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ORIGINAL ARTICLE

Safety of dietary supplementation with arginine in adult humans

Catherine J. McNeal[1] · Cynthia J. Meininger[2] · Colin D. Wilborn[3] · Carmen D. Tekwe[4] · Guoyao Wu[2,5]

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Abstract

Previous studies with animals and humans have shown beneficial effects of dietary supplementation with L-arginine (Arg) on reducing white fat and improving health. At present, a long-term safe level of Arg administration to adult humans is unknown. The objective of this study was to conduct a randomized, placebo-controlled, clinical trial to evaluate the safety and tolerability of oral Arg in overweight or obese but otherwise healthy adults with a body mass index of ≥ 25 kg/m^2. A total of 142 subjects completed a 7-day wash-in period using a 12 g Arg/day dose. All the remaining eligible 101 subjects who tolerated the wash-in dose (45 men and 56 women) were assigned randomly to ingest 0, 15 or 30 g Arg (as pharmaceutical-grade Arg-HCl) per day for 90 days. Arg was taken daily in at least two divided doses by mixing with a flavored beverage. At Days 0 and 90, blood pressures of study subjects were recorded, their physical examinations were performed, and their blood and 24-h urine samples were obtained to measure: (1) serum concentrations of amino acids, glucose, fatty acids, and related metabolites; and (2) renal, hepatic, endocrine and metabolic parameters. Our results indicate that the serum concentration of Arg in men or women increased ($P < 0.05$) progressively with increasing oral Arg doses from 0 to 30 g/day. Dietary supplementation with 30 g Arg/day reduced ($P < 0.05$) systolic blood pressure and serum glucose concentration in females, as well as serum concentrations of free fatty acids in both males and females. Based on physiological and biochemical variables, study subjects tolerated oral administration of 15 and 30 g Arg/day without adverse events. We conclude that a long-term safe level of dietary Arg supplementation is at least 30 g/day in adult humans.

Keywords Arginine · Health · Nutrition · Obesity · Safety · Supplementation

Abbreviations

ALT	Alanine transaminase
Arg	L-Arginine
AST	Aspartate transaminase
BMI	Body mass index
BW	Body weight
GI	Gastrointestinal
NO	Nitric oxide
OSL	Observed safe level
TSH	Thyroid stimulating hormone

Introduction

L-Arginine (Arg) was traditionally been classified as a nutritionally nonessential amino acid for healthy adults because it is synthesized in the body to maintain nitrogen balance (Hou et al. 2016a; Hou and Wu 2017). Since the discovery in 1988 that Arg is the nitrogenous precursor for the biosynthesis of nitric oxide (NO), there has been growing interest in the roles of Arg in the functions of multiple systems [including

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✉ Guoyao Wu
g-wu@tamu.edu

[1] Department of Internal Medicine, Baylor Scott & White Health, Temple, TX 76508, USA

[2] Department of Medical Physiology, College of Medicine, Texas A&M Health Science Center, Temple, TX 76504, USA

[3] Department of Exercise and Sport Science, University of Mary Hardin-Baylor, Belton, TX 76513, USA

[4] Department of Epidemiology and Biostatistics, School of Public Health, Texas A&M Health Science Center, College Station, TX 77843, USA

[5] Department of Animal Science and Faculty of Nutrition, Texas A&M University, College Station, TX 77843, USA

the circulatory, gastrointestinal (GI), immune, and reproductive systems] in humans (Blachier et al. 2011; Clarkson et al. 1996; Creager et al. 1992; Kayacelebi et al. 2015; Luiking et al. 2012; McNeal et al. 2010, 2016; Morris 2007; Pahlavani et al. 2017). Additionally, Arg has been reported to reduce fat accretion in white adipose tissue and improve metabolic profiles in adult mammals, including obese rats (Fu et al. 2005; Jobgen et al. 2009a, b) and humans (Hurt et al. 2014; Lucotti et al. 2006), and in growing swine (Tan et al. 2009). Thus, Arg is now recognized to be an important ingredient in dietary supplements, functional foods, and beverages.

Despite its versatile metabolic functions, Arg supplementation in humans has been limited due to the concerns of regulatory agencies, policymakers, and consumers regarding the safety of its long-term use (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 Arg administration in adults and children, 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, biomedical 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).

We have previously reviewed the literature describing the safety, pharmacokinetics, and effectiveness of oral Arg in adults (McNeal et al. 2016). An OSL value for oral administration of Arg to healthy adults has been suggested to be 20 g/day (Shao and Hathcock 2008). However, higher levels of Arg [up to 30 g/day for 7 days (Evans et al. 2004)] have been tested in short-term studies without serious adverse effects (McNeal et al. 2016). This limits our confidence in the 20 g/day dose as the OSL value for oral administration of Arg to adult humans, and underscores the need for larger and longer studies. Thus, because the safe upper level for Arg supplementation to adults remains unknown, the objective of the present study was to determine the OSL of oral administration of Arg in overweight or obese adults without any other health problems.

Study subjects and methods

This clinical trial involved a randomized, placebo-controlled, double-blinded trial. It was approved by the Institutional Review Boards (IRB) of Baylor Scott & White Health and Texas A&M University. The investigators received funding for this study from the International Council of Amino Acid Science (Brussels, Belgium).

Human subjects

The intent of this study was to assess the safety of Arg supplementation in human adults and secondarily to assess its effects on their metabolic status (including fat mass). Therefore, we recruited overweight or obese but otherwise healthy subjects. According to the medical literature, some obese subjects may be classified as metabolically healthy (Muñoz-Garach et al. 2017; Stefan et al. 2013). To date, there is no universally accepted definition of metabolically healthy obesity, and most studies have defined this condition as a body mass index (BMI) of ≥ 30 kg/m^2 without the presence of metabolic diseases, such as type-2 diabetes, dyslipidemia and hypertension (Muñoz-Garach et al. 2017; Stefan et al. 2013; Zheng et al. 2016). A total of 142 adults (men and women) with a BMI of ≥ 25 kg/m^2 were recruited (see Fig. 1). To be included, subjects could not be taking any chronic medications, except for oral contraceptives. Exclusion criteria were: (1) chronic or acute infections at the time of enrollment or during the study (if they required antibiotic therapy); (2) active inflammatory or autoimmune disorders; (3) active malignancy, tumors, or pathological angiogenesis; (4) any GI disease (with the exception of occasional, intermittent problems, such as gastroesophageal reflux not requiring chronic treatment); (5) any tobacco use (cigarettes, cigars, pipes, or use of smokeless tobacco or snuff) over the past year; (6) clinical evidence of hypertension, hypotension, or atherosclerosis documented in the medical record; (7) previous medical history resulting in hospitalization and/or use of medications for > 28 consecutive days in the past 5 years (e.g., cardiovascular, renal, hepatic, GI, neuropsychiatric, allergic or endocrine disease); (8) use of any chronic medications (other than oral contraceptives); and (9) women who were pregnant or nursing or women who were planning conception during the course of the study. To minimize drop-outs among individuals unable to tolerate oral Arg, all subjects took 12 g Arg (as Arg-HCl) per day in at least two divided doses as a powder mixed with a sugar-free beverage of choice (typically artificially sweetened lemon flavoring; $n = 140$) or in a capsule form ($n = 2$) for 7 days. After the wash-in period, 41 subjects were excluded for the following reasons: no time—11; loss to follow-up—12; lack of interest—4; dislike of product taste—5; development of exclusions—5; intolerable GI symptoms—3; and uncomfortable thought—1. All the remaining eligible 101 subjects (45 men and 56 women) were randomized into one of the three treatment groups: 0, 15, and 30 g oral Arg (as Arg-HCl) per day (Fig. 1). Their physical characteristics are summarized

