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ORIGINAL ARTICLE

Safety of long-term dietary supplementation with L-arginine in pigs

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Abstract This study was conducted with a swine model to determine the safety of long-term dietary supplementation with L-arginine–HCl or L-arginine free base. Beginning at 30 days of age, pigs were fed a corn- and soybean meal-based diet (31.5 g/kg body weight/day) supplemented with 0, 1.21, 1.81 or 2.42 % L-arginine–HCl (Experiment 1) or with 0, 1, 1.5 or 2 % L-arginine (Experiment 2). The supplemental doses of 0, 1, 1.5, and 2 % L-arginine provided pigs with 0, 315, 473, and 630 mg L-arginine/kg body weight/day, respectively, which were equivalent to 0, 286, 430, and 573 mg L-arginine/kg body weight/day, respectively, in humans. At 121 days of age (91 days after initiation of supplementation), blood samples were obtained from the jugular vein of pigs at 1 and 4 h after feeding for hematological and clinical chemistry tests. Dietary supplementation with L-arginine increased plasma concentrations of arginine, ornithine, proline, albumin and reticulocytes, while reducing plasma concentrations of ammonia, free fatty acids, triglyceride, cholesterol, and neutrophils. L-Arginine supplementation enhanced protein gain and reduced white-fat deposition in the body. Other variables in standard hematology and clinical chemistry tests, serum concentrations of insulin, growth hormone and insulin-like growth factor-I did not differ among all the groups of pigs. These results indicate that dietary supplementation with L-arginine (up to 630 mg/kg body weight/day) is safe in pigs for at least 91 days. Our findings help guide clinical studies to determine the safety of long-term oral administration of L-arginine to humans.

Keywords Arginine · Humans · Nutrition · Safety · Swine

Abbreviations

Arg	L-Arginine
HPLC	High-performance liquid chromatography
NO	Nitric oxide

Introduction

L-Arginine has traditionally been classified as a nutritionally nonessential amino acid for healthy adults because it is synthesized in most mammals, including humans, rats and pigs (Flynn et al. 2002; Wu 2010; Wu and Knabe 1995). However, as the common precursor for the synthesis of nitric oxide (NO), creatine, polyamines, and other molecules with enormous physiological importance (Morris 2007; Wu et al. 2009), L-arginine has versatile roles in metabolism and physiology and, therefore, is beneficial for the functions of multiple systems (including the circulatory, gastrointestinal, immune, and reproductive systems) in humans and animals (Creager et al. 1992; Hoang et al. 2013; Wu and Meininger 2000; Wu et al. 2012). Additionally, L-arginine has recently been reported to reduce fat accretion in white adipose tissue of obese rats (Fu et al. 2005; Jobgen et al. 2009; Wu et al. 2007b), obese humans (Hurt et al. 2014; Lucotti et al. 2006), obese sheep (Satterfield et al. 2012), and growing-finishing pigs (Tan et al. 2012). Thus, L-arginine is now recognized to be a potentially attractive ingredient in dietary

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supplements, functional foods, and beverages (Wu 2013). However, the use of L-arginine for these purposes has been limited primarily due to the concerns of regulatory agencies, policymakers, and consumers over the safety of long-term supplementation because of (1) the lack of clinical data in the literature (Shao and Hathcock 2008); and (2) a reportedly possible increase in the risk of adverse cardiovascular events in patients with acute myocardial infarction (Schulman et al. 2006). At present, the safe upper limit for arginine supplementation to healthy adults is unknown (Boger and Bode-Boger 2001; Evans et al. 2004; Hurt et al. 2014; Tanghao et al. 1999; Wu et al. 2013).

The present study was conducted with pigs to determine their physiological responses to dietary supplementation with graded levels of L-arginine or L-arginine–HCl. The pig is similar to the human with respect to nutrient digestion, absorption, and metabolism, as well as physiology and immunology, and, therefore, is a widely used animal model for studying human nutrition and physiology (Burrin et al. 2014; Suryawan and Davis 2014; Wu et al. 1996). When food protein is hydrolyzed by proteases and peptidases in the gastrointestinal tract, L-arginine or its dipeptide/tripeptides is released and subsequently absorbed by intestinal mucosal cells (Closs et al. 2004; Wu 1998). L-Arginine–HCl, the hydrochloric salt of L-arginine, is often manufactured in human-food, animal-feed, or pharmaceutical grades (McKnight et al. 2010; McNeal et al. 2010; Wu 2009). Thus, we used both L-arginine and L-arginine–HCl in our study because they are commercially available for oral or intravenous administration into humans and animals.

Materials and methods

L-Arginine (free base), L-arginine–HCl, and L-alanine were products of Ajinomoto Inc. (Tokyo, Japan). Their purity was >99.9 %, as analyzed by high-performance liquid chromatography (Wu and Meininger 2008). Pigs used for this study were the offspring of Yorkshire × Landrace dams and Duroc × Hampshire sires, and were maintained at the Texas A&M University Swine Center. The experimental procedures were approved by the Institutional Agricultural Animal Care and Use Committee of Texas A&M University.

Experiment 1: Effects of long-term dietary L-arginine–HCl supplementation on physiological responses in pigs

Animals and diets

During the suckling period, pigs were nursed by sows fed an 18.4 % crude-protein diet (Mateo et al. 2008). Piglets were weaned at 21 days of age to a conventional corn- and soybean meal-based diet (Table 1). This basal diet contained 21.0 % crude protein and 1.35 % L-arginine, which were analyzed, as we described previously (Dai et al. 2014; Li et al. 2011). At 30 days of age, four pigs with similar body weights were selected from each of 12 litters and assigned randomly to one of four treatment groups (0, 1.21, 1.81, and 2.42 % L-arginine–HCl) on the basis of litter origin to reduce variation among the experimental groups. The four different diets were prepared by adding 0, 1.21, 1.81, or 2.42 % L-arginine–HCl to the corn- and soybean meal-based diet at the expense of cornstarch. These doses of L-arginine–HCl provided 0, 1, 1.5, and 2 % L-arginine, respectively. Isonitrogenous amounts of L-alanine were added to the diets containing 0, 1.21 and 1.81 % L-arginine–HCl (Table 2). There were 12 pigs (6 males and 6 females) per dietary group, which were housed individually in pens (23–25 °C and relative humidity of 55–60 %). During the entire experimental period, pigs had free access to drinking water and were offered their respective diets

Table 1 Composition of the basal experimental diet

Ingredient	Percentage (g/100 g)
Corn	52.28
Soybean meal	37.0
Soybean oil	2
Cornstarch	4.09
Lysine–HCl, 98.8 (%)	0.15
Dicalcium phosphate	1.56
Limestone	1.02
Medication[a]	1
Vitamin premix[b]	0.5
Trace mineral premix[c]	0.15
NaCl	0.25
Calculated content (%)	
Crude protein[d]	21.0
Digestible energy (kcal/kg)	3,630
Calcium	0.85
Phosphorus	0.70

