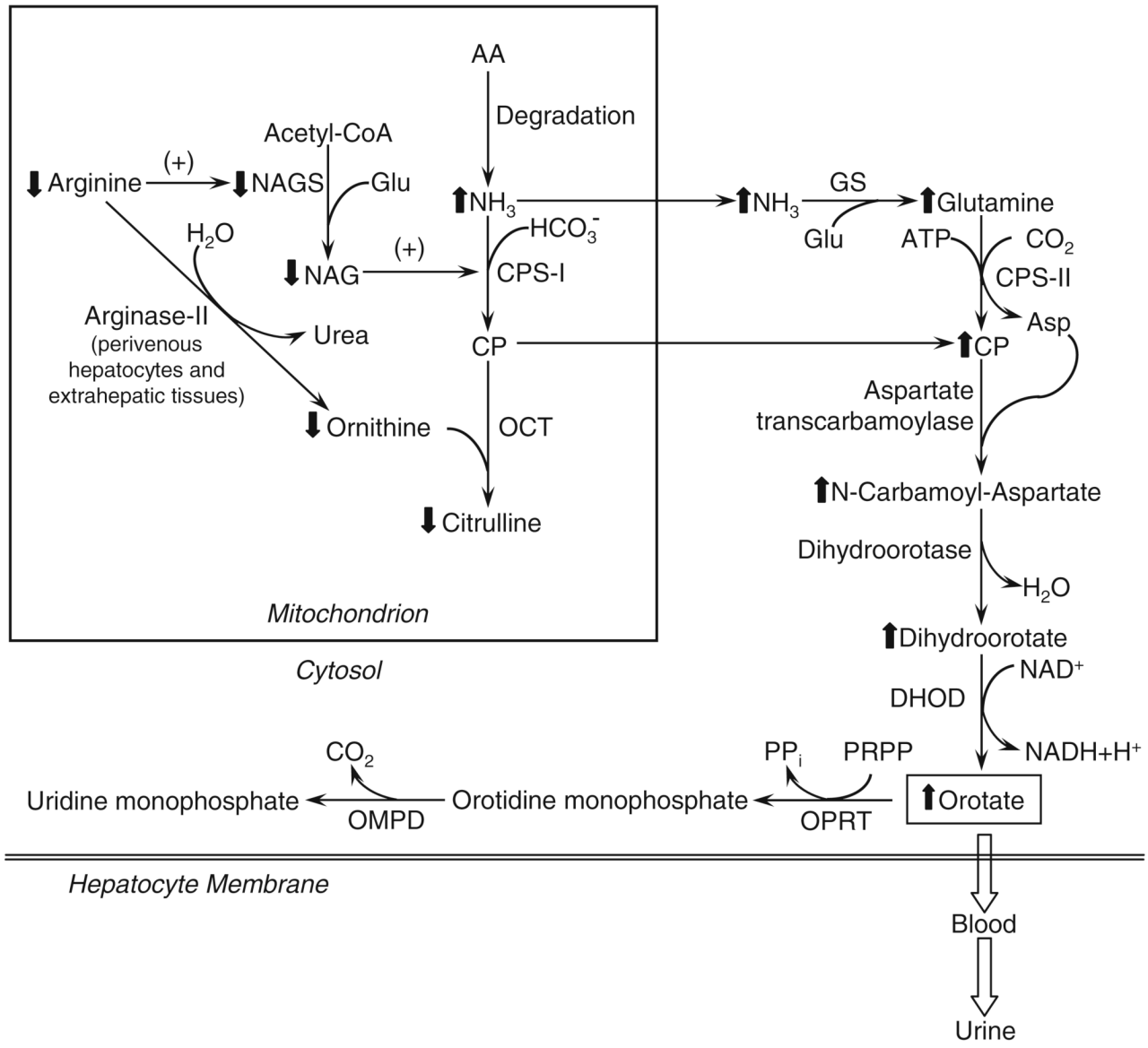

Fig. 3.