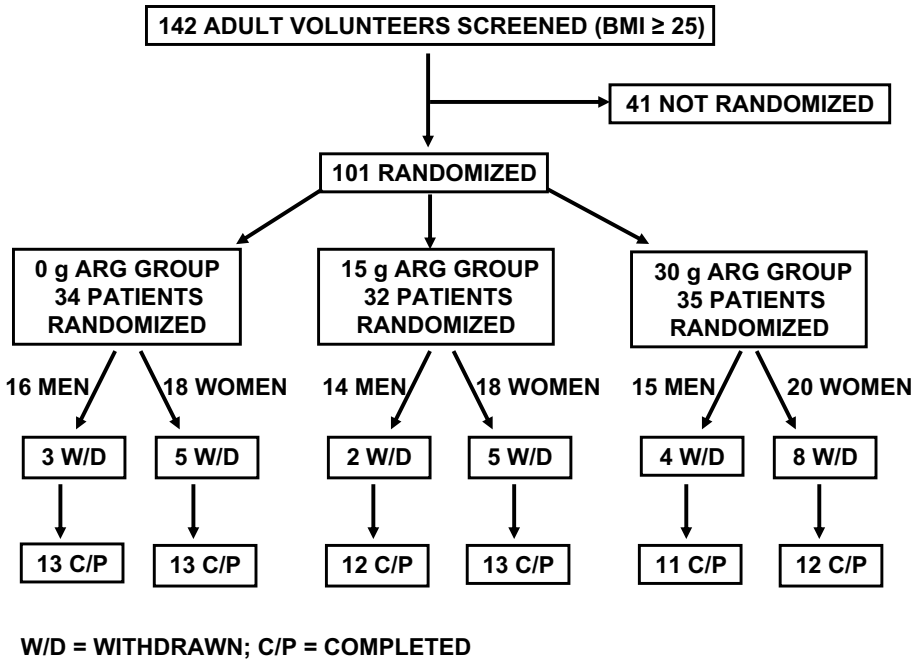

Fig. 1 Experimental design of the study. A total of 142 overweight or obese but otherwise healthy adult humans (men and women) with a body mass index (BMI) of ≥ 25 kg/m^2 were recruited. An initial screening, in which all subjects took 12 g Arg (as Arg-HCl) per day in at least two divided doses as a powder mixed with a beverage or as capsules for 7 days, was conducted to determine their interest in the study and their tolerance to Arg. After this preliminary screening, 41 subjects were not randomized into the study for a variety of reasons. One hundred and one subjects met the study criteria and were randomized into one of the three treatment groups: 0, 15, and 30 g oral Arg (as Arg-HCl) per day. *W/D* withdrew from the study, *C/P* completed the study

in Table 1. Each subject participated in only one treatment group.

As with any clinical trial involving a dietary supplement, the first step would be to evaluate its safety and tolerability. Based on the results of the present work, subsequent studies can expand the inclusion criteria to a broader population. Because GI tolerability is a significant side-effect limitation, Arg would likely not be recommended for any patients with coexisting GI problems (Grimble 2007). For example, metformin is a drug often used for treating obese individuals with insulin resistance but would likely not be prescribed if a patient was already having GI issues that metformin would exacerbate (McCreight et al. 2016).

Oral administration of Arg

Pharmaceutical-grade Arg-HCl (99.9% purity; Lot no. 12051091) was obtained from Kyowa Hakko Bio Co., Ltd (Tokyo, Japan). Arg was dispensed into jars with scoops (using 100% rice maltodextrin to achieve the same total weight of material per jar) by the compounding pharmacy (Medicine Shoppe, Belton, TX, USA). The placebo group was supplemented only with 100% rice maltodextrin. Rice maltodextrin was chosen to have the same density and solubility as Arg, so that both substances appear to be indistinguishable. Randomization to each dose was determined by the Baylor Scott & White Biostatistics Department. Compliance was monitored: (1) using a log book to document missed doses; and (2) accounting for unused product.

Selection of the Arg dose was based on (1) average intake of Arg from regular foods (~ 5 g/day) by adults in the US (Flynn et al. 2002); (2) results of previous human studies suggesting that a safe upper limit for short-term oral administration of Arg to healthy adults is 40 g/day for 6 days (Beaumier et al. 1995); (3) published work involving healthy adults pigs (Hu et al. 2015; Wu et al. 2016) and rats (Tsubuku et al. 2004; Yang et al. 2015) which can tolerate at least 0.21–0.42 and 2.1–5.7 g Arg/kg body weight/day, respectively; and (4) consideration that a supplemental dose of Arg ≥ 30 g/day is unlikely to generate additional health benefits to adult people (Buchman et al. 1999; Evans et al. 2004; Grimble 2007; Wu et al. 2009).

All study subjects were advised to take the study medication in equally divided portions at least twice daily with food, mixing 1 scoop into the beverage of their choice. Subjects returned unused drug every 45 days. They were instructed to eat healthy diets and maintain exercise habits throughout the study. At each visit, study subjects also completed a food frequency questionnaire (Block et al. 1992), which