Values are expressed on an as-fed basis. Dry matter content was 89.8 %

[a] Containing the following (mg/kg diet): carbadox, 55

[b] Containing the following (mg/kg diet): retinyl palmitate 4.24, cholecalciferol 0.019, all-rac-α-tocopheryl acetate 44, menadione sodium bisulfate complex 9, riboflavin 7.7, D-calcium pantothenate 33, niacin 33, choline 287, vitamin B-12 0.044, and D-biotin 0.22

[c] Containing the following (mg/kg diet): Cu 15, Fe 100, I 0.6, Mn 20, Zn 100, and Se 0.1

[d] The analyzed contents (% of diet; on an as-fed basis) of amino acids were as follows: arginine 1.35, alanine 1.26, aspartate 1.37, asparagine 0.96, glutamate 1.93, glutamine 1.62, glycine 0.86, histidine 0.57, isoleucine 0.87, leucine 1.76, lysine 1.41, methionine 0.36, phenylalanine 1.01, proline 1.65, serine 0.77, threonine 0.85, tryptophan 0.24, tyrosine 0.75, and valine 0.97

Table 2 Amounts of L-arginine or L-arginine–HCl and L-alanine supplemented to the basal diet

L-Arg or L-Arg–HCl supplementation to the basal diet[a]	L-Alanine supplementation to the basal (%)[b]	Total L-arginine content in the whole diet (%)[c]
Experiment 1 (L-Arg–HCl supplementation)		
0.0	4.09	1.35
1.21 % L-Arg–HCl	2.05	2.35
1.81 % L-Arg–HCl	1.02	2.85
2.42 % L-Arg–HCl	0.0	3.35
Experiment 2 (L-Arg supplementation)		
0.0	4.09	1.35
1.0 % L-Arg	2.05	2.35
1.5 % L-Arg	1.02	2.85
2.0 % L-Arg	0.0	3.35

As-fed basis. L-Arginine content in the basal diet was 1.35 %. The basal diet was supplemented with L-arginine (free base) or L-arginine–HCl at the expense of cornstarch

L-Arg, L-arginine

[a] The amount of L-arginine (molecular wt, 174.2) was calculated on the basis of that of L-arginine–HCl (molecular wt, 210.7). On molar basis, 1.21 % L-Arg–HCl is equivalent to 1.0 % L-Arg

[b] L-Alanine (molecular wt, 89.1) was provided as the isonitrogenous control for L-arginine

[c] The sum of L-arginine in the basal diet plus supplemental L-arginine in the free base or hydrochloric form

at 31.5 g feed per kg body weight per day (divided in two equal meals at 8:00 am and 5:00 pm). The body weights of pigs were determined weekly. The pigs consumed all the feed provided each day.

Collection of blood samples

At 121 days of age (i.e., 91 days after initiation of arginine supplementation), blood samples (10 ml) were obtained from the jugular vein of pigs at 1 and 4 h after feeding. Hematological and clinical chemistry tests of 6-ml blood samples were conducted at Texas A&M Veterinary Medical Diagnostic Laboratory (College Station, Texas). The remaining 4-ml blood samples in tubes with or without EDTA were immediately centrifuged at 10,000 g for 1 min, and the supernatant fluid (plasma or serum) was stored at −80 °C for subsequent analysis of metabolites and hormones.

Determination of body composition

At the end of the second blood collection, six pigs (3 males and 3 females) were selected randomly from 0 and 2 % L-arginine groups to be humanely euthanized by intracardiac administration of 10 ml saturated KCl after anesthesia induced by intramuscular administration of Telazol (5 mg/kg body weight). Immediately after euthanasia, the abdomen of individual pigs was opened and the whole gastrointestinal tract was isolated. The contents of the digestive tract were removed and its lumen was washed three times with saline. The whole body of each pig (including the gastrointestinal tract) was homogenized using a Seydelmann Cutter K64 (Strasser; Stuttgart, Germany), as described by Satterfield et al. (2012, 2013). The content of water, crude protein, crude fat, ash (minerals), and carbohydrate was determined, as previously described (Dai et al. 2014; Jobgen et al. 2009; Wu et al. 1999).

Analyses of amino acids, glucose, ammonia and urea in plasma

Plasma (0.5 ml) were deproteinized with an equal volume of 1.5 M $HClO_4$, followed by addition of 0.25 ml 2 M K_2CO_3 (Wu et al. 1994). Amino acids in the neutralized extract were determined by fluorometric HPLC methods involving pre-column derivatization with *o*-phthaldialdehyde as described previously (Wu et al. 1997; Wu and Meininger 2008). The integration of chromatographic peaks was performed using Millenium-32 Software (Waters, Milford, MA, USA). Glucose was determined enzymatically by a spectrophotometric method involving hexokinase and glucose-6-phosphate dehydrogenase (Satterfield et al. 2013). Ammonia and urea were determined using glutamate dehydrogenase and urease plus glutamate dehydrogenase, respectively (Wu 1995).

Analysis of free fatty acids, triglyceride, and hormones in plasma

Free fatty acids and triglyceride in plasma were analyzed using chloroform extraction and assay kits from Wako Chemicals (Richmond, VA, USA), as we previously described (Jobgen et al. 2009). Serum insulin and growth hormone were determined, respectively, using radioimmunoassay kits for porcine insulin and growth hormone (Linco, St. Louis, MO, USA). Insulin-like growth factor-1 (IGF-1) was analyzed using an assay kit from Diagnostic Systems Laboratories, Inc. (Webster, Texas) for porcine serum.

Experiment 2: Effects of long-term dietary L-arginine supplementation on physiological responses in pigs

Experiment 2 was conducted as Experiment 1, except that 0, 1, 1.5, or 2 % L-arginine was supplemented to the basal diet instead of L-arginine–HCl (Table 2).

