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INVITED REVIEW

Catabolism and safety of supplemental L-arginine in animals

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Abstract L-arginine (Arg) is utilized via multiple pathways to synthesize protein and low-molecular-weight bioactive substances (e.g., nitric oxide, creatine, and polyamines) with enormous physiological importance. Furthermore, Arg regulates cell signaling pathways and gene expression to improve cardiovascular function, augment insulin sensitivity, enhance lean tissue mass, and reduce obesity in humans. Despite its versatile roles, the use of Arg as a dietary supplement is limited due to the lack of data to address concerns over its safety in humans. Data from animal studies are reviewed to assess arginine catabolism and the safety of long-term Arg supplementation. The arginase pathway was responsible for catabolism of 76–85 and 81–96 % Arg in extraintestinal tissues of pigs and rats, respectively. Dietary supplementation with Arg–HCl or the Arg base [315- and 630-mg Arg/(kg BW d) for 91 d] had no adverse effects on male or female pigs. Similarly, no safety issues were observed for male or female rats receiving supplementation with 1.8- and 3.6-g Arg/(kg BW d) for at least 91 d. Intravenous administration of Arg–HCl to gestating sheep at 81 and 180 mg Arg/(kg BW d) is safe for at least 82 and 40 d, respectively. Animals fed conventional diets can well tolerate large amounts of supplemental Arg [up to 630-mg Arg/(kg BW d) in pigs or 3.6-g Arg/(kg BW d) in rats] for 91 d, which are equivalent to 573-mg Arg/(kg BW d) for humans. Collectively, these results can help guide studies to determine the safety of long-term oral administration of Arg in humans.

Keywords Amino acids · Nutrition · Catabolism · Health · Dietary supplementation

Abbreviations

AA	Amino acid(s)
Arg	L-arginine
AUC	Area-under-the curve
BW	Body weight
CAT	Cationic amino acid transporter
d	Day(s)
hArg	Homoarginine
MRSD	Maximum recommended starting dose
NO	Nitric oxide
NOS	Nitric oxide synthase

Introduction

L-arginine (Arg; free base) is a naturally occurring amino acid (AA) in animals and plants (Wu 2013). The content of this nutrient is relatively high in foods originating from animals (e.g., meat and seafood), plants (e.g., cottonseed, peanut, rapeseed, rice protein concentrate, soy protein isolate, and watermelon), and other photosynthetic organisms (e.g., microalgae and macroalgae), but is relatively

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low in the milk of most mammals, including humans, cows, and sows, as compared with most other AAs (Collins et al. 2007; Davis et al. 1994; Li et al. 2011; Wu et al. 2009, 2016). Although Arg had traditionally been classified on the basis of nitrogen balance, as a "nutritionally nonessential AA" for healthy adults (Rose 1957), this AA is now considered to be a conditionally essential nutrient in the diet for meeting specific needs (e.g., spermatogenesis in males and embryonic growth and survival in gestating females) beyond protein synthesis (Bazer et al. 2015; Hou et al. 2015, 2016a, b; Kong et al. 2012).

Arg is now recognized to be a beneficial ingredient in dietary supplements, functional foods, and beverages for humans (Brown et al. 2015; Lan et al. 2015; Popolo et al. 2014; Wu 2013). However, the use of Arg for these purposes has been limited largely owing to the concerns of government regulatory agencies, policymakers, and consumers over the safety of long-term supplementation. This is primarily because there is a lack of relevant clinical data in the literature (Calabrò et al. 2014; Shao and Hathcock 2008) and because oral administration of Arg (3 × 3 g per day) was reported to cause adverse cardiovascular events in patients with acute myocardial infarction (Schulman et al. 2006). At present, a safe upper limit for Arg supplementation to healthy adults is unknown (Böger 2014; Shao and Hathcock 2008). Studies with animal models can be used to estimate the maximum recommended starting dose (MRSD) for designing initial clinical trials with men and women after a safety factor of at least 10 is taken into consideration (FDA 2005).

Metabolic and regulatory functions of Arg

The first step for the utilization of extracellular Arg in animals is its uptake by cells. Among cationic AA transporters 1, 2, and 3 (CAT-1, 2, and 3), CAT-1 is the main transporter of Arg and other cationic AAs (e.g., lysine, ornithine, and histidine) in most cell types (Hatzoglou et al. 2004). Extrahepatic cells have high rates of transport of Arg and its immediate precursor, citrulline (Breuillard et al. 2015; Monné et al. 2015; Morris 2006). In contrast, the plasma membranes of mammalian hepatocytes do not express CAT-1 (Closs et al. 2004) or citrulline transporters (Windmueller and Spaeth 1981). Thus, the livers of both pigs (Wu et al. 2007b) and rats (Windmueller and Spaeth 1981) extract only a limited amount of Arg from the blood, and do not take up citrulline.

Arg serves as a substrate for syntheses of protein, creatine, nitric oxide (NO), agmatine, polyamines, proline, glutamate, and homoarginine in mammals, thereby playing important roles in nutrition and physiology (Luiking et al. 2012; Tsikas and Wu 2015; Wu and Morris 1998). For example, creatine participates in ATP storage in skeletal muscle and nerves (Brosnan and Brosnan 2007). Second, NO regulates endothelium-dependent relaxation of blood and lymph vessels, immunity, neurotransmission, and energy metabolism (Benhar 2015; Dai et al. 2013). Third, polyamines are necessary for DNA and protein syntheses, as well as proliferation and differentiation of all cell types (Agostinelli 2014). Fourth, agmatine binds α2-adrenergic and imidazoline receptors and blocks N-methyl-D-aspartate receptor and other cation ligand-gated channels, while inhibiting or modulating NO synthase (NOS) and inducing secretion of some peptide hormones (Moretti et al. 2014). Fifth, homoarginine may antagonize asymmetric dimethylarginine in cardiovascular and neurological systems to beneficially improve NO synthesis (Bernstein et al. 2015; Pilz et al. 2015). The metabolic versatility of Arg is the basis for great interest in its use over the past 25 years to prevent and treat dysfunction of multiple systems (e.g., circulatory, gastrointestinal, immune, and reproductive systems) in mammals and birds (Dashtabi et al. 2015; Wu et al. 2009).