Table 1 Physical characteristics of the human subjects who completed the study

Variable	Day of study	Male participants			Female participants		
		0 g Arg/day ($n=13$)	15 g Arg/day ($n=12$)	30 g Arg/day ($n=11$)	0 g Arg/day ($n=13$)	15 g Arg/day ($n=13$)	30 g Arg/day ($n=12$)
Age, year (minimum, maximum)		35 ± 2.5 (23, 50)	33 ± 2.6 (22, 55)	33 ± 1.5 (25, 42)	40 ± 2.6 (29, 53)	40 ± 3.2 (23, 57)	34 ± 2.6 (22, 50)
Height (cm)		176.4 ± 1.1	178.1 ± 2.3	177.5 ± 2.3	163.6 ± 1.4	163.1 ± 1.8	165.0 ± 2.5
Waist circumference (cm)	0	117.6 ± 7.1	115.3 ± 4.3	112.8 ± 2.5	109.5 ± 3.3	108.2 ± 4.1	110.0 ± 3.8
	90	119.6 ± 8.1	114.8 ± 5.3	113.3 ± 2.8	108.7 ± 3.3	107.4 ± 4.3	111.3 ± 4.1
Body weight (kg)	0	116.3 ± 8.5	108.3 ± 6.8	107.8 ± 2.9	100.1 ± 4.2	93.6 ± 5.8	100.2 ± 5.4
	90	117.1 ± 9.5	107.9 ± 7.2	108.1 ± 2.2	99.4 ± 4.1	95.4 ± 6.6	98.7 ± 5.7
Body mass index (kg/m^2)	0	36.1 ± 2.5	32.9 ± 1.7	32.9 ± 0.8	36.6 ± 1.8	34.3 ± 1.9	35.4 ± 1.6
	90	36.6 ± 2.9	32.9 ± 1.8	32.9 ± 0.9	36.3 ± 1.8	34.1 ± 1.8	35.0 ± 1.7
Lean mass (kg)	0	68.0 ± 2.9	65.1 ± 3.2	67.6 ± 1.5	49.2 ± 1.5	45.9 ± 2.5	50.6 ± 2.7
	90	67.3 ± 3.2	65.2 ± 3.5	67.6 ± 1.6	47.9 ± 1.3†	45.7 ± 2.3	50.2 ± 2.5
Fat mass (kg)	0	37.4 ± 6.1	32.4 ± 3.5	28.5 ± 2.4	42.2 ± 3.5	41.7 ± 4.3	38.7 ± 3.0
	90	38.8 ± 6.5	32.5 ± 3.7	30.4 ± 2.3	42.6 ± 3.5	41.5 ± 4.0	39.5 ± 3.3
Blood pressure							
Systolic (mmHg)	0	130.8 ± 2.5	130.3 ± 2.6	130.4 ± 2.1	123.0 ± 2.8	118.5 ± 3.1	130.7 ± 3.4
	90	134.1 ± 3.0	135.7 ± 3.1	135.2 ± 2.5	125.2 ± 2.6	116.5 ± 1.9	125.2 ± 3.5*
Diastolic (mmHg)	0	77.1 ± 1.9	78.1 ± 2.3	76.7 ± 2.7	73.4 ± 2.2	73.3 ± 1.7	77.0 ± 2.0
	90	80.8 ± 2.4	78.7 ± 1.7	80.6 ± 3.1	74.8 ± 2.5	74.0 ± 1.9	74.9 ± 2.6

Values are mean ± SEM, with the number (n) of subjects given in the parentheses

*$P < 0.05$ vs the value for d 0 (the beginning of the trial)

†$P < 0.01$ vs the value for d 0 (the beginning of the trial)

was analyzed with a nutrient analysis program designed by the Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School (http://www.Harvardsffq.date). The analysis included dietary intake of protein, amino acids, lipids, carbohydrates, calories, vitamins and minerals (Harvard School of Public Health 2018). Data were exported into an Excel spreadsheet. Each volunteer was also asked to complete a daily diary in which they noted any medication taken, missed doses, and report any changes in health status to the study coordinator. The physical activity of study subjects was assessed at each visit using the Physical Activity History questionnaire, a tool that has been used in the Coronary Artery Risk Development in young Adults study (Sidney et al. 1991). Blinding to the dose allocation was maintained until all subjects had completed the study; abnormal laboratory values > 3 ULN (upper limit normal) were repeated within 2 weeks and the data were reviewed to resolve any study-related problems.

On Days 0 and 90 of the study, a medical history was obtained and a general physical exam was performed. Body weight (BW), height, and waist circumference (using a Gulick tape measure) were measured, and BMI calculated. Blood pressure was measured after a 15 min rest using a DynapMap in the left and right arms and the two values were averaged (Jani et al. 2006). Blood samples (15 ml) were obtained after a 12-h fasting to tubes containing no coagulants; samples were immediately centrifuged at 600 g and 4 °C for 10 min to obtain serum. Urine was collected over a 24-h period prior to the blood draw. Serum and urine samples were divided into three aliquots and stored at − 80 °C until analysis. On Days 0 and 90, the body fat composition was estimated with the use of dual-energy X-ray absorptiometry (DXA) (Urbina et al. 2017). On Day 45 of the study, blood pressures and body weights were also recorded.

Laboratory analyses

For amino acid analysis, serum (1 ml) was immediately acidified with 1 ml of 1.5 M $HClO_4$, followed by neutralization with 0.5 ml of 2 M K_2CO_3 (Wu and Meininger 2008). The neutralized extracts were stored at − 80 °C. Serum samples were analyzed for thyroid stimulating hormone (TSH), glucose, lipid levels (total cholesterol and triglyceride, total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol, HDL-C), electrolytes, albumin, total protein, and total bilirubin by Quest Diagnostics (Irvine, Texas, USA). Urea and ammonia in serum and urine were analyzed by enzymatic methods (Wu 1995),

while amino acids (including homoarginine and methylarginines) and nitrite plus nitrate (stable products of NO oxidation) in both types of samples were determined by high-performance liquid chromatography (Hou et al. 2015b; Li et al. 2000; Marliss et al. 2006; Wu and Meininger 2008). Free fatty acids and creatinine were determined using assay kits from Wako Chemicals (Richmond, VA, USA) and Sigma Chemicals (St. Louis, MO, USA), respectively (Hu et al. 2015). Growth hormone and insulin in serum were measured using ELISA assay kits (Abcam, Cambridge, MA, USA). Enzymatic activities of alanine transaminase (ALT) and aspartate transaminase (AST) in serum were determined with the use of a Microplate reader (Wu et al. 2000).

Statistical analysis

Results are expressed as mean ± SEM. All the statistical analyses were performed using the SAS software (SAS Institute, Cary, NC, USA). We first obtained the means at both baseline and Day 90 by gender and treatment group assignment (0, 15 or 30 g Arg/day) for each of the variables analyzed. ANOVA models were used to perform the analysis of the data collected at the baseline to test for treatment group differences in each outcome variable by gender. Similar analyses were also performed for all the data collected on Day 90. The ANOVA models for the Day 90 data were all adjusted for their baseline values. Differences among all possible pairs of the treatment groups were determined using the Tukey's test for multiple pairwise comparisons. In addition to the ANOVA models, paired differences between Days 0 and 90 were also analyzed using the paired *t* test. A probability value of 0.05 was used to determine statistical significance in all the analyses conducted. Log transformation was used when needed to obtain approximate normality prior to conducting the statistical analyses.