Statistical analyses

Results are expressed as mean ± SEM. Statistical analyses of data were performed by one-way analysis of variance

Table 3 Effects of dietary supplementation with L-arginine–HCl or L-arginine on body weights of pigs

Days post arginine supplementation	Supplemental L-arginine in diet (%)			
	0	1.0	1.5	2.0
Exp. 1 (L-arginine–HCl supplementation)				
Day 0	9.42 ± 0.11	9.44 ± 0.10	9.41 ± 0.09	9.45 ± 0.10
Day 28	20.0 ± 0.19^{c}	20.6 ± 0.18^{b}	21.0 ± 0.16^{a,b}	21.5 ± 0.20^{a}
Day 56	40.2 ± 0.32^{c}	41.1 ± 0.35^{b}	41.7 ± 0.29^{a,b}	42.2 ± 0.30^{a}
Day 91	64.3 ± 0.40^{c}	65.5 ± 0.43^{b}	66.3 ± 0.38^{a,b}	67.1 ± 0.42^{a}
Exp. 2 (L-arginine supplementation)				
Day 0	9.50 ± 0.13	9.52 ± 0.15	9.46 ± 0.12	9.51 ± 0.13
Day 28	20.3 ± 0.24^{c}	21.0 ± 0.25^{b}	21.5 ± 0.20^{a,b}	21.9 ± 0.23^{a}
Day 56	40.6 ± 0.35^{c}	41.6 ± 0.37^{b}	42.2 ± 0.33^{a,b}	42.8 ± 0.32^{a}
Day 91	64.6 ± 0.44^{c}	65.9 ± 0.47^{b}	66.7 ± 0.42^{a,b}	67.5 ± 0.45^{a}

Values, expressed as kg, are mean ± SEM, $n = 12$. Day 0 = 30 days of age

[a–c] Within a row, means sharing different superscript letters differ ($P < 0.05$)

using the General Linear Models procedures of the statistical analysis (Assaad et al. 2014). Differences among treatment means were determined using the Student–Newman–Keuls multiple comparison method (Assaad et al. 2014). Comparisons of means between 1- and 4-h time points were analyzed by the paired *t* test. A probability value ≤0.05 was taken to indicate statistical significance (Wei et al. 2012).

Results

Feed intake of pigs supplemented with L-arginine–HCl or L-arginine

Feed intake by pigs in the experimental groups was 31.5 g/kg body weight per day based on the study's design. This level of feed intake supplied 425 mg L-arginine/kg body weight/day in the basal diet. The supplemental doses of 0, 1.21, 1.81, and 2.42 % L-arginine–HCl provided the pigs with 0, 315, 472.5, and 630 mg L-arginine/kg body weight/day, respectively. Similarly, the supplemental doses of 0, 1, 1.5, and 2 % L-arginine provided the pigs with 0, 315, 472.5, and 630 mg L-arginine/kg body weight/day, respectively. Based on the conversion ratio of 1.1:1.0 (pigs vs. humans; FDA 2005), the human-equivalent doses of the supplemental arginine were 0, 286, 430, and 573 mg L-arginine/kg body weight/day, respectively, or 20, 30, and 40 g L-arginine/day for a 70-kg person, respectively.

Effects of dietary supplementation with L-arginine–HCl or L-arginine on the growth of pigs

In both Exp. 1 and Exp. 2, supplementing 1.21, 1.81 or 2.42 % L-arginine–HCl (equivalent to 1, 1.5 or 2 % L-arginine) or 1, 1.5 or 2 % L-arginine to the basal diet increased ($P < 0.05$) the body weight of pigs, as compared with the control group (Table 3). The body weights of pigs did not differ ($P > 0.05$) either between the 1 and 1.5 % arginine groups or between the 1.5 and 2 % arginine groups. However, in both experiments, the pigs supplemented with 2 % arginine were heavier ($P < 0.05$) than the pigs supplemented with 1 % arginine.

Effects of dietary supplementation with L-arginine–HCl or L-arginine on concentrations of amino acids, ammonia, and lipids in pig plasma

Plasma concentrations of amino acids, ammonia and lipids in control and arginine-supplemented pigs at 1 and 4 h after feeding are summarized in Table 4 (Exp. 1) and Table 5 (Exp. 2). Compared with values obtained at 4 h after feeding, plasma concentrations of amino acids were higher ($P < 0.05$) at 1 h after feeding. Dietary supplementation with 0.5–2 % arginine dose-dependently increased plasma concentrations of arginine, ornithine and proline, while reducing plasma concentrations of glutamine, glycine, ammonia, cholesterol, free fatty acids, and triglyceride in both Exp. 1 and Exp. 2. Dietary supplementation with L-arginine as either L-arginine-HCl or L-arginine free base did not affect ($P > 0.05$) concentrations of lysine, histidine, citrulline, and other amino acids in the plasma of pigs at either 1 or 4 h after feeding in Exp. 1 (Table 6) or Exp. 2 (Table 7).

Effects of dietary supplementation with L-arginine–HCl or L-arginine on the hematology of pigs

Compared with the control group, dietary supplementation with 2 % L-arginine increased ($P < 0.05$) concentrations of total serum protein, albumin, alkaline phosphatase, mean corpuscular volume, reticulocytes, as well as the percentages of monocytes and eosinophils in blood obtained from pigs at both 1 and 4 h after feeding, while reducing the percentage of neutrophils in blood (Tables 8 and 9). Dietary supplementation with L-arginine as either L-arginine-HCl or L-arginine free base did not affect ($P > 0.05$) concentrations

Table 4 Effects of dietary supplementation with L-arginine–HCl on concentrations of amino acids, ammonia, and lipids in the plasma of pigs at 1 and 4 h after feeding (Exp. 1)

Variable	Time after feeding (h)	Supplemental L-arginine (as Arg–HCl) in diet (%)			
		0	1.0	1.5	2.0
Alanine	1	$1{,}150 \pm 37^{a}$	782 ± 26^{b}	649 ± 20^{c}	552 ± 15^{d}
	4	433 ± 16*	405 ± 15*	397 ± 15*	388 ± 13*
Arginine	1	282 ± 9^{d}	470 ± 11^{c}	567 ± 12^{b}	641 ± 15^{a}
	4	194 ± 7*	$261 \pm 8^{c,*}$	$342 \pm 10^{b,*}$	$443 \pm 11^{a,*}$
Glutamine	1	622 ± 12^{a}	594 ± 10^{b}	$577 \pm 8^{b,c}$	554 ± 8^{c}
	4	$568 \pm 10^{a,*}$	$557 \pm 9^{a,b*}$	$546 \pm 7^{a,b,*}$	$535 \pm 6^{b,*}$
Glycine	1	$1{,}026 \pm 16^{a}$	882 ± 14^{b}	814 ± 11^{c}	750 ± 10^{d}
	4	$857 \pm 12^{a,*}$	$790 \pm 10^{b*}$	$735 \pm 8^{c,*}$	$684 \pm 8^{d,*}$
Ornithine	1	95 ± 3^{d}	126 ± 4^{c}	150 ± 4^{b}	176 ± 5^{a}
	4	$80 \pm 2^{d,*}$	$104 \pm 3^{c,*}$	$131 \pm 3^{b,*}$	$154 \pm 4^{a,*}$
Proline	1	286 ± 6^{d}	312 ± 6^{c}	345 ± 7^{b}	382 ± 9^{a}
	4	$261 \pm 5^{d,*}$	$283 \pm 5^{c,*}$	$317 \pm 6^{b,*}$	$346 \pm 7^{a,*}$
Ammonia	1	68.3 ± 2.1^{a}	61.0 ± 1.8^{b}	53.4 ± 1.5^{c}	50.6 ± 1.4^{c}
	4	75.9 ± 2.5^{a}	66.4 ± 2.2^{b}	54.2 ± 2.0^{c}	51.8 ± 1.7^{c}
FFA	1	261 ± 6.3^{a}	214 ± 5.5^{b}	182 ± 5.1^{c}	154 ± 4.3^{d}
	4	274 ± 7.0^{a}	220 ± 6.1^{b}	186 ± 5.7^{c}	159 ± 4.5^{d}
TG	1	523 ± 12^{a}	479 ± 10^{b}	420 ± 8.2^{c}	388 ± 6.3^{d}
	4	538 ± 14^{a}	485 ± 12^{b}	433 ± 10^{c}	390 ± 8.6^{d}
Cholesterol	1	69.3 ± 2.1^{a}	$66.2 \pm 1.9^{a,b}$	$60.2 \pm 1.8^{b,c}$	56.0 ± 1.6^{c}
	4	68.4 ± 2.0^{a}	$64.0 \pm 1.7^{a,b}$	$59.3 \pm 1.5^{b,c}$	54.7 ± 1.4^{c}