Much evidence shows that Arg has regulatory functions in gene expression, cell signaling, and enzyme activity (Wu 2013). For example, physiological concentrations of Arg allosterically activate N-acetylglutamate synthase to maintain the hepatic urea cycle in an active state, stimulate the expression of peroxisome proliferator-activated receptor γ coactivator-1α (a master activator of mitochondrial biogenesis), enhance the phosphorylation of AMP-activated protein kinase, and activate the mechanistic target of rapamycin cell signaling pathway in multiple tissues (Caldovic and Tuchman 2003; Kim et al. 2013; Tan et al. 2012). Thus, Arg promotes the oxidation of long-chain fatty acids and glucose in insulin-target tissues, protein synthesis in skeletal muscle, and brown adipose tissue development, thereby enhancing lean tissue mass and reducing white adipose tissue in obese rats (Fu et al. 2005; Jobgen et al. 2009), obese sheep (Satterfield et al. 2012), growing–finishing pigs (Tan et al. 2009), and obese humans (Hurt et al. 2014; Lucotti et al. 2006). These effects of Arg have important implications for improving human health and well-being, as well as global animal production.

Catabolism and safety of supplemental Arg in pigs

The pig is a widely used animal model for studying human nutrition because of anatomical and functional similarities (e.g., digestion, absorption, and metabolism of nutrients, as well as physiology, immune response, and toxicology) between these two species (Burrin et al. 2014; Suryawan and Davis 2014). Therefore, we used pigs fed a conventional diet to determine their physiological responses to dietary supplementation with graded levels of Arg or

Arg–HCl (Hu et al. 2015). In the lumen of the gastrointestinal tract, the hydrolysis of food protein by proteases and peptidases generates Arg and Arg-containing dipeptide/tripeptides, followed by the absorption of those digestion products into small-intestinal mucosal cells (the major route) and luminal bacteria (Wu 2013). Using 35-d-old pigs (weaned at 21 d of age) that were individually fitted with a simple T-cannula in the mid-duodenum (Wu et al. 1996b), we found no measurable catabolism of Arg in the stomach and the upper part of duodenum in the pigs fed a corn- and soybean meal-based diet supplemented with 1 or 2 % Arg. Thus, compared with the pigs without Arg supplementation, there were no increases in the content of ornithine, proline, citrulline, agmatine, homoarginine, polyamines or nitrate in the duodenal digesta of the Arg-supplemented pigs. Furthermore, the supplemental Arg was fully recovered in the duodenal digesta of the pigs. For oral or intravenous administration into humans and animals, a neutral salt of Arg (e.g., Arg–HCl or Arg-α-ketoglutarate) is used to prevent an acid–base imbalance. Arg can be used as an ingredient in a pelleted enteral diet for some species (e.g., swine and rats) without inducing feed aversion (Wu et al. 2014). Supplemental Arg–HCl, Arg-α-ketoglutarate, or Arg is fully available for absorption by enterocytes and luminal microbes of the small intestine (Wu 2014).

Enterocytes of post-weaning pigs express type-I and type-II arginases (Flynn et al. 1999) to actively degrade Arg into ornithine and urea, with ornithine being further converted into proline by ornithine aminotransferase and pyrroline-5-carboxylate reductase (Wu et al. 1996a). Bacteria of the lumen of the pig small intestine are also capable of metabolizing some dietary Arg via these pathways (Dai et al. 2012). Intestinal catabolism of Arg reduces the amount of dietary Arg entering the portal vein. The availability of an oral substance in the plasma can be calculated by dividing the area under the curve (AUC) of the substance in the plasma for oral administration by that for i.v. administration (Jacquez 1996; Ritschel 1986). Based on this principle, we found that approximately 40 % of oral Arg is utilized (through catabolism and protein synthesis) by the small intestine in growing and adult pigs, with the remaining portion of Arg entering the portal vein (Wu et al. 2007a). Results of our studies indicate: (1) the absolute availability of oral Arg in the blood did not differ among the male or female pigs supplemented with 315- and 630-mg Arg/(kg BW d); and (2) that similar proportions of enteral Arg enter the blood of pigs in these dietary groups (Table 1).

In extraintestinal tissues, the Arg that is not used for protein synthesis enters catabolic pathways initiated by arginase, arginine:glycine amidinotransferase, arginine decarboxylase, and NOS (Fig. 1). These pathways are used to synthesize ornithine, creatine, agmatine, and NO, respectively (Tsikas and Wu 2015; Wu et al. 2009). Based on our published work (Hu et al. 2015), we estimated that the small intestine and extraintestinal tissues can catabolize: (1) 153- and 365-mg Arg/(kg BW d), respectively, in the 64.3-kg pig fed a basal diet containing 1.35 % Arg without Arg supplementation; and (b) 405- and 743-mg Arg/(kg BW d) in the 67.1-kg pig fed the basal diet supplemented with 2 % Arg (Table 2).