Results

Physical characteristics of study subjects

Baseline characteristics of study participants did not differ ($P > 0.05$) among the three groups of males or females (Table 1), indicating the good randomization of our experimental subjects. Compared with values at Day 0, the BW of either male or female subjects at Day 90 did not differ ($P > 0.05$) among the 0, 15 and 30 g Arg/day groups (Table 1). Dietary supplementation with up to 30 g Arg/day for 90 days did not affect ($P > 0.05$) the waist circumference, body weight, lean mass, fat mass, BMI, or blood pressure (systolic or diastolic) of male subjects at Day 90. Similar results were obtained for waist circumference, body weight, BMI in female subjects. However, females in the control group lost 1.3 kg lean mass over a 90-day period ($P < 0.01$), but those in the 15 and 30 g Arg/day groups maintained their lean masses during the entire experimental period. Of note, the systolic pressure of female subjects in the 30 g Arg/day group was 5.5 mmHg lower ($P < 0.05$) at Day 90 than at Day 0. The systolic pressure of female subjects in the 0 or 15 g Arg/day group did not differ ($P > 0.05$) between Days 0 and 90. On Day 45 of the study, the blood pressures or body weights of non-fasting subjects did not differ ($P > 0.05$) among the 0, 15 and 30 g Arg/day groups (data not shown). At the end of the 90-day study, all subjects appeared healthy based on their physical characteristics.

Food intake of study subjects

Food intake is difficult to measure in free-living human subjects. Thus, we estimated food intake by having subjects complete a food frequency questionnaire in the 24-h prior to each visit. Results are shown in Table 2. Among the three study groups in males or females, nutrient intake did not differ ($P > 0.05$) at Day 0 or 90. In each study group, nutrient intake did not differ ($P > 0.05$) between Days 0 and 90 of the study.

Concentrations of hormones, protein and electrolytes, and enzymatic activities of transaminases in serum

Concentrations of insulin, growth hormone, TSH, total protein, potassium, and total bilirubin in the serum of male or female subjects did not differ ($P > 0.05$) among the 0, 15 and 30 g Arg/day groups (Table 3). Compared with Day 0, the concentration of sodium in the serum of male subjects at Day 90 was slightly lower [by 1.5 mM ($P < 0.05$)] and that of chloride at Day 90 was slightly greater [by 2.6 mM ($P < 0.05$)] in response to oral administration of 30 g Arg/day. A slight increase of 1.6 mM in serum chloride was observed at Day 90 in male subjects taking 15 g Arg/day ($P < 0.05$). In all male subjects, serum concentrations of bicarbonate at Day 90 were all lower ($P < 0.05$) than those at Day 0, and the decreases were slightly greater ($P < 0.05$) in the 15 and 30 g Arg/day group than in the 0 g Arg/day group. Interestingly, no change in serum concentrations of chloride and bicarbonate occurred over a 90-day period in female subjects taking 0, 15 or 30 g Arg/day. In contrast, dietary supplementation with 30 g Arg/day for 90 days increased ($P < 0.05$) the serum concentration of albumin in female subjects. In both males and females, dietary supplementation with 15 and 30 g Arg/day slightly reduced ($P < 0.05$) the enzymatic activities of AST and ALT in serum at Day 90, in comparison with Day 0.

Table 2 Energy and nutrient intakes of male and female participants in different treatment groups at Days 0 and 90 of the study

Variable	Day of study	Male participants			Female participants		
		0 g Arg/day ($n=13$)	15 g Arg/day ($n=12$)	30 g Arg/day ($n=11$)	0 g Arg/day ($n=13$)	15 g Arg/day ($n=13$)	30 g Arg/day ($n=12$)
Calorie, kcal/day	0	2443 ± 295	2188 ± 164	2298 ± 254	2020 ± 168	2031 ± 194	1916 ± 169
	90	1866 ± 218	2103 ± 239	1694 ± 168	1660 ± 292	1720 ± 263	1659 ± 212
Crude protein, g/day	0	116 ± 19	97 ± 10	101 ± 11	86 ± 7.4	97 ± 9.4	82 ± 6.2
	90	94 ± 17	106 ± 18	79 ± 9.5	80 ± 18	77 ± 10	74 ± 10
Arginine, g/day	0	6.8 ± 1.2	5.6 ± 0.63	5.9 ± 0.67	5.0 ± 0.46	5.7 ± 0.57	4.7 ± 0.35
	90	5.4 ± 1.0	6.3 ± 1.2	4.6 ± 0.55	4.7 ± 1.1	4.5 ± 0.69	4.3 ± 0.62
Lysine, g/day	0	8.4 ± 1.5	7.0 ± 0.78	7.2 ± 0.72	6.2 ± 0.59	7.1 ± 0.74	5.9 ± 0.46
	90	7.0 ± 1.4	8.1 ± 1.6	5.6 ± 0.75	5.8 ± 1.3	5.5 ± 0.65	5.2 ± 0.72
Carbohydrates, g/day	0	286 ± 34	266 ± 26	258 ± 30	266 ± 29	233 ± 24	231 ± 25
	90	203 ± 24	219 ± 26	188 ± 19	201 ± 30	203 ± 29	200 ± 28
Fiber, g/day	0	24 ± 3.2	22 ± 2.9	22 ± 2.5	21 ± 1.8	21 ± 2.3	21 ± 2.4
	90	19 ± 2.4	16 ± 2.4	19 ± 2.7	20 ± 3.4	19 ± 3.3	21 ± 4.6
Lipids, g/day	0	90 ± 12	82 ± 8.0	91 ± 12	71 ± 6.5	78 ± 7.8	74 ± 6.1
	90	73 ± 10	87 ± 10	70 ± 7.9	61 ± 12	67 ± 13	62 ± 8.4
Calcium, g/day	0	1.1 ± 0.19	1.1 ± 0.15	1.1 ± 0.13	1.0 ± 0.11	0.91 ± 0.10	918 ± 80
	90	0.88 ± 0.13	0.87 ± 0.09	0.87 ± 0.09	0.74 ± 0.11	0.82 ± 0.14	1034 ± 158
Phosphorous, g/day	0	1.8 ± 0.28	1.6 ± 0.16	1.6 ± 0.16	1.4 ± 0.10	1.5 ± 0.14	1332 ± 102
	90	1.4 ± 0.21	1.6 ± 0.23	1.3 ± 0.14	1.2 ± 0.22	1.3 ± 0.18	1207 ± 166
Vitamin A, mg/day	0	1.4 ± 0.36	1.1 ± 0.23	1.2 ± 0.27	1.0 ± 0.14	1.1 ± 0.14	1373 ± 155
	90	1.3 ± 0.19	0.77 ± 0.21	1.0 ± 0.17	1.2 ± 0.23	1.1 ± 0.18	1077 ± 195
Vitamin D, IU/day	0	428 ± 123	372 ± 103	347 ± 71	303 ± 71	233 ± 44	366 ± 89
	90	411 ± 71	292 ± 89	262 ± 47	342 ± 69	335 ± 55	332 ± 84
Vitamin C, mg/day	0	280 ± 84	156 ± 34	153 ± 31	193 ± 55	134 ± 23	125 ± 14
	90	199 ± 96	108 ± 22	134 ± 22	223 ± 77	121 ± 21	177 ± 56
Vitamin B6, mg/day	0	1.2 ± 0.11	1.1 ± 0.14	1.1 ± 0.07	1.3 ± 0.17	1.4 ± 0.15	1.2 ± 0.14
	90	1.3 ± 0.19	1.0 ± 0.00	1.3 ± 0.21	1.3 ± 0.19	1.2 ± 0.11	1.3 ± 0.23
Zinc, mg/day	0	28 ± 7.7	20 ± 3.9	21 ± 5.1	14 ± 1.5	15 ± 1.6	14 ± 1.2
	90	19 ± 5.3	17 ± 2.9	13 ± 2.1	21 ± 6.5	16 ± 5.2	14 ± 2.1
Copper, mg/day	0	2.2 ± 0.50	1.9 ± 0.31	2.0 ± 0.36	1.6 ± 0.14	1.8 ± 0.20	1.8 ± 0.21
	90	1.6 ± 0.23	1.6 ± 0.27	1.6 ± 0.23	1.8 ± 0.32	1.5 ± 0.21	1.5 ± 0.24
Iron, mg/day	0	16 ± 2.1	14 ± 1.2	15 ± 2.0	14 ± 1.3	15 ± 1.5	13 ± 1.2
	90	12 ± 1.2	14 ± 1.7	11 ± 1.3	14 ± 2.8	12 ± 1.8	12 ± 1.8