Blood samples were obtained from 121-day-old pigs (91 days after initiation of arginine supplementation) at 1 and 4 h post feeding. Values, expressed as μM for amino acids, ammonia, FFA, and TG, and as mg/dL for cholesterol, are mean ± SEM, $n = 12$/group

FFA free fatty acids, *TG* triglyceride

* $P < 0.05$ vs. the corresponding value for the 1-h group

[a–d] Within a row, means sharing different superscript letters differ ($P < 0.05$)

of glucose, electrolytes, select enzymes, insulin, growth hormone, insulin-like growth factor-1 (Table 8), or the numbers of white blood cells, red blood cells, and platelets in blood obtained at both 1 and 4 h after feeding in Exp. 1 or Exp. 2 (Table 9).

Body composition of pigs

Protein was the most abundant component of dry matter in pigs (Table 10). When data from Exp. 1 and Exp. 2 were combined, the body composition of nutrients as the percentage of body weight did not differ ($P > 0.05$) between male and female pigs at 121 days of age. Dietary supplementation with L-arginine as either L-arginine-HCl or L-arginine free base did not statistically affect ($P > 0.05$) the percentages of water, crude protein, crude fat, minerals or carbohydrate in the whole body. The total amounts of these nutrients in the pigs were calculated on the basis of their body weights (Table 3). Compared with the control group, dietary supplementation with 2 % L-arginine (either as L-arginine–HCl or L-arginine) increased the total amounts of water ($P < 0.01$), protein ($P < 0.01$), and minerals ($P < 0.05$), while reducing ($P < 0.01$) the total amounts of crude fat, in the pigs (Table 10). The total amounts of carbohydrate did not differ ($P > 0.05$) between the control and L-arginine supplemented pigs.

Discussion

L-Arginine is a substrate for the synthesis of protein, NO, ornithine (the precursor of polyamines, proline, and glutamate), creatine, and agmatine in mammals (Wu and Morris 1998). As a major building block for polypeptides, arginine represents 14 % of total nitrogen in body proteins (Wu et al. 1999). Of particular note, each of the L-arginine-derived metabolites has enormous physiological importance (Wu et al. 2009). First, NO regulates many of the cellular and organ functions in the body, including endothelium-dependent relaxation of blood vessels, the immune response, and neurotransmission (Dai et al. 2013; Wu and Meininger 2009). Second, polyamines are required for the synthesis of DNA and protein as well as the proliferation and differentiation of all cell types (Agostinelli 2014). Third, proline is a major amino acid for collagen synthesis and, therefore, plays a key role in remodeling of the extracellular matrix (Phang and Liu 2012). Fourth, glutamate is a neurotransmitter in the central nervous system and the gastrointestinal tract (San Gabriel and Uneyama 2013). Finally, creatine is an antioxidant and participates in energy metabolism in skeletal muscle and nerves (Brosnan and Brosnan 2007).

Besides serving as a substrate for synthetic pathways, arginine also has regulatory functions. For example,

Table 5 Effects of dietary supplementation with L-arginine on concentrations of amino acids, ammonia, and lipids in the plasma of pigs at 1 and 4 h after feeding (Exp. 2)

Variable	Time after feeding (h)	Supplemental L-arginine in diet (%)			
		0	1.0	1.5	2.0
Alanine	1	1,108 ± 34[a]	764 ± 23[b]	633 ± 17[c]	548 ± 13[d]
	4	425 ± 14*	412 ± 13*	404 ± 13*	392 ± 10*
Arginine	1	275 ± 7[d]	462 ± 9[c]	553 ± 10[b]	646 ± 13[a]
	4	188 ± 5[d,*]	240 ± 7[c,*]	331 ± 8[b,*]	432 ± 10[a,*]
Glutamine	1	630 ± 10[a]	602 ± 9[b]	585 ± 9[b,c]	561 ± 8[c]
	4	571 ± 8a*	560 ± 8[a,b,*]	552 ± 7[a,b,*]	539 ± 7[b,*]
Glycine	1	1,026 ± 16[a]	882 ± 14[b]	814 ± 11[c]	750 ± 10[d]
	4	857 ± 12a*	790 ± 10[b,*]	735 ± 8[c*]	684 ± 8[d,*]
Ornithine	1	95 ± 3[d]	126 ± 4[c]	150 ± 4[b]	176 ± 5[a]
	4	80 ± 2[d*]	104 ± 3[c,*]	131 ± 3[b*]	154 ± 4[a,*]
Proline	1	286 ± 6[d]	312 ± 6[c]	345 ± 7[b]	382 ± 9[a]
	4	261 ± 5[d,*]	283 ± 5[c,*]	317 ± 6[b*]	346 ± 7[a,*]
Ammonia	1	70.4 ± 2.1[a]	62.4 ± 1.8[b]	55.1 ± 1.6[c]	52.8 ± 1.5[c]
	4	77.3 ± 2.3[a]	64.8 ± 2.0[b]	56.3 ± 1.8[c]	53.5 ± 1.6[c]
FFA	1	271 ± 6.8[a]	223 ± 6.2[b]	191 ± 4.6[c]	158 ± 3.8[d]
	4	278 ± 7.0[a]	230 ± 6.1[b]	194 ± 5.2[c]	162 ± 4.1[d]
TG	1	535 ± 10[a]	484 ± 9.3[b]	437 ± 8.6[c]	390 ± 7.1[d]
	4	546 ± 12[a]	492 ± 9.7[b]	450 ± 8.4[c]	401 ± 7.2[d]
Cholesterol	1	70.5 ± 2.3[a]	67.0 ± 2.1[a,b]	61.4 ± 1.7[b,c]	57.3 ± 1.5[c]
	4	71.7 ± 2.5[a]	68.3 ± 2.4[a,b]	63.0 ± 2.2[b,c]	58.2 ± 1.8[c]