Based on the daily excretion of creatinine, homoarginine (hArg), agmatine, and NOx in the urine (Table 3), creatine synthesis is the major pathway for Arg catabolism in pigs supplemented with 0-, 315-, and 630-mg Arg/(kg BW d) (Table 4). Note that although the liver and kidney can synthesize homoarginine from Arg and lysine, this pathway is quantitatively minor for Arg utilization in the animals. Based on the production of creatine + hArg + agmatine + NOX via the non-arginase pathway (Table 4), the arginase pathway (calculated as rates of Arg catabolism via arginase and non-arginase pathways minus rates of Arg catabolism via the non-arginase pathway) contributed to 76, 82, and 85 % of Arg catabolized in extraintestinal tissues of the 65-kg pig supplemented with 0-, 315-, and 630-mg Arg/(kg BW d), respectively. Rates of urinary excretion of Arg, which are negligible in comparison with dietary Arg intake, or concentrations of Arg in urine, did not differ among these groups of pigs (Hou et al. 2016b). These results indicate that dietary Arg underwent extensive degradation in pigs.

We assessed the safety of long-term Arg–HCl or Arg supplementation in pigs between 30 and 121 d of age, based on general observations (e.g., behavior, skin health, and hair), feed intake, growth, body composition, as well as hematological and blood chemistry tests (Hu et al. 2015). In this study, male and female pigs were fed a typical corn- and soybean meal-based diet (containing 1.35 % Arg) supplemented with 0, 1, 1.5, and 2 % Arg (as either Arg–HCl or Arg) for 91 d. The supplemental doses of 0, 1, 1.5, and 2 % Arg provided pigs with 0-, 315-, 473-, and 630-mg Arg/(kg BW d), respectively, beyond the amount of digestible Arg in the basal diet [382 mg/(kg BW d)] at the constant feed intake of 31.5 g/(kg BW d). Hematological variables at 1 and 4 h after feeding were: (1) the numbers of white blood cells, red blood cells, and platelets; (2) blood hemoglobin, blood pH, plasma protein, and fibrinogen; (3) mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean corpuscular volume; and (4) percentages of neutrophils, lymphocytes, monocytes, and eosinophils. Serum chemistry at 1 and 4 h after feeding included concentrations of: (1) total serum protein, albumin, and globulins; (2) total bilirubin, amino acids, glucose, urea, ammonia, creatinine, free fatty acids, triglyceride, and cholesterol; (3) sodium, chloride, calcium, phosphorus, and magnesium; (4) insulin, growth hormone, alkaline phosphatase, alanine transaminase, aspartate transaminase,

Table 1 Absolute availability of oral L-arginine (Arg) in the blood of pigs and rats

Animals	Dose of administered L-arginine-HCl (mg/kg body weight)	Area under the curve of Arg in plasma for i.v. administration (h μmol/L)	Area under the curve of Arg in plasma for oral administration (h μmol/L)	Absolute availability of oral Arg (%, g/g)
Male pigs[1]	50	898 ± 19	546 ± 16	60.7 ± 1.2
	100	1884 ± 101	1147 ± 58	61.0 ± 1.6
	150	2864 ± 71	1762 ± 92	61.4 ± 2.4
Female pigs[1]	50	910 ± 20	552 ± 14	60.8 ± 2.0
	100	1946 ± 71	1170 ± 65	61.2 ± 2.2
	150	2907 ± 85	1791 ± 81	61.6 ± 1.7
Male rats[2]	200	504 ± 15	305 ± 18	60.3 ± 1.9
	400	1071 ± 66	655 ± 49	61.0 ± 1.5
	800	2209 ± 79	1357 ± 53	61.5 ± 2.1
Female rats[2]	200	515 ± 12	310 ± 14	60.1 ± 2.2
	400	1109 ± 89	669 ± 54	60.6 ± 1.8
	800	2248 ± 102	1387 ± 86	61.7 ± 2.5

Values are mean ± SEM, $n = 6$. Data were analyzed by 1-way ANOVA and the Student–Newman Keuls multiple comparison test (Assaad et al. 2014). Within a column for males or females of each species, mean values for area-under-the curve (AUC) of Arg in plasma differ ($p < 0.05$)

At 5 h after i.v. or oral administration of Arg, plasma concentrations of Arg returned to the baseline values in all dose groups of pigs and rats. Thus, within 24 h, pigs or rats can dispose of 4.8 times (i.e., 24/5 = 4.8) the administered Arg doses. For example, in pigs, the single doses of 50-, 100-, and 150-mg Arg/kg body weight are equivalent to 240-, 480-, and 720-mg Arg/(kg BW d), which cover the range of our supplemental doses of Arg in pigs [i.e., 315 and 630 mg/(kg BW d)]. The absolute availability of oral Arg in the plasma did not differ among the different groups of pigs or rats

[1] Male or female pigs were fed a corn- and soybean meal-based diet between d 110 and d 117 of age, as described by Hu et al. (2015). At d 118 of age, pigs received single i.v. administration of L-arginine-HCl (50-, 100-, or 150-mg Arg/kg body weight). Blood samples (25 μl) were obtained from the ear vein of pigs immediately before and at 15, 30, 60, 120, 180, 240, and 300 min after L-arginine-HCl administration. Plasma Arg concentrations were analyzed to calculate the area under the curve (AUC) of Arg (Wu et al. 2007a). On d 120 of age, individual pigs received a single oral administration of L-arginine-HCl (50-, 100-, or 150-mg Arg/kg body weight); blood samples were obtained, and plasma Arg concentrations were analyzed to calculate the AUC of Arg, as described previously. The absolute availability (%) of oral Arg was calculated as (AUC of Arg in plasma for i.v. administration ÷ AUC of Arg in plasma for oral administration) × 100 (Wu et al. 2007a). For male pigs, the ratio of AUC (h μmol/L) to oral Arg dose (mg/kg body weight) was 10.9 ± 0.32, 11.5 ± 0.58, and 11.7 ± 0.61; $p = 0.541$ for 50-, 100-, and 150-mg Arg/kg body weight doses, respectively. Similar results were obtained for female pigs