Values are mean ± SEM, with the number (n) of subjects given in the parentheses

Concentrations of urea, ammonia, creatinine, and amino acids in serum

Dietary supplementation with 15 and 30 g Arg/day for 90 days increased ($P < 0.05$) serum concentrations of Arg, ornithine and homoarginine in both males and females, but did not affect ($P > 0.05$) those of urea, ammonia, creatinine, histidine, lysine and methylarginines, in male or female subjects, as compared with Day 0 (Table 4). In male and female subjects taking 30 g Arg/day, serum concentrations of proline were greater ($P < 0.01$) at Day 90 than those at Day 0 (Table 4). Oral administration of 30 g Arg/day reduced ($P < 0.05$) serum concentrations of glutamine and glycine in both male and female subjects, whereas oral administration of 15 g Arg/day had no effect ($P > 0.05$) on these variables. Dietary supplementation with 15 or 30 g Arg/day did not affect ($P > 0.05$) serum concentrations of other amino acids in male or female subjects (Supplemental Table 1).

Concentrations of glucose and lipids in serum

Dietary supplementation with 15 or 30 g Arg/day for 90 days did not affect ($P > 0.05$) the concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, or triglycerides in the serum of male and female subjects (Table 5). While the

Table 3 Concentrations of hormones, protein and electrolytes, and enzymatic activities of transaminases in the serum of the human subjects who completed the study

Variable	Day of study	Male participants			Female participants		
		0 g Arg/day ($n=13$)	15 g Arg/day ($n=12$)	30 g Arg/day ($n=11$)	0 g Arg/day ($n=13$)	15 g Arg/day ($n=13$)	30 g Arg/day ($n=12$)
TSH (mIU/L)	0	2.24 ± 0.22	1.67 ± 0.20	1.48 ± 0.14	1.51 ± 0.16	1.76 ± 0.42	1.83 ± 0.23
	90	2.00 ± 0.24	1.56 ± 0.15	1.78 ± 0.23	1.52 ± 0.13	1.81 ± 0.50	2.04 ± 0.24
Insulin (pM)	0	110 ± 8.3	98 ± 12	101 ± 7.8	114 ± 7.1	103 ± 7.8	106 ± 9.2
	90	105 ± 10	107 ± 9.2	112 ± 8.2	108 ± 7.4	110 ± 7.9	112 ± 8.3
Growth hormone (ng/ml)	0	1.06 ± 0.26	1.14 ± 0.31	1.25 ± 0.35	2.48 ± 0.33	2.75 ± 0.42	2.60 ± 0.48
	90	1.18 ± 0.30	1.27 ± 0.29	1.31 ± 0.32	2.52 ± 0.36	2.80 ± 0.40	2.72 ± 0.46
Total protein (g/100 ml)	0	7.17 ± 0.18	7.31 ± 0.08	7.27 ± 0.07	7.17 ± 0.10	7.19 ± 0.11	7.08 ± 0.11
	90	7.25 ± 0.20	7.28 ± 0.10	7.24 ± 0.16	7.23 ± 0.07	7.05 ± 0.14	7.18 ± 0.11
Albumin (g/100 ml)	0	4.35 ± 0.12	4.69 ± 0.09	4.72 ± 0.07	4.22 ± 0.08	4.35 ± 0.11	4.21 ± 0.05
	90	4.46 ± 0.10	4.66 ± 0.08	4.69 ± 0.09	4.26 ± 0.08	4.25 ± 0.09	4.33 ± 0.06*
AST (nmol/ml per min)	0	18.1 ± 1.6	16.9 ± 1.5	16.6 ± 1.2	19.3 ± 1.1	19.4 ± 1.2	19.5 ± 0.9
	90	16.3 ± 1.5	15.2 ± 1.4*	15.4 ± 1.2*	17.8 ± 1.0	17.4 ± 1.1*	17.4 ± 1.0*
ALT (nmol/ml per min)	0	17.9 ± 1.8	17.6 ± 1.6	16.0 ± 1.3	18.7 ± 1.0	20.5 ± 1.2	19.3 ± 1.2
	90	17.6 ± 1.8	16.8 ± 1.7*	15.5 ± 1.2*	18.2 ± 0.9	19.4 ± 1.2*	18.4 ± 1.1*
Total bilirubin (mg/100 ml)	0	0.74 ± 0.10	0.63 ± 0.08	0.63 ± 0.07	0.47 ± 0.03	0.57 ± 0.06	0.44 ± 0.05
	90	0.63 ± 0.07	0.69 ± 0.09	0.60 ± 0.04	0.44 ± 0.04	0.48 ± 0.04	0.43 ± 0.03
Na^+ (mM)	0	138.7 ± 0.5	139.5 ± 0.5	139.6 ± 0.7	139.5 ± 0.4	138.2 ± 0.4	138.9 ± 0.5
	90	138.5 ± 0.4	139.1 ± 0.8	138.1 ± 0.7*	139.1 ± 0.7	138.4 ± 0.5	138.4 ± 0.6
K^+ (mM)	0	4.26 ± 0.02	4.40 ± 0.07	4.33 ± 0.10	4.25 ± 0.09	4.26 ± 0.05	4.21 ± 0.07
	90	4.27 ± 0.04	4.34 ± 0.07	4.18 ± 0.09	4.26 ± 0.11	4.21 ± 0.05	4.19 ± 0.08
Cl^- (mM)	0	103.9 ± 0.9	104.2 ± 0.4	104.1 ± 0.8	105.9 ± 0.4	105.8 ± 0.4	105.7 ± 0.6
	90	104.3 ± 0.6	105.8 ± 0.5*	106.7 ± 0.8*	105.6 ± 0.6	106.3 ± 0.5	106.7 ± 0.8
HCO_3^- (mM)	0	25.2 ± 0.5	25.8 ± 0.6	25.4 ± 0.6	24.5 ± 0.4	23.8 ± 0.7	23.7 ± 0.5
	90	24.3 ± 0.6*	22.9 ± 1.0*	22.1 ± 0.9*	23.6 ± 0.7	22.8 ± 0.7	22.3 ± 0.7