Blood samples were obtained from 121-day-old pigs (91 days after initiation of arginine supplementation) at 1 and 4 h post feeding. Values, expressed as μM for amino acids, ammonia, FFA, and TG, and as mg/dL for cholesterol, are mean ± SEM, $n = 12$/group

FFA free fatty acids, *TG* triglyceride

* $P < 0.05$ vs. the corresponding value for the 1-h group

[a–d] Within a row, means sharing different superscript letters differ ($P < 0.05$)

arginine is an allosteric activator of *N*-acetylglutamate synthase (Wu 2013). This enzyme converts glutamate and acetyl-CoA into *N*-acetylglutamate, which is an essential allosteric activator of carbamoylphosphate synthase-I, a key enzyme in the hepatic urea cycle for detoxification of ammonia (Meijer et al. 1985). Thus, L-arginine is required for maintaining hepatic urea synthesis in an active state. Also, pharmacological levels of L-arginine stimulate the secretion of growth hormone and insulin in mammals, thereby playing an important role in regulating protein metabolism (Flynn et al. 2002; Grimble 2007). Furthermore, through stimulating the expression of peroxisome proliferator-activated receptor γ coactivator-1α (a master activator of mitochondrial biogenesis) and the phosphorylation of AMP-activated protein kinase, L-arginine increases the oxidation of fatty acids and glucose in insulin-sensitive tissues, thereby reducing accretion of fat in white adipose tissue (Fu et al. 2005; McKnight et al. 2010). Finally, L-arginine activates the mammalian target of rapamycin cell signaling pathway and, therefore, protein synthesis in multiple tissues, including skeletal muscle (Yao et al. 2008) and small intestine (Tan et al. 2010). Thus, L-arginine can spare muscle protein in obese subjects consuming a weight-reducing diet (Lucotti et al. 2006).

As noted previously, L-arginine had traditionally been classified as a nutritionally nonessential amino acid for healthy adults because it can be synthesized in the body to maintain nitrogen balance (Visek 1986; Wu et al. 2014). However, since the discovery in 1988 that L-arginine is the isonitrogenous precursor for the synthesis of nitric oxide (a signaling molecule and a major vasodilator) in mammalian cells, there has been growing interest in the roles of L-arginine in the functions of multiple systems (including the circulatory, gastrointestinal, immune, and reproductive systems) in humans (Wu et al. 2009). Mean arginine intake by the US adult population is ~5 g/day (Flynn et al. 2002). Approximately 40 % of L-arginine in the diet is catabolized in the first pass by the small intestine, and the remaining 60 % (3 g arginine/day) enters the portal circulation (Castillo et al. 1993; Dai et al. 2011). For comparison, endogenous synthesis of L-arginine from dietary glutamine, glutamate, and proline as well as arterial glutamine provides 1.5 g L-arginine/day to a 70-kg healthy subject (Wu and Morris 1998). Among this total amount of 4.5 g L-arginine from exogenous and endogenous sources, 2.3 g (~50 %) is utilized to synthesize creatine via inter-organ cooperation (the kidneys and liver) (Wu and Morris 1998). The remaining amount of L-arginine (2.2 g/day) is utilized to synthesize protein, ornithine, and nitric oxide. Thanks to the recognition that enhancing the circulating levels of L-arginine can activate key signaling pathways that are beneficial to health, particularly improving cardiovascular function and reducing excessive white adipose tissue in humans and animals (McKnight et al. 2010), L-arginine has now become

Table 6 Concentrations of free amino acids in the plasma of control and L-arginine–HCl supplemented pigs obtained at 1 and 4 h after feeding (Exp. 1)

Amino acid	Blood sample obtained at 1 h after feeding		Blood sample obtained at 4 h after feeding	
	Control	Arginine	Control	Arginine
Asn	130 ± 6.2	122 ± 5.5	119 ± 4.6	126 ± 6.5
Asp	12.1 ± 0.45	11.9 ± 1.2	10.8 ± 1.8	10.6 ± 1.1
β-Ala	14.3 ± 1.0	15.6 ± 1.2	14.8 ± 0.7	15.1 ± 0.6
Cit	67.4 ± 2.8	69.1 ± 3.7	70.3 ± 2.4	70.8 ± 2.2
Glu	92.3 ± 4.8	94.0 ± 6.0	90.7 ± 4.4	91.6 ± 4.5
His	112 ± 4.6	115 ± 2.9	107 ± 2.8	109 ± 5.6
Ile	125 ± 7.4	121 ± 7.9	113 ± 3.7	118 ± 5.8
Leu	236 ± 8.1	241 ± 6.8	233 ± 7.4	227 ± 7.9
Lys	208 ± 13	192 ± 12	194 ± 9.6	189 ± 10
Met	49.9 ± 3.8	48.3 ± 2.8	46.4 ± 1.9	44.8 ± 4.2
Cys	214 ± 12	210 ± 13	208 ± 15	226 ± 17
Phe	106 ± 5.8	104 ± 4.6	97.7 ± 4.1	98.2 ± 5.6
Ser	152 ± 7.2	151 ± 6.9	147 ± 3.7	145 ± 4.8
Taurine	101 ± 7.5	93.3 ± 6.5	99.5 ± 8.2	96.0 ± 4.6
Thr	175 ± 8.5	167 ± 7.9	163 ± 5.8	161 ± 10
Trp	98.2 ± 8.4	107 ± 4.0	96.8 ± 4.8	91.4 ± 4.7
Tyr	121 ± 5.4	116 ± 4.2	107 ± 3.5	112 ± 4.5
Val	242 ± 8.2	234 ± 7.5	236 ± 4.2	229 ± 8.8

Blood samples were obtained from 121-day-old pigs (91 days after initiation of arginine supplementation) at 1 and 4 h post feeding. Values, expressed as μM, are mean ± SEM, $n = 12$/group

Table 7 Concentrations of free amino acids in the plasma of control and L-arginine supplemented pigs obtained at 1 and 4 h after feeding (Exp. 2)