[2] Male or female rats were fed a fed a casein-based semi-purified diet between d 122 and d 129 of age, as described by Yang et al. (2015). At d 130 of age, rats received a single i.v. administration of L-arginine-HCl (200, 400 or 800 mg Arg/kg body weight). Blood samples (25 μl) were obtained from the tail vein of conscious rats immediately before and at 15, 30, 60, 120, 180, 240, and 300 min after L-arginine-HCl administration. The absolute availability (%) of oral Arg in rats was calculated as described for pigs. For male rats, the ratio of AUC (h μmol/L) to oral Arg dose (mg/kg body weight) was 1.53 ± 0.09, 1.64 ± 0.12, and 1.70 ± 0.07; $p = 0.462$) for 200-, 400-, and 800-mg Arg/kg body weight doses, respectively. Similar results were obtained for female rats

gamma-glutamyl transpeptidase, insulin-like growth factor-I, and lactate dehydrogenase. Results of all of the measured variables in pigs fed diets supplemented with 0, 1, 1.5, and 2 % Arg were within physiological ranges and were not adversely affected by Arg administration (Hu et al. 2015). Of note, dietary supplementation with Arg to pigs did not affect concentrations of lysine or histidine in the plasma (indicating a lack of antagonism among basic amino acids), but beneficially increased lean tissue mass and reduced white fat, as well as concentrations of ammonia, free fatty acids, triglyceride, and cholesterol in the plasma (Hu et al. 2015). These results indicate that dietary supplementation with 315-to-630-mg Arg/(kg BW d) is safe in pigs fed corn- and soybean meal-based diets supplemented with 1 or 2 % Arg for at least 91 d. This conclusion, however, may not apply to low-birth-weight neonatal pigs (Getty et al. 2015). Of note, supplementing 4 % Arg (as Arg base) to a corn- and soybean meal-based diet had adverse effects on young pigs, as indicated by AA imbalance and reduced growth performance (Edmonds et al. 1987).

Catabolism and safety of supplemental Arg in rats

The rat is a standard laboratory animal model for studying digestion, absorption, and metabolism of nutrients, as well as physiology, immune response, and toxicology in humans (FDA 2005; Tsubuku et al. 2004; Wu et al. 1999). Like pigs, the small intestine of rats also extensively catabolizes Arg in the enteral

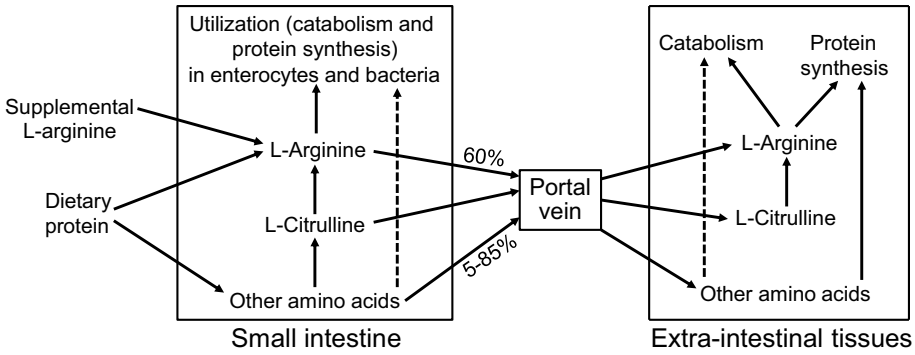

Fig. 1 Metabolism of L-arginine in animals. In the gastrointestinal tract, the hydrolysis of dietary protein by extracellular proteases and peptidases releases L-arginine, other amino acids, and small peptides into the lumen. Enterocytes take up these digestion products, degrade arginine, and release L-citrulline. Catabolism of amino acids also occurs in the luminal bacteria of the gut. In post-weaning pigs and rats, approximately 40 % of L-arginine and 15 % (e.g., tryptophan) to 95 % (e.g., glutamate) of other amino acids in the lumen of the small intestine are utilized through catabolism and protein synthesis, during the first pass into the portal vein. Thus, 5 % (e.g., glutamate) to 85 % (e.g., tryptophan) of amino acids in the lumen of the small intestine enters the portal vein. In extraintestinal tissues, the L-arginine that is not used for protein synthesis enters catabolic pathways initiated by arginase, arginine:glycine amidinotransferase, arginine decarboxylase, and nitric oxide synthase

diet (Wu and Morris 1998), such that 40 % of orally administered Arg is utilized (through catabolism and protein synthesis) in the gut during the first pass, with the remaining portion of Arg entering the portal vein (Wu et al. 2007a). Based on our published work (Yang et al. 2015), we estimated that the small intestine and extraintestinal tissues can catabolize: (1) 106- and 405-mg Arg/(kg BW d), respectively, in the 0.45-kg male rat fed a casein-based semi-purified diet containing 0.61 % Arg without Arg supplementation; and (b) 1.55- and 2.56-g Arg/(kg BW d), respectively, in 0.45-kg rats fed the basal diet supplemented with 3.6 g Arg/(kg BW d) via drinking water (Table 5). Adding Arg–HCl to drinking water is a simple and convenient way to supplement this AA to rats.

The metabolic patterns of Arg in rats are similar to those in pigs (Tables 3, 4). Overall, the arginase pathway (rates of Arg catabolism via both arginase and nonarginase pathways given in Table 1 minus rates of Arg the non-arginase pathway) contributed to 81, 94, and 96 % of Arg catabolized in extraintestinal tissues of the adult rat supplemented with 0, 1.8, and 3.6 g Arg/(kg BW d), respectively. Based on the negligible rates of urinary excretion of Arg among these groups of rats (Table 4), we conclude that rats have a high capacity to degrade dietary Arg as pigs do.