Values are mean ± SEM, with the number (n) of subjects given in the parentheses

TSH thyroid stimulating hormone, *ALT* alanine transaminase, *AST* aspartate transaminase

*$P < 0.05$ vs the value for day 0 (the beginning of the trial)

concentrations of glucose in serum did not differ ($P > 0.05$) among male subjects taking 0, 15 and 30 g Arg/day for 90 days, dietary supplementation with 15 or 30 g Arg/day reduced ($P < 0.05$) the concentration of glucose in the serum of female subjects. In both male and female subjects taking 30 g Arg/day, serum concentrations of free fatty acids were reduced ($P < 0.05$) by 6% and 9%, respectively, at Day 90, as compared with Day 0. Oral administration of 15 g Arg/day did not affect ($P > 0.05$) serum concentrations of free fatty acids in male or female subjects.

Urine volume and urinary excretion of metabolites

Dietary supplementation with 15 and 30 g Arg/day for 90 days did not affect ($P > 0.05$) the volume of urine voided by male and female subjects or their urinary excretion of Arg, glutamine, glycine, histidine and methylarginines (Table 6). In both male and female subjects taking 30 g Arg/day, daily urinary excretion of urea, ammonia, ornithine, lysine and creatinine was greater at Day 90 than that at Day 0 (Table 6). These variables did not differ ($P > 0.05$) between Days 0 and 90 for subjects taking 0 or 15 g Arg/day. In both male and female subjects supplemented with 15 and 30 g Arg/day, urinary excretion of homoarginine was increased ($P < 0.05$) at Day 90, in comparison with Day 0. However, urinary excretion of other amino acids in male and female subjects was not affected ($P > 0.05$) by dietary supplementation with 15 or 30 g Arg/day (Supplemental Table 2).

Discussion

Mean Arg intake by the US adult population is ~ 5 g/day (Flynn et al. 2002). Approximately 40% of Arg in the diet is catabolized in the first pass by the small intestine, and the remaining 60% (3 g Arg/day) enters the portal circulation

Table 4 Concentrations of amino acids and nitrogenous metabolites in the serum of the human subjects who completed the study

Variable	Day of study	Male participants			Female participants		
		0 g Arg/day ($n=13$)	15 g Arg/day ($n=12$)	30 g Arg/day ($n=11$)	0 g Arg/day ($n=13$)	15 g Arg/day ($n=13$)	30 g Arg/day ($n=12$)
Urea nitrogen (mM)	0	5.93 ± 0.23	5.33 ± 0.22	5.81 ± 0.24	5.41 ± 0.18	5.24 ± 0.14	5.56 ± 0.17
	90	5.84 ± 0.26	5.81 ± 0.23	5.88 ± 0.26	5.26 ± 0.14	5.15 ± 0.17	5.84 ± 0.24
Ammonia (μM)	0	22.2 ± 1.3	25.2 ± 3.2	22.7 ± 1.6	23.6 ± 1.0	23.8 ± 1.4	22.7 ± 1.0
	90	22.8 ± 1.5	25.5 ± 2.1	25.3 ± 2.0	24.2 ± 1.4	24.4 ± 1.6	26.0 ± 2.6
Creatinine (μM)	0	68.2 ± 1.8	67.5 ± 1.7	68.8 ± 2.2	64.5 ± 2.0	65.2 ± 1.6	63.1 ± 2.3
	90	67.0 ± 2.0	68.1 ± 1.5	69.3 ± 2.1	65.3 ± 2.1	65.9 ± 1.7	64.6 ± 2.5
Arginine (μM)	0	103 ± 4.5	100 ± 5.7	104 ± 4.6	90 ± 8.0	93 ± 4.7	89 ± 4.7
	90	104 ± 7.2	123 ± 8.0†	153 ± 15†	82 ± 6.1	114 ± 7.8*	138 ± 16†
Glutamine (μM)	0	634 ± 33	650 ± 55	642 ± 37	580 ± 22	556 ± 33	542 ± 28
	90	635 ± 45	618 ± 52	579 ± 36*	578 ± 25	549 ± 43	503 ± 24*
Glycine (μM)	0	212 ± 13	204 ± 11	221 ± 12	226 ± 28	253 ± 29	227 ± 28
	90	204 ± 15	193 ± 10	196 ± 14*	217 ± 30	243 ± 28	200 ± 22*
Histidine (μM)	0	77.8 ± 3.0	74.8 ± 2.6	85.2 ± 5.0	70.7 ± 2.4	75.0 ± 2.8	78.9 ± 2.3
	90	77.1 ± 4.2	78.1 ± 3.6	83.3 ± 3.1	71.3 ± 2.7	71.8 ± 2.9	72.7 ± 3.7
Homoarginine (μM)	0	2.40 ± 0.12	2.45 ± 0.14	2.37 ± 0.11	2.32 ± 0.13	2.38 ± 0.15	2.26 ± 0.12
	90	2.31 ± 0.15	2.80 ± 0.16*	2.95 ± 0.13*	2.39 ± 0.14	2.71 ± 0.17*	2.88 ± 0.15*
Lysine (μM)	0	199 ± 17	198 ± 13	188 ± 7.6	180 ± 11	185 ± 12	175 ± 5.5
	90	206 ± 16	201 ± 12	178 ± 8.5	164 ± 7.2	177 ± 15	163 ± 11
Ornithine (μM)	0	69 ± 7.1	63 ± 4.5	53 ± 2.5	58 ± 6.0	58 ± 6.6	54 ± 4.5
	90	71 ± 8.3	82 ± 4.8†	93 ± 12†	52 ± 3.8	68 ± 6.0*	84 ± 10†
Proline (μM)	0	246 ± 11	232 ± 9.5	235 ± 10	230 ± 14	221 ± 16	218 ± 13
	90	238 ± 13	251 ± 12	277 ± 12*	225 ± 16	237 ± 19	253 ± 15*
ADMA (μM)	0	1.28 ± 0.09	1.22 ± 0.11	1.36 ± 0.12	0.95 ± 0.07	0.98 ± 0.06	0.96 ± 0.07
	90	1.30 ± 0.10	1.35 ± 0.12	1.32 ± 0.13	1.01 ± 0.08	0.96 ± 0.07	0.95 ± 0.08
NMMA (μM)	0	0.24 ± 0.02	0.22 ± 0.02	0.23 ± 0.03	0.22 ± 0.02	0.24 ± 0.03	0.22 ± 0.03
	90	0.25 ± 0.03	0.24 ± 0.03	0.22 ± 0.02	0.21 ± 0.02	0.23 ± 0.03	0.21 ± 0.02
SDMA (μM)	0	0.41 ± 0.05	0.38 ± 0.04	0.43 ± 0.04	0.39 ± 0.04	0.38 ± 0.03	0.41 ± 0.04
	90	0.42 ± 0.04	0.40 ± 0.05	0.41 ± 0.04	0.40 ± 0.03	0.37 ± 0.04	0.39 ± 0.04