Amino acid	Blood sample obtained at 1 h after feeding		Blood sample obtained at 4 h after feeding	
	Control	Arginine	Control	Arginine
Asn	123 ± 5.2	117 ± 6.4	112 ± 5.9	118 ± 4.8
Asp	11.5 ± 1.03	10.6 ± 0.93	12.0 ± 0.88	11.2 ± 0.95
β-Ala	13.7 ± 0.62	14.4 ± 1.0	13.5 ± 0.94	14.0 ± 0.83
Cit	68.9 ± 2.0	70.2 ± 2.6	67.4 ± 1.8	69.1 ± 1.6
Glu	90.6 ± 3.5	92.8 ± 4.8	93.5 ± 2.9	94.3 ± 3.7
His	108 ± 2.9	112 ± 3.3	110 ± 2.8	114 ± 3.5
Ile	118 ± 6.6	120 ± 5.4	125 ± 4.6	122 ± 4.0
Leu	240 ± 7.3	236 ± 6.1	229 ± 6.8	232 ± 8.2
Lys	212 ± 11	204 ± 9.6	207 ± 8.4	195 ± 12
Met	50.2 ± 2.4	49.3 ± 2.6	47.7 ± 2.0	46.9 ± 3.3
Cys	227 ± 10	216 ± 13	214 ± 11	220 ± 14
Phe	103 ± 4.8	105 ± 5.3	101 ± 5.2	103 ± 5.0
Ser	160 ± 6.4	157 ± 6.1	155 ± 4.9	152 ± 5.3
Taurine	113 ± 8.0	104 ± 7.7	108 ± 8.6	102 ± 6.5
Thr	171 ± 7.3	173 ± 8.4	169 ± 6.4	168 ± 9.1
Trp	96.0 ± 6.1	101 ± 5.5	98.3 ± 5.6	95.8 ± 6.4
Tyr	115 ± 4.9	112 ± 4.6	110 ± 4.4	109 ± 3.7
Val	238 ± 7.3	231 ± 6.8	227 ± 6.2	225 ± 6.5

Blood samples were obtained from 121-day-old pigs (91 days after initiation of arginine supplementation) at 1 and 4 h post feeding. Values, expressed as μM, are mean ± SEM, $n = 12$/group

a potentially attractive ingredient in dietary supplements, functional foods, and beverages (Wu 2013; Hurt et al. 2014). Notably, there are reports that, in healthy adults, increasing L-arginine provision (through supplementation) beyond that in the regular diet can reduce adhesion of platelets to the wall of blood vessels and improve flow-mediated dilation of blood vessels (Gornik and Creager 2004), while enhancing exercise capacity (Colgan 1993), muscle protein synthesis (Cynober 2007; Paddon-Jones et al. 2004), collagen synthesis (Barbul 1986), and fertility (Li et al. 2014; Mateo et al. 2007). This improvement of health and well-being is expected to reduce the risk for chronic disease, including cardiovascular disease (the number one killer in developed countries), obesity, and cancer, further underscoring the importance of L-arginine supplementation in global human health.

Despite its versatile metabolic functions, the use of L-arginine as a dietary or beverage supplement has been limited due to the concerns of regulatory agencies, policymakers, and consumers over the safety of its long-term use as a supplement (i.e., >2 months), because of (1) the lack of clinical data in the literature (Shao and Hathcock 2008); and (2) a possible increase in the risk of adverse cardiovascular events in patients with acute myocardial infarction; Schulman et al. 2006). Results of previous studies indicate the absence of a systematic pattern of adverse effects of oral L-arginine administration in adult humans, which precludes the selection of "No Observed Adverse Effect Level" or "Lowest Observed Adverse Effect Level" as the usual approach to identify a tolerable Upper Level of intake for this dietary supplement (Hayashi 2003; Shao and Hathcock 2008). Thus, investigators have developed a newer method for risk assessment, named the observed safe level (OSL) or the highest observed intake, which is defined as the highest intake level with sufficient evidence of safety (FAO/WHO 2006). In a double-blind, placebo-controlled trial with 16 healthy adult males, oral administration of 20 g L-arginine/day for 4 weeks did not result in any adverse effect as determined using standard clinical chemistry indices (Chin-Dusting et al. 1996). Likewise, healthy adults could tolerate oral administration of 40 g L-arginine/day for 1 week (duration of the study; Beaumier et al. 1995). Similarly, results from other trials indicated no side effects of oral administration of 21 and 42 g L-arginine/day to patients with hypercholesterolemia (Clarkson

Table 8 Concentrations of total protein, metabolites, hormones, and enzymes in the serum of control and L-arginine-supplemented pigs at 1 and 4 h after feeding

Variable	Time after feeding (h)	Exp. 1		Exp. 2	
		Control	2.42 % Arg–HCl	Control	2 % L-Arginine
Total serum protein (g/dl)	1	5.43 ± 0.16	5.80 ± 0.17*	5.52 ± 0.14	5.91 ± 0.15*
	4	5.40 ± 0.18	5.74 ± 0.15*	5.56 ± 0.15	5.84 ± 0.16*
Albumin (g/dl)	1	3.60 ± 0.15	3.94 ± 0.12*	3.67 ± 0.13	4.02 ± 0.12*
	4	3.62 ± 0.13	3.88 ± 0.10*	3.69 ± 0.12	3.91 ± 0.11*
Globulins (g/dl)	1	1.83 ± 0.09	1.86 ± 0.08	1.85 ± 0.08	1.89 ± 0.09
	4	1.78 ± 0.09	1.86 ± 0.10	1.87 ± 0.09	1.93 ± 0.10
Total bilirubin (mg/dl)	1	<0.10	<0.10	<0.10	<0.10
	4	<0.10	<0.10	<0.10	<0.10
Calcium (mg/dl)	1	10.6 ± 0.11	10.7 ± 0.07	10.8 ± 0.10	10.9 ± 0.08
	4	10.6 ± 0.12	10.6 ± 0.19	10.7 ± 0.11	10.8 ± 0.12
Phosphorus (mg/dl)	1	8.16 ± 0.26	8.23 ± 0.16	8.22 ± 0.23	8.25 ± 0.19
	4	7.49 ± 0.12†	7.74 ± 0.22†	7.61 ± 0.14†	7.65 ± 0.18†
Glucose (mg/dl)	1	106 ± 3.49	108 ± 2.48	109 ± 3.71	107 ± 3.26
	4	101 ± 2.06	102 ± 4.59	105 ± 2.81	103 ± 4.02
Urea (mM)	1	2.43 ± 0.17	2.65 ± 0.13	2.36 ± 0.15	2.51 ± 0.16
	4	2.77 ± 0.15	2.78 ± 0.16	2.60 ± 0.16	2.63 ± 0.17
Creatinine (mg/dl)	1	1.59 ± 0.05	1.51 ± 0.08	1.63 ± 0.07	1.56 ± 0.09
	4	1.57 ± 0.06	1.47 ± 0.09	1.61 ± 0.08	1.58 ± 0.10
Sodium (mM)	1	141 ± 0.95	142 ± 0.20	140 ± 0.91	141 ± 0.84
	4	141 ± 1.41	141 ± 1.24	141 ± 1.06	141 ± 1.45
Potassium (mM)	1	5.18 ± 0.12	5.13 ± 0.13	5.12 ± 0.14	5.16 ± 0.15
	4	5.65 ± 0.18	5.62 ± 0.17	5.57 ± 0.16	5.54 ± 0.14
Chloride (mM)	1	104 ± 0.33	103 ± 0.71	105 ± 0.42	106 ± 0.66
	4	103 ± 0.77	104 ± 0.55	104 ± 0.61	103 ± 0.83
Magnesium (mM)	1	1.54 ± 0.04	1.62 ± 0.04	1.58 ± 0.05	1.64 ± 0.06
	4	1.63 ± 0.04	1.69 ± 0.02	1.66 ± 0.06	1.70 ± 0.05
Insulin (pM)	1	70.5 ± 6.2	73.1 ± 6.6	71.3 ± 5.8	74.0 ± 6.0
	4	72.8 ± 6.4	75.4 ± 6.9	74.6 ± 7.5	76.8 ± 6.2
Growth hormone (pM)	1	248 ± 13	260 ± 16	236 ± 11	239 ± 14
	4	256 ± 15	268 ± 17	242 ± 13	261 ± 15
IGF-I (ng/ml)	1	201 ± 10	207 ± 11	204 ± 12	218 ± 14
	4	213 ± 12	220 ± 14	206 ± 14	215 ± 16
ALP (U/l)	1	126 ± 8.36	159 ± 9.04*	130 ± 7.15	166 ± 8.23*
	4	122 ± 8.24	157 ± 9.22*	128 ± 7.44	161 ± 8.60*
ALT (U/l)	1	31.2 ± 1.35	31.8 ± 2.11	32.6 ± 1.50	31.4 ± 1.88
	4	32.3 ± 1.73	31.6 ± 2.14	32.9 ± 2.01	32.0 ± 1.65
AST (U/l)	1	35.1 ± 2.65	37.0 ± 3.11	36.4 ± 3.28	36.1 ± 4.02
	4	36.8 ± 2.89	38.0 ± 4.07	37.2 ± 3.51	37.7 ± 4.63
LDH (IU/l)	1	606 ± 25.9	623 ± 31.7	618 ± 21.4	612 ± 27.9
	4	612 ± 32.1	646 ± 38.5	626 ± 26.6	633 ± 30.1
GGT (U/l)	1	34.4 ± 6.04	38.7 ± 5.19	35.1 ± 4.84	40.6 ± 4.73
	4	36.8 ± 2.67	40.0 ± 4.24	38.0 ± 3.76	42.3 ± 5.15