Mechanisms for hyperammonemia and increased oroate production in arginine-deficient mammals. *AA* amino acids, *Asp* aspartate, *CP* carbamoylphosphate, *CPS-I* carbamoylphosphate synthetase-I (ammonia), *CPS-II* carbamoylphosphate synthetase-II (glutamine), *DHOD* dihydroorotate dehydrogenase, *Glu* glutamate; *GS*, glutamine synthetase; *NAG*, *N*-acetylglutamate; *NAGS*, *N*-acetylglutamate synthase; *OCT*, ornithine carbamoyltransferase, *OMPD* orotidine monophosphate decarboxylase, *OPRT* orotate phosphoribosyltransferase, *PRPP* 5-phosphoribosyl-1-pyrophosphate, ↑ Increase, ↓ Decrease. Reprinted from Biomedicine and Pharmacotherapy Flynn et al. (2002) with permission from Elsevier

Table 1

Roles of arginine in growth, health and disease

Roles of arginine	Effect	Mediators
Cardiovascular disorders		
Coronary and peripheral arterial diseases	↓	NO
Heart failure, stroke, and ischemia/reperfusion injury	↓	NO
Sickle cell anemia and vasculopathy	↓	NO
Endothelial dysfunction in patients with CVRF		
Aging and hyperhomocysteinemia	↓	NO
Diabetes, hypertension, and smoking	↓	NO
Hypercholesterolemia and high fat feeding	↓	NO
Hormone secretion		
Growth hormone, glucagon, insulin, and prolactin	↑	NO and ornithine
Placental lactogen and progesterone	↑	NO and ornithine
Immune function		
B-cell maturation and antibody production	↑	NO, PA, and PS
Killing pathogens (bacteria, fungi, parasites and virus)	↑	NO
T-cell proliferation and cytokine production	↑	NO, PA, and PS
Metabolism		
BAT growth and energy-substrate oxidation	↑	cGMP, PA, cAMP, and NO
Cell signaling (AMPK, mTOR, and cGMP)	↑	NO and Arg
Lactogenesis and neonatal growth and development	↑	Arg, NO, mTOR, PA, and proline
Mitochondrial biogenesis and function	↑	cGMP, PA, and NO
Protein synthesis and muscle growth	↑	mTOR and PA
Ammonia detoxification via the urea cycle	↑	NAG and ornithine
Obesity, insulin resistance, and dyslipidemia	↓	AMPK signaling, Arg, and NO
Orotic aciduria and gouts	↓	NAG and ornithine
Production of ROS and oxidative stress	↓	Arg, creatine, PA, and NO
Protein degradation and apoptosis	↓	mTOR, NO, and autophagy
Reproduction		
Embryo implantation, survival, and growth	↑	NO, PA, and PS
Fetal survival, growth, and health	↑	NO, PA, and PS
Ovulation, ovarian steroidogenesis and oocyte quality	↑	NO and PA
Placental angiogenesis, growth, and function	↑	NO, PA, and PS
Spermatogenesis, sperm quality, and male fertility	↑	NO, PA, and PS
Uterine contractility and preterm labor	↓	NO
Erectile dysfunction	↓	NO
Preeclampsia in human pregnancy and animal models	↓	NO
Skeletal muscle and brain function	↑	Creatine, NO, and PS
Tissue injury and repair		
Cystic fibrosis and lung injury	↓	NO, PA, and proline
Gastrointestinal, liver and vessel injury	↓	NO, PA, and proline
Necrotizing enterocolitis in infants	↓	NO and PA
Renal disease with systemic hypertension	↓	NO

Roles of arginine	Effect	Mediators
Severe malaria, ulcers, and mitochondrial myopathy	↓	NO
Tissue integrity, wound healing, and angiogenesis	↑	NO, PA, proline, and PS
Tumor growth		
Tumorigenesis at early stages	↓	NO
Tumorigenesis at late stages	↑	PA, proline, ornithine, and PS

The symbols "↑" and "↓" denote enhancement and inhibition (or prevention), respectively

AMPK AMP-activated protein kinase; *BAT* brown adipose tissue, *CVRF* cardiovascular risk factors, *mTOR* mammalian target of rapamycin (protein kinase, *NAG N*-acetylglutamate, *NO* nitric oxide, *PA* polyamines, *PS* protein synthesis, *ROS* reactive oxygen species