Values are mean ± SEM, with the number (n) of subjects given in the parentheses

ADMA asymmetric dimethylarginine, *NMMA* NG-mono-methyl-arginine, *SDMA* symmetric dimethylarginine

*$P < 0.05$ vs the value for day 0 (the beginning of the trial)

†$P < 0.01$ vs the value for day 0 (the beginning of the trial)

(Castillo et al. 1993). For comparison, endogenous synthesis of Arg from dietary glutamine, glutamate, and proline, as well as arterial glutamine provides 1.5 g Arg/day to a 70-kg healthy subject (Wu and Morris 1998). Among this total amount of 4.5 g Arg from exogenous and endogenous sources, 2.3 g (~50%) is utilized to synthesize creatine via inter-organ cooperation (the kidneys and liver) (Brosnan and Brosnan 2007). The remaining amount of Arg (2.2 g/day) is utilized to synthesize protein, ornithine, nitric oxide, polyamines, creatine, and homoarginine. In addition, a very small amount of the protein-bound Arg is methylated to form methylarginines in proteins, which, upon degradation, release ADMA, NMMA and SDMA (Tsikas et al. 2018; Haghikia et al. 2017; Kayacelebi et al. 2017). In adult humans ingesting 15 or 30 g Arg/day, most of this AA was actively catabolized to its various products (Tables 4, 6). In those subjects, based on urinary excretion of metabolites (Table 6), the supplemental Arg was metabolized primarily via arginase (~80%; the formation of urea, ornithine and its derivatives, and CO_2), and to a lesser extent, via Arg:glycine amidotransferase (~19%; the production of creatine) and other pathways (~1%; including the syntheses of NO, homoarginine, and methylarginines), as we reported for pigs and rats (Wu et al. 2016). Thanks to the recognition that enhancing the circulating levels of Arg can activate key signaling pathways that are beneficial to white-fat reduction and health (McKnight et al. 2010), Arg has now become a potentially attractive ingredient in dietary

Table 5 Concentrations of glucose and lipids in the serum of the human subjects who completed the study

Variable	Day of study	Male participants			Female participants		
		0 g Arg/day ($n=13$)	15 g Arg/day ($n=12$)	30 g Arg/day ($n=11$)	0 g Arg/day ($n=13$)	15 g Arg/day ($n=13$)	30 g Arg/day ($n=12$)
Glucose (mM)	0	5.68 ± 0.16	5.41 ± 0.19	5.72 ± 0.19	5.41 ± 0.17	5.29 ± 0.14	5.56 ± 0.15
	90	5.57 ± 0.15	5.38 ± 0.17	5.59 ± 0.20	5.36 ± 0.15	5.01 ± 0.13*	5.30 ± 0.14*
Total cholesterol (mg/100 ml)	0	198 ± 9.3	188 ± 8.2	190 ± 13	181 ± 8.3	179 ± 8.6	174 ± 7.5
	90	198 ± 9.7	202 ± 12	196 ± 10	191 ± 6.5	175 ± 8.6	171 ± 7.4
HDL cholesterol (mg/100 ml)	0	48.0 ± 14.2	42.3 ± 2.9	48.0 ± 2.1	56.2 ± 5.3	57.0 ± 2.4	53.3 ± 3.6
	90	49.5 ± 4.0	41.2 ± 2.7	48.7 ± 2.5	57.2 ± 4.6	55.4 ± 3.6	55.2 ± 3.7
LDL cholesterol (mg/100 ml)	0	126 ± 8.8	115 ± 7.9	117 ± 11	104 ± 7.5	102 ± 7.7	97 ± 6.1
	90	126 ± 5.6	127 ± 9.9	122 ± 8.5	111 ± 5.6	99 ± 6.7	97 ± 6.6
Triglycerides (mg/100 ml)	0	119 ± 18	153 ± 18	124 ± 23	104 ± 11	99 ± 12	116 ± 16
	90	111 ± 13	170 ± 23	123 ± 21	115 ± 13	103 ± 11	94 ± 10
Free fatty acids (μM)	0	448 ± 46	431 ± 50	443 ± 42	502 ± 48	494 ± 53	507 ± 51
	90	451 ± 49	425 ± 44	417 ± 37*	506 ± 49	473 ± 51	462 ± 47*

Values are mean ± SEM, with the number (n) of subjects given in the parentheses

*$P < 0.05$ vs the value for day 0 (the beginning of the trial)

supplements, functional foods, and beverages. Notably, there are reports that, in human adults, increasing Arg 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 in males and females (Wu et al. 2009). These improvements of health and well-being are expected to reduce the risk for chronic disease, including cardiovascular disease (the number one killer in developed countries) and cancer, further underscoring the importance of Arg supplementation in global human health.

After Arg enters cells in the body, this amino acid or its metabolites fulfill multiple regulatory and signaling functions besides serving as a substrate for synthetic pathways (Hou et al. 2015a; Li et al. 2016). For example, as a major vasodilator, NO relaxes the vascular smooth muscle cells and facilitates the flow of blood into peripheral tissues. In support of this view, oral administration of 30 g Arg/day reduced systolic blood pressure in female subjects (Table 1). In addition, Arg is an allosteric activator of *N*-acetylglutamate synthase. 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, Arg is required for maintaining hepatic urea synthesis in an active state. In addition, physiological levels of Arg may enhance the secretion of growth hormone and insulin in mammals, thereby playing an important role in regulating protein metabolism (Blachier et al. 1989; Flynn et al. 2002). Furthermore, through stimulating the expression of peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α, a master activator of mitochondrial biogenesis) and the phosphorylation of 5′-adenosine monophosphate-activated protein kinase (AMPK), Arg increases the oxidation of fatty acids in insulin-sensitive tissues, thereby reducing the accretion of fat in white adipose tissue (Fu et al. 2005; McKnight et al. 2010). This explains our observations that oral administration of 30 g Arg/day reduced the serum concentrations of free fatty acids in both males and females (Table 5). Likewise, as reported for healthy adult rats (Jobgen et al. 2009a; Hu et al. 2015; Wu et al. 2016), oral administration of 15 or 30 g Arg/day beneficially reduced the concentrations of serum glucose (an indicator of improved metabolic control), while increasing the concentrations of serum albumin (an indicator of improved hepatic function) in female subjects (Table 3). Finally, Arg activates the mammalian target of rapamycin (mTOR) cell signaling pathway, and therefore, protein synthesis in multiple tissues, including skeletal muscle (Yao et al. 2008), small intestine (Tan et al. 2010), brown adipose tissue (Ma et al. 2017), and other tissues (Kong et al. 2012). Thus, Arg can spare muscle protein in subjects consuming a weight-reducing diet (Lucotti et al. 2006) and maintain lean masses in overweight or obese subjects (Table 1).