We assessed the safety of long-term Arg–HCl or Arg supplementation in rats between 42 and 133 d of age, based on general observations (e.g., behavior, skin health, and hair), feed intake, growth, weight of tissues (including brain, pancreas, spleen, skeletal muscle, and stomach), body composition, rectal temperature, systolic blood pressure, oxygen consumption, carbon-dioxide production, energy expenditure, and blood chemistry (e.g., blood pH) tests (Yang et al. 2015). In this experiment that lasted for 91 d, male and female rats were fed a casein-based semi-purified diet (containing 0.61 % Arg) and received drinking water containing Arg–HCl, which provided 0-, 1.8-, or 3.6-g Arg/(kg BW d) beyond the amount of digestible Arg in the basal diet [264 mg/(kg BW d)]. Variables measured in the plasma included: (1) amino acids, glucose, urea, ammonia, creatinine, free fatty acids, triglyceride, and cholesterol; (2) insulin, growth hormone, insulin-like growth factor-I, corticosterone, adiponectin, leptin, total triiodothyronine, total thyroxine, alanine transaminase, aspartate transaminase, alkaline phosphatase, and lactate dehydrogenase. Results of all the measured variables (including hematological and chemistry tests) in male and female rats receiving oral administration of 0-, 1.8-, and 3.6-g Arg/(kg BW d) were within physiological ranges and were not adversely affected by Arg administration (Yang et al. 2015). Dietary supplementation with Arg to rats had no effect on concentrations of lysine or histidine in plasma [an indicator of the balance among basic AAs (Holecek and Sispera 2016)], but reduced white fat mass and concentrations of ammonia, free fatty acids, and triglycerides in plasma, while increasing lean tissue mass (Yang et al. 2015). Our results indicate that dietary supplementation with Arg [1.8–3.6 g/(kg BW d)] is safe in rats for at least 91 d. Similarly, based on histological examination of tissues, food intake, and overall health status, Tsubuku et al. (2004) reported that adult male and female rats of the same age as the rats used in our study (Yang et al. 2015) can tolerate at least 3.3- and 3.9-g Arg/(kg BW d), respectively, for 13 weeks.

Table 2 Catabolism of supplemental L-arginine (Arg) in extraintestinal tissues of 121-d-old pigs

Supplemental Arg dose via the diet mg/(kg body weight·d)	Digestible Arg in dietary protein[1] mg/(kg body weight·d)	Arg catabolism in the small intestine[2] mg/(kg body weight·d)	Total Arg entering the portal vein from the basal diet plus supplementation[3] mg/(kg body weight·d)	Arg accretion in extraintestinal tissues[4] mg/(kg body weight·d)	Arg catabolism via arginase and non-arginase pathways in extraintestinal tissues[5] mg/(kg body weight·d)
0	382	153	229	136	365
315	382	279	418	136	554
473	382	342	513	136	649
630	382	405	607	136	743

Data were calculated from the published work (Hu et al. 2015). Values are means for 12 pigs per dietary group (6 males and 6 females). Between 30 and 121 d of age, male and female pigs were fed a corn- and soybean meal-based diet supplemented with 0, 1.21, 1.81, or 2.42 % Arg–HCl (0, 1, 1.5, or 2 % Arg). The supplemental doses of Arg were 0-, 315-, 473-, or 630-mg/(kg BW d)

[1] Calculated according to the body weight (64.3–67.1 kg) of pigs, their food intake [31.5 g/(kg BW d)], Arg content in the basal diet (1.35 %), and the true digestibility of Arg (89.9 %) in the dietary protein (Wu et al. 2014). The basal diet provided 425-mg Arg/(kg BW d)]

[2] Calculated according to the rate of first-pass utilization of luminal Arg (40 %) by the small intestine (Wu et al. 2007a, b). Namely, Arg catabolism in the small intestine = (Supplemental Arg + digestible Arg in dietary protein) × 40 %; for example, in the 630 mg/(kg BW d) supplemental Arg group, the value = (630 + 382) × 40 % = 405 mg/(kg BW d)

[3] Calculated according to the rate of first-pass utilization of luminal Arg (40 %) by the small intestine (Wu et al. 2007a). Namely, total Arg entering the portal vein from the basal diet plus supplementation = (Supplemental Arg + digestible Arg in dietary protein) × (1–40 %); for example, in the 630-mg/(kg BW d) supplemental Arg group, the value = (630 + 382) × (1–40 %) = 607 mg/(kg BW d)

[4] Calculated according to the gain of extraintestinal tissues and Arg content in extraintestinal tissues (1.06-g Arg/100-g wet tissue weight) (Wu et al. 2014). The pooled standard error of the mean is 6 mg/(kg BW d). At d 121 of age, the mean body weights of pigs were 64.3, 65.5, 66.3, and 67.3 kg in the 0-, 315-, 473-, and 630-mg/(kg BW d) supplemental Arg groups, respectively (Hu et al. 2015), whereas the mean weight gains of extraintestinal tissues were 827, 841, 848, and 858 g/d in those groups, respectively. The accretion of Arg in extraintestinal tissues was calculated on the basis of Arg accumulation in all non-intestinal tissues (including the blood) and, therefore, included proteins secreted by these tissues

[5] Calculated as total Arg entering the portal vein from the basal diet plus supplementation + endogenous Arg synthesis [i.e., 272 mg/(kg BW d)] (Wu et al. 1997)—Arg accretion in proteins of extraintestinal tissues; for example, in the 630 mg/(kg BW d) supplemental Arg group, the value = (607 + 272−136) = 743 mg/(kg BW d). The pooled standard error of the mean is 14 mg/(kg BW d). Within the range of oral Arg supplementation, synthesis of citrulline from glutamine and proline by pig enterocytes was not affected (our unpublished data)

Table 3 Urinary excretion of metabolites in pigs and rats supplemented with or without L-arginine for 13 weeks