Blood samples were obtained from 121-day-old pigs (91 days after initiation of arginine supplementation) at 1 and 4 h post feeding. Values are mean ± SEM, $n = 6$

ALP alkaline phosphatase, *ALP* alkaline phosphatase, *ALT* alanine transaminase, *AST* aspartate transaminase, *GGT* gamma-glutamyl transpeptidase, *IGF-I* insulin-like growth factor, *LDH* lactate dehydrogenase

* $P < 0.05$ vs the corresponding control group

† $P < 0.05$ vs the corresponding 1 h group

Table 9 Hematology variables in control and L-arginine-supplemented pigs at 1 and 4 h after feeding

Variable	Time after feeding (h)	Exp. 1		Exp. 2	
		Control	2.42 % Arg–HCl	Control	2 % Arginine
Total WBC, $\times 10^3/\mu l$	1	15.3 ± 0.89	15.3 ± 1.15	15.1 ± 1.03	15.2 ± 1.22
	4	16.6 ± 1.21	16.9 ± 1.36	16.4 ± 1.15	16.6 ± 1.08
Total RBC, $\times 10^6/\mu l$	1	6.80 ± 0.22	7.08 ± 0.20	6.93 ± 0.31	7.15 ± 0.38
	4	6.76 ± 0.25	6.84 ± 0.36	6.88 ± 0.26	7.01 ± 0.42
Platelets, $\times 10^3/\mu l$	1	349 ± 14.3	334 ± 25.6	337 ± 12.9	340 ± 16.4
	4	366 ± 18.3	350 ± 24.2	353 ± 15.2	358 ± 19.6
Blood HG (g/dl)	1	11.5 ± 0.34	12.2 ± 0.38	11.7 ± 0.28	11.9 ± 0.33
	4	11.4 ± 0.37	11.2 ± 0.66	12.0 ± 0.25	11.6 ± 0.40
Plasma protein (g/dl)	1	6.12 ± 0.15	6.44 ± 0.18	6.16 ± 0.17	6.41 ± 0.16
	4	6.05 ± 0.14	6.22 ± 0.17	6.11 ± 0.16	6.36 ± 0.19
Fibrinogen (mg/dl)	1	291 ± 18.0	274 ± 16.5	295 ± 15.2	283 ± 19.4
	4	283 ± 19.7	269 ± 17.4	287 ± 18.9	280 ± 20.1
MCV (fl)	1	47.6 ± 0.36	49.6 ± 0.39*	47.3 ± 0.32	49.4 ± 0.37*
	4	47.5 ± 0.34	49.2 ± 0.37*	47.1 ± 0.33	49.0 ± 0.40*
MCH (pg)	1	16.9 ± 0.14	17.3 ± 0.26	17.1 ± 0.20	17.2 ± 0.23
	4	16.8 ± 0.13	16.9 ± 0.14	17.2 ± 0.17	17.0 ± 0.19
MCHC (g/dl)	1	35.5 ± 0.15	35.0 ± 0.24	35.2 ± 0.20	35.4 ± 0.26
	4	35.6 ± 0.29	34.9 ± 0.42	35.4 ± 0.23	35.1 ± 0.31
Reticulocytes (%)	1	1.88 ± 0.22	2.28 ± 0.16*	1.93 ± 0.15	2.20 ± 0.17*
	4	1.75 ± 0.17	2.14 ± 0.20*	1.78 ± 0.14	2.10 ± 0.16*
Percent of WBC in blood					
Neutrophils (%)	1	20.8 ± 1.24	14.6 ± 1.33*	21.4 ± 1.18	15.0 ± 0.96*
	4	22.2 ± 1.06	16.0 ± 1.21*	20.9 ± 1.13	16.3 ± 1.14*
Lymphocytes (%)	1	72.8 ± 1.49	74.2 ± 1.53	73.5 ± 1.37	73.8 ± 1.42
	4	73.3 ± 3.44	72.4 ± 3.06	74.1 ± 2.38	73.5 ± 2.56
Monocytes (%)	1	4.17 ± 0.46	5.32 ± 0.51*	4.25 ± 0.38	5.18 ± 0.41*
	4	2.50 ± 0.32†	3.84 ± 0.38*,†	2.69 ± 0.25†	3.76 ± 0.29*,†
Eosinophils (%)	1	2.50 ± 0.24	3.14 ± 0.37*	2.62 ± 0.18	3.07 ± 0.22*
	4	2.00 ± 0.19†	2.57 ± 0.26*,†	2.13 ± 0.12†	2.60 ± 0.24*,†
Blood pH	1	7.39 ± 0.01	7.40 ± 0.01	7.40 ± 0.01	7.40 ± 0.01
	4	7.40 ± 0.01	7.40 ± 0.01	7.40 ± 0.01	7.39 ± 0.01