The safe upper limit of long-term oral administration of Arg to healthy adults with a normal BMI and to overweight or obese but otherwise healthy subjects is unknown. The lack of this knowledge has limited the global use of Arg as a functional amino acid to improve human health and well being. Additionally, as noted by Shao and Hathcock (2008), the absence of a safe upper limit for Arg supplementation

Table 6 Urine volumes and urinary excretion of metabolites in the human subjects who completed the study

Variable	Day of study	Male participants			Female participants		
		0 g Arg/day ($n=13$)	15 g Arg/day ($n=12$)	30 g Arg/day ($n=11$)	0 g Arg/day ($n=13$)	15 g Arg/day ($n=13$)	30 g Arg/day (n = 12)
Urine volume (L/day)	0	1.87 ± 0.22	1.80 ± 0.25	1.91 ± 0.21	1.55 ± 0.17	1.43 ± 0.16	1.52 ± 0.14
	90	1.93 ± 0.28	1.84 ± 0.31	1.94 ± 0.25	1.61 ± 0.23	1.47 ± 0.20	1.54 ± 0.16
Urinary excretion of metabolites							
Urea (mmol/day)	0	470 ± 59	452 ± 50	428 ± 43	311 ± 30	293 ± 31	283 ± 29
	90	462 ± 62	478 ± 66	492 ± 56*	281 ± 35	316 ± 24	337 ± 32*
Ammonia (mmol/day)	0	14.3 ± 2.3	15.2 ± 2.7	14.8 ± 2.8	8.6 ± 1.0	8.4 ± 1.0	8.26 ± 1.4
	90	13.9 ± 1.6	16.1 ± 3.1	20.5 ± 3.4*	8.0 ± 0.6	8.8 ± 0.7	12.0 ± 1.6*
Arginine (μmol/day)	0	184 ± 25	152 ± 20	137 ± 21	99 ± 15	96 ± 13	93 ± 13
	90	182 ± 34	188 ± 34	172 ± 26	113 ± 14	106 ± 19	110 ± 24
Creatinine (mmol/day)	0	12.7 ± 1.8	13.0 ± 1.6	13.3 ± 1.7	9.24 ± 1.0	9.37 ± 1.3	9.10 ± 1.2
	90	12.4 ± 1.9	14.2 ± 1.5	16.8 ± 1.8*	9.36 ± 1.3	10.2 ± 1.5	11.8 ± 1.6*
Glutamine (μmol/day)	0	636 ± 82	524 ± 67	618 ± 74	484 ± 82	533 ± 62	506 ± 60
	90	643 ± 102	522 ± 87	576 ± 90	550 ± 110	508 ± 67	491 ± 66
Glycine (μmol/day)	0	1485 ± 304	1247 ± 228	1383 ± 253	1360 ± 283	1409 ± 272	1421 ± 232
	90	1399 ± 261	1106 ± 153	1204 ± 192	1275 ± 260	1328 ± 284	1213 ± 266
Histidine (μmol/day)	0	1261 ± 164	1089 ± 162	1492 ± 325	836 ± 145	935 ± 163	769 ± 121
	90	1152 ± 204	1178 ± 187	1527 ± 246	919 ± 206	720 ± 120	942 ± 156
Homoarginine (μmol/day)	0	2.12 ± 0.30	2.07 ± 0.26	2.16 ± 0.24	2.16 ± 0.25	2.12 ± 0.27	2.09 ± 0.24
	90	2.04 ± 0.32	2.35 ± 0.29*	2.68 ± 0.30*	2.10 ± 0.27	2.47 ± 0.28*	2.60 ± 0.27*
Lysine (μmol/day)	0	290 ± 47	161 ± 34	208 ± 68	273 ± 53	407 ± 144	212 ± 61
	90	256 ± 61	197 ± 39	266 ± 72*	284 ± 63	370 ± 128	250 ± 62*
Ornithine (μmol/day)	0	88 ± 32	85 ± 31	120 ± 38	54 ± 15	62 ± 16	68 ± 20
	90	63 ± 17	107 ± 40	165 ± 46*	47 ± 13	61 ± 23	108 ± 27*
Proline (μmol/day)	0	213 ± 28	198 ± 25	176 ± 20	164 ± 18	160 ± 22	157 ± 20
	90	205 ± 31	210 ± 29	193 ± 24	155 ± 17	171 ± 24	179 ± 21
ADMA (μmol/day)	0	28.6 ± 3.4	27.0 ± 3.8	27.9 ± 2.7	18.1 ± 2.6	17.8 ± 2.4	18.4 ± 2.2
	90	30.5 ± 3.7	29.9 ± 3.5	30.2 ± 3.3	17.7 ± 2.3	18.3 ± 2.6	17.1 ± 2.5
NMMA (μmol/day)	0	20.0 ± 2.5	18.7 ± 2.3	19.5 ± 2.2	14.9 ± 2.0	15.5 ± 2.2	14.3 ± 1.7
	90	21.6 ± 2.8	20.1 ± 2.6	20.3 ± 2.4	14.3 ± 2.3	15.0 ± 2.5	14.8 ± 2.1
SDMA (μmol/day)	0	27.2 ± 3.3	25.8 ± 3.5	26.4 ± 3.0	19.0 ± 2.7	20.2 ± 2.8	19.7 ± 2.5
	90	28.1 ± 3.4	26.3 ± 3.2	27.6 ± 3.2	18.4 ± 2.5	19.1 ± 3.0	18.8 ± 2.7

Values are mean ± SEM, with the number (n) of subjects given in the parentheses

ADMA asymmetric dimethylarginine, *NMMA* NG-mono-methyl-arginine, *SDMA* symmetric dimethylarginine

*$P < 0.05$ vs the value for day 0 (the beginning of the trial)

has frequently been interpreted erroneously to indicate insufficient evidence for its safety in humans. This may lead to the unwarranted issuing of warnings by regulatory agencies against dietary supplementation with Arg or Arg-containing products (e.g., Health Canada 2006). The present clinical study was conducted to address this significant issue in amino acid nutrition and supplementation. We determined many clinical and biochemical variables for safety monitoring and possible efficacy in improving health, with the rationale being explained in Table 7. The variables included cardiovascular function (systolic and diastolic blood pressures), renal function (urinary excretion of creatinine, urea and ammonia), hepatic function (concentrations of ammonia, urea, albumin, total protein, bilirubin, AST, and ALT in serum), endocrine status (concentrations of glucose, insulin, thyroid stimulating hormone, and growth hormone in serum), and metabolic parameters [e.g., total cholesterol, HDL, triglyceride, and LDL-cholesterol, as well as ADMA, NMMA and SDMA (risk factors for cardiovascular disease (Tsikas et al. 2018; Haghikia et