Animals	Supplemental dose of Arg mg Arg/(kg body weight d)	Excretion of Arg and its metabolites in urine mg Arg or its metabolite/(kg body weight·d)				
		Arg	hArg	Creatinine	NOx	Agmatine
Pigs[1]	0	0.258 ± 0.017	0.095 ± 0.004[c]	54.5 ± 2.4[c]	1.02 ± 0.06[c]	0.027 ± 0.002[c]
	315	0.281 ± 0.018	0.149 ± 0.006[b]	62.8 ± 2.8[b]	1.55 ± 0.08[b]	0.035 ± 0.002[b]
	630	0.296 ± 0.018	0.210 ± 0.009[a]	71.0 ± 3.2[a]	2.02 ± 0.11[a]	0.045 ± 0.003[a]
Rats[2]	0	0.514 ± 0.030	0.109 ± 0.005[c]	48.2 ± 1.9[c]	1.60 ± 0.06[c]	0.028 ± 0.002[c]
	1800	0.550 ± 0.035	0.178 ± 0.007[b]	54.8 ± 2.3[b]	2.38 ± 0.09[b]	0.037 ± 0.003[b]
	3600	0.598 ± 0.033	0.286 ± 0.011[a]	62.0 ± 2.6[a]	3.16 ± 0.12[a]	0.053 ± 0.004[a]

Data were calculated from the published work (Hou et al. 2016b). Values are mean ± SEM, $n = 12$ pigs (121 d of age; 6 males and 6 females) or 12 rats (133 d of age; 6 males and 6 females) per dietary group. The measured variables did not differ between males and females in either animal species

[1] Between 30 and 121 d of age, male and female pigs were fed a corn- and soybean meal-based diet supplemented with 0, 1.21, or 2.42 % Arg–HCl (0, 1 or 2 % Arg) to provide intake of 0-, 315-, or 630-mg Arg/(kg BW d). On d 120 of age, 24-h urine collection was made from pigs in metabolism cages

[2] Between 42 and 133 d of age, male and female Sprague–Dawley rats were fed a casein-based semi-purified diet and received drinking water supplemented with or without Arg–HCl to provide the supplemental intake of 0-, 1.8-, or 3.6-g Arg/(kg BW d). On d 132 of age, 24-h urine collection was made from rats in metabolism cages

[a–c] Within a column for each species, means sharing different superscript *letters* differ ($p < 0.05$)

Table 4 Extraintestinal catabolism of arginine via arginase and non-arginase pathways in pigs and rats supplemented with or without L-arginine for 13 weeks

Animals	Supplemental dose of Arg mg Arg/(kg body weight d)	Extraintestinal catabolism of Arg to form its metabolites via non-arginase pathways[1] mg Arg/(kg body weight d)				Extraintestinal catabolism of Arg via arginase[2] mg Arg/(kg body weight d)
		hArg	Creatinine	NOx	Agmatine	
Pigs	0	0.088 ± 0.004[c]	83.9 ± 3.7[c]	2.86 ± 0.17[c]	0.036 ± 0.003[c]	278 ± 5.6[c]
	315	0.138 ± 0.005[b]	96.7 ± 4.3[b]	4.35 ± 0.22[b]	0.047 ± 0.003[b]	453 ± 8.1[b]
	630	0.194 ± 0.008[a]	109 ± 4.9[a]	5.67 ± 0.31[a]	0.060 ± 0.004[a]	628 ± 11[a]
Rats	0	0.100 ± 0.005[c]	74.2 ± 2.9[c]	4.49 ± 0.17[c]	0.037 ± 0.003[c]	335 ± 12[c]
	1800	0.165 ± 0.006[b]	84.4 ± 3.5[b]	6.68 ± 0.25[b]	0.050 ± 0.004[b]	1402 ± 38[b]
	3600	0.265 ± 0.010[a]	95.5 ± 4.0[a]	8.87 ± 0.34[a]	0.071 ± 0.005[a]	2466 ± 47[a]

Calculated from data in Table 3 according to the molecular weights of each metabolite. Values are mean ± SEM, $n = 12$ pigs (121 d of age; 6 males and 6 females) or 12 rats (133 d of age; 6 males and 6 females) per dietary group. Rates of extraintestinal Arg catabolism were 365, 554, and 743 mg/(kg BW d) in pigs supplemented with 0, 315, and 630 mg Arg/(kg BW d), respectively (Hu et al. 2015). Rates of extraintestinal Arg catabolism were 414 ± 18, 1494 ± 50, and 2571 ± 64 mg/(kg BW d) in rats supplemented with 0, 1.8, and 3.6 g Arg/(kg BW d), respectively (Hou et al. 2016a, b)

[1] hArg, creatinine, NOx and agmatine were produced from Arg via non-arginase pathways (Wu and Morris 1998)

[2] These values are calculated as the rates of Arg catabolism via arginase plus non-arginase pathways (Table 2) minus the rates of Arg catabolism via the non-arginase pathway. The values of extraintestinal Arg catabolism via the arginase pathway do not include proteins excreted by extraintestinal tissues

[a–c] Within a column for each species, means sharing different superscript *letters* differ ($p < 0.05$)

Catabolism and safety of supplemental Arg in sheep

The sheep is an excellent animal model to study fetal growth and maternal physiological adaptation to changes in environmental factors (including nutrition and toxins) during human pregnancy (Reynolds et al. 2015; Sawant et al. 2015). Uterine-derived Arg is essential for the growth, development, and survival of ovine embryos (Wang et al. 2014a, b, c). Because bacteria in the ovine rumen completely degrade dietary Arg (Wu 2013), intravenous administration of Arg was adopted to evaluate its safety in these

Table 5 Catabolism of supplemental L-arginine (Arg) in extraintestinal tissues of 133-d-old rats