Blood samples were obtained from 121-day-old pigs (91 days after initiation of arginine supplementation) at 1 and 4 h post feeding. Values are mean ± SEM, $n = 6$

HG hemoglobin, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *MCV* mean corpuscular volume, *RBC* red blood cells, *WBC* white blood cells

* $P < 0.05$ vs the corresponding control group

† $P < 0.05$ vs the corresponding 1 h group

et al. 1996) or cystic fibrosis (Grasemann et al. 2005) for 4 and 6 weeks, respectively. Based on these findings, an OSL value for oral administration of L-arginine to healthy adults has been suggested to be 20 g/day (Shao and Hathcock 2008). However, the published studies with healthy subjects involved a short duration of L-arginine supplementation (1–4 weeks) and a small number of subjects (5–16) (Beaumier et al. 1995; Chin-Dusting et al. 1996). These concerns limit our confidence in the 20 g/day dose as the OSL value for oral administration of L-arginine to healthy adults and underscore the need for larger and longer studies.

Data from animal studies are much needed to help design clinical trials with humans. Pigs are very similar to humans with regard to digestion, metabolism, and physiology; thus, the pig is a widely used animal model to study human nutrition (Kim and Wu 2004; Pond and Mersmann 2001). The half-lives of intravenously and orally

Table 10 Effects of dietary supplementation with L-arginine–HCl or L-arginine on the body composition of pigs

Variable	Exp. 1		Exp. 2	
	Control	2.42 % Arg–HCl	Control	2 % Arginine
Percentage of nutrients, %				
Water	68.0 ± 0.64	68.6 ± 0.61	68.1 ± 0.61	68.8 ± 0.65
Crude protein	16.0 ± 0.52	16.2 ± 0.54	15.9 ± 0.55	16.1 ± 0.57
Crude fat	12.6 ± 0.45	11.8 ± 0.42	12.5 ± 0.41	11.7 ± 0.48
Minerals	3.02 ± 0.15	3.03 ± 0.14	3.01 ± 0.13	3.02 ± 0.14
Carbohydrate	0.38 ± 0.03	0.37 ± 0.02	0.39 ± 0.03	0.38 ± 0.03
Amounts of nutrients, kg/pig				
Water	43.7 ± 0.32	46.0 ± 0.35**	43.9 ± 0.34	46.4 ± 0.37**
Crude protein	10.3 ± 0.08	10.9 ± 0.09**	10.2 ± 0.07	10.9 ± 0.08**
Crude fat	8.10 ± 0.06	7.92 ± 0.05*	8.08 ± 0.05	7.90 ± 0.05*
Minerals	1.94 ± 0.02	2.03 ± 0.02*	1.95 ± 0.02	2.04 ± 0.02*
Carbohydrate	0.245 ± 0.01	0.248 ± 0.01	0.252 ± 0.01	0.256 ± 0.01

Pigs were euthanized at 121 days of age (91 days after initiation of arginine supplementation) to determined their body compositions. Values are mean ± SEM, $n = 6$

* $P < 0.05$ and ** $P < 0.01$ vs the corresponding control group

administered L-arginine in the plasma of nonpregnant pigs are 1.06 and 1.86 h, respectively (Wu et al. 2007a). We also reported that healthy adult pigs and rats can tolerate large amounts of supplemental L-arginine (at least 0.21–0.42 and 2.1–5.7 g/kg body weight/day, respectively) (Wu et al. 2007a). Tsubuku et al. (2004) reported that adult rats can tolerate at least 3.6 g L-arginine/kg body weight per day. On the basis of the finding that the intake of dry matter by adult humans is ~ 10 % of that by adult rats, an adult human can likely tolerate an enteral supplemental dose of at least 0.21–0.57 g/kg body weight/day (or 15–40 g/kg body weight/day for a 70-kg subject; Wu et al. 2007a).

Results from the present student provide further evidence that pigs can tolerate high intake of dietary L-arginine. The corn- and soybean meal-based diet used for this research contained 1.35 % L-arginine, supplying 425 mg L-arginine/kg body weight/day. The supplemental doses of 0, 1, 1.5, and 2 % L-arginine provided pigs with 0, 315, 473, and 630 mg L-arginine/kg body weight/day, respectively. Based on the conversion factor adopted by the Food and Drug Administration of the United States (2005), the human-equivalent doses of the supplemental L-arginine were 0, 286, 430, and 573 mg L-arginine/kg body weight/day, respectively, or 20, 30, and 40 g L-arginine/day for a 70-kg person, respectively. Dietary supplementation with L-arginine dose-dependently increased plasma concentrations of arginine, ornithine and proline, while reducing plasma concentrations of ammonia, glycine, glutamine, ammonia, cholesterol, free fatty acids, and triglycerides. This is consistent with the roles for L-arginine in enhancing ornithine formation, ammonia detoxification and muscle protein synthesis in mammals (Wu and Morris 1998; Yao et al. 2008), while stimulating oxidation of fatty acids and inhibiting synthesis of both long-chain fatty acids and triglycerides in a tissue-specific manner (McKnight et al. 2010; Wu et al. 2012). Accordingly, L-arginine-supplemented pigs gained more protein but less fat, as compared with the control group (Table 10). Interestingly, serum levels of alanine transaminase, aspartate transaminase, and lactate dehydrogenase (indicators of integrity of the liver and other tissues) did not differ between control and arginine-supplemented pigs, but serum levels of alkaline phosphatase (released by bone and liver) were elevated in the L-arginine group than in the control. Our study reveals that L-arginine supplementation increases not only tissue protein synthesis but also possibly bone growth in pigs. Of note, all of other variables in standard hematology and clinical chemistry tests did not differ among control and L-arginine-supplemented pigs. Collectively, these results indicate that dietary supplementation with L-arginine or L-arginine–HCl (up to 630 mg L-arginine/kg body weight/day) is safe in pigs for at least 91 days. Our findings help guide clinical studies to determine the safety of long-term oral administration of L-arginine to humans.

In conclusion, supplementing up to 2 % L-arginine (as either L-arginine–HCl or L-arginine base) to the diet for 91 days did not have any adverse effect on postweaning pigs. Our results also indicate a promising effect of L-arginine on improving nutritional status and lean tissue mass, while beneficially reducing plasma levels of ammonia, free fatty acids, triglyceride, and cholesterol, as well as white fat in the body. The data from the present study are helpful in predicting a safe upper limit for oral administration of L-arginine to healthy adults and in guiding clinical studies to determine long-term safety of L-arginine supplementation in humans.

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

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