Sex	Supplemental Arg dose via drinking water mg Arg/(kg body weight d)	Arg intake from the basal diet[1] mg Arg/(kg body weight·d)	Digestible Arg in dietary protein[2] mg Arg/(kg body weight·d)	Arg extraction in the small intestine[3] mg Arg/(kg body weight·d)	Total Arg entering the portal vein from the basal diet plus water[4] mg Arg/(kg body weight·d)	Arg accretion in extra intestinal tissues[5] mg Arg/(kg body weight·d)	Arg catabolism in extra intestinal tissues[6] mg Arg/(kg body weight·d)
Male	0	278 ± 5.2	264 ± 4.9	106 ± 2.0^{c}	158 ± 9.4^{c}	25 ± 1.3	405 ± 26^{c}
	1800	279 ± 6.8	265 ± 6.5	826 ± 23^{b}	1239 ± 63^{b}	26 ± 1.4	1485 ± 72^{b}
	3600	276 ± 5.4	262 ± 5.1	1545 ± 38^{a}	2317 ± 82^{a}	26 ± 1.4	2563 ± 93^{a}
Female	0	302 ± 6.0	287 ± 5.7	115 ± 2.3^{c}	172 ± 10^{c}	23 ± 1.2	422 ± 28^{c}
	1800	303 ± 6.3	288 ± 6.0	835 ± 21^{b}	1253 ± 64^{b}	23 ± 1.2	1502 ± 74^{b}
	3600	300 ± 6.7	285 ± 6.4	1554 ± 36^{a}	2331 ± 88^{a}	24 ± 1.3	2579 ± 96^{a}

Data were calculated from the published work (Yang et al. 2015). Values are means for 12 rats per group (6 males and 6 females)

Between 6 and 19 weeks of age, male or female Sprague–Dawley rats were fed a casein-based semi-purified diet and received drinking water supplemented with Arg–HCl to provide 0, 1.8, or 3.6 g Arg/(kg BW d)

[1] Calculated according to the body weight (0.45 kg for males and 0.29 kg for females) of rats, their food intake [46 and 50 g/(kg BW d), for males and females, respectively], and Arg content in the basal diet (0.61 %)

[2] Calculated according to the true digestibility of Arg in the diet (95 %)

[3] Calculated according to the rate of first-pass utilization of luminal Arg (40 %) by the small intestine (Wu et al. 2007a). Namely, total Arg entering the portal vein from the basal diet plus supplementation = (Supplemental Arg + Digestible Arg in dietary protein) × (40 %); for example, in the 3600-mg/(kg BW d) supplemental Arg group, the value = (3600 + 285) × 40 % = 1554 mg/(kg BW d)

[4] Calculated according to the rate of first-pass utilization of luminal Arg (40 %) by the small intestine (Wu et al. 2007a). Namely, total Arg entering the portal vein from the basal diet plus supplementation = (Supplemental Arg + Digestible Arg in dietary protein) × (1–40 %); for example, in the 3600-mg/(kg BW d) supplemental Arg group, the value = (3600 + 285) × (1–40 %) = 2331 mg/(kg BW d)

[5] Calculated according to the gain of extraintestinal tissues and Arg content in extraintestinal tissues (1.06-g Arg/100-g wet tissue weight) (Wu 2013). At d 133 of age, the mean body weights of male and female rats were 0.49 and 0.29 kg, respectively (Yang et al. 2015), whereas the mean weight gains of extraintestinal tissues were 1.0 and 0.6 g/d in male and female rats, respectively

[6] Calculated as total Arg entering the portal vein from the basal diet plus supplementation + endogenous Arg synthesis [i.e., 272 mg/(kg BW d)] (Windmueller and Spaeth 1981)—Arg accretion in extraintestinal tissues; for example, in the 3600-mg/(kg BW d) supplemental Arg group, the value = (2331 + 272−24) = 2579 mg/(kg BW d)

[a–c] Within a column for each sex, mean values not sharing the same superscript *letters* differ ($p < 0.05$)

The values of extraintestinal Arg catabolism do not include proteins excreted by extraintestinal tissues

Table 6 Human-equivalent doses of supplemental L-arginine (Arg) in pigs and rats

Animal	Sex	Age (d)	Body weight (BW) kg	Oral dose of administration mg Arg/(kg BW·d)	Form of Arg	Human equivalent dose mg Arg/(kg BW·d)	References
Pig[1]	Male	30–121	9.41–67.1	630	Arg–HCl	573	Hu et al. 2015
	Female	30–121	9.41–67.1	630	Arg–HCl	573	Hu et al. 2015
Pig[2]	Male	30–121	9.46–67.5	630	Arg	573	Hu et al. 2015
	Female	30–121	9.46–67.5	630	Arg	573	Hu et al. 2015
Rat[3]	Male	42–133	0.20–0.45	3,600	Arg–HCl	573	Yang et al. 2015
	Female	42–133	0.17–0.30	3,600	Arg–HCl	573	Yang et al. 2015
Rat[4]	Male	42–133	0.18–0.53	3,300	Arg	525	Tsubuku et al. 2004
	Female	42–133	0.15–0.33	3,900	Arg	621	Tsubuku et al. 2004

[1] Arg–HCl was added to enteral diets for pigs

[2] Arg base was added to enteral diets for pigs

[3] Arg–HCl was added to drinking water for rats

[4] Arg base was added to enteral diets for rats

ruminants. The rate of turnover of Arg in the plasma of pregnant ewes is high, with the half-life being 0.75 h on d 105–135 of gestation (Lassala et al. 2009; Wu et al. 2007a). This half-life value is similar to those reported for adult pigs (0.83 h) and rats (0.76 h) (Wu et al. 2007a), indicating that Arg is rapidly metabolized in both ruminants and nonruminants.

We (Lassala et al. 2009, 2010, 2011; Satterfield et al. 2012, 2013) and others (McCoard et al. 2013) conducted a series of experiments to assess the safety of administration of Arg in gestating sheep. Between d 60 of gestation and parturition, i.v. infusions of 81-mg Arg (as Arg–HCl)/(kg BW d) into underfed ewes, 3 times daily (0800, 1500, and 2200 h), for 82 or 87 d did not affect feed intake, concentrations of lysine, histidine, insulin, or growth hormone in the maternal serum or maternal and fetal health, but reduced concentrations of ammonia, free fatty acids, and triglycerides, and enhanced birth weights of lambs, compared with saline-infused underfed ewes (Lassala et al. 2010). In this study, blood samples were obtained from ewes immediately before Arg–HCl infusion at 0800 (Lassala et al. 2010). Similar results were obtained for i.v. infusions of: (1) 180-mg Arg (as Arg–HCl)/(kg BW d) into Booroola Rambouillet ewes carrying 2–4 fetuses between d 100 and 121 of gestation (Lassala et al. 2011); (2) 180-mg Arg (as Arg–HCl)/(kg BW d) into ewes with twin fetuses between d 100 and 140 of gestation (McCoard et al. 2013); or (3) 81-mg Arg (as Arg–HCl)/(kg BW d) into underfed or obese ewes between d 100 and 125 of gestation (Satterfield et al. 2013). These results indicate that a large amount of supplemental Arg provided via i.v. administration is well tolerated by pregnant sheep, and can improve fetal growth and development without adverse effects on mother or fetus. Future studies are warranted to determine the catabolism, efficacy, and safety of oral rumen-protected Arg or Arg precursors in sheep and other ruminant species (e.g., cattle and goats).

Human-equivalent doses for supplemental Arg in animals

As noted previously (Table 2), male and female pigs can tolerate high intake of dietary Arg (Hu et al. 2015). The corn- and soybean meal-based diet contained 1.35 % Arg and supplied 425-mg Arg/(kg BW d) or 382 digestible Arg/(kg BW d). The supplemental doses of 1, 1.5, and 2 % Arg provided pigs with 315-, 473-, and 630-mg Arg/(kg BW d), respectively. According to the conversion ratio of 1.1:1.0 (pigs vs humans) established by the Food and Drug Administration (FDA 2005), the human-equivalent doses of the supplemental Arg are 286-, 430-, and 573-mg Arg/(kg BW d), respectively. These doses translate into 20-, 30-, and 40-g Arg/d for a 70-kg human subject, respectively (Table 6). Considering a safety factor of 10, the MRSD for initial clinical trial would be 2-, 3-, and 4-g Arg/d for a 70-kg human subject, which is below the average Arg intake of 4.4 g/d for the adult U.S. population (King et al. 2008). Dietary supplementation with the dose of 4 g Arg/d is safe for adult humans (Shao and Hathcock 2008). Similarly, based on the conversion ratio of 1:0.16 [rats vs humans (FDA 2005)], the human-equivalent doses for the supplemental Arg of 1.8 and 3.6 g/(kg BW d) in rats are 286- and 573-mg Arg/(kg BW d), respectively. These doses translated into 20 and 40 g Arg/d for a 70-kg human subject, respectively. At present, the human-equivalent doses for supplemental Arg of 180 mg/(kg BW d) in gestating sheep cannot be calculated, because a conversion ratio has not been established by the Food and Drug Administration. As approximately 40 % of oral Arg is metabolized in the

small intestine during the first pass (Castillo et al. 1993; Wu et al. 2007a), this i.v. dose is equivalent to 300-mg Arg/(kg BW d) for oral administration.

Data from animal studies have relevance to the safety of arginine supplementation in humans. Mean intake of dietary arginine by the U.S. adult population is 4.4 g/d (King et al. 2008), as noted previously. Oral administration of Arg (10 g/d) for 3 or 6 months to humans with peripheral arterial occlusive disease or coronary artery disease was safe and did not disturb metabolic profiles in the plasma and urine (Kayacelebi et al. 2015). Chin-Dusting et al. (1996) reported that oral administration of 20 g Arg/d to healthy adult males for 4 weeks did not result in any adverse effect as determined by standard clinical chemistry variables. Likewise, healthy adults could tolerate oral administration of 40-g Arg/d for 1 week (duration of the study) (Beaumier et al. 1995). In addition, 4-week oral administration of 21-g Arg/d had no side effects on patients with hypercholesterolemia (Clarkson et al. 1996). Likewise, 6-week oral administration of 42-g Arg/(70-kg BW d) to patients with cystic fibrosis did not cause undesirable effects (Grasemann et al. 2005). It is recommended that this amount of Arg be taken in equally divided doses within a day to prevent any abrupt increase in NO production by the gastrointestinal tract, the vasculature, and cells of the immune system (Flynn et al. 2002; Grimble 2007; Wu and Meininger 2000).

Conclusion

Dietary supplementation with up to 630-mg Arg/(kg BW d) or 3.6-g Arg/(kg BW d) for 91 d has no adverse effects on conventionally fed pigs or rats, respectively. These doses of supplemental arginine are equivalent to 573-mg Arg/(kg BW d) for humans, or 40-g Arg/d for a 70-kg subject. Both pigs and rats can consume Arg–HCl or Arg as an ingredient of their enteral diets. Oral or i.v. administration of Arg to animals is safe and beneficial for improving whole-body metabolic profile, fetal growth and development, insulin sensitivity, and lean tissue mass, as well as reducing white fat mass and concentrations of ammonia, free fatty acids, triglyceride, and cholesterol in the plasma. Data from animal studies are helpful for predicting a safe upper limit for oral administration of Arg to healthy adults and in guiding clinical studies to determine long-term safety of L-arginine supplementation in humans.

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Compliance with ethical standards

The use of animals for our research described in this review article was approved by the Institutional Animal Care and Use Committee of Texas A&M University.

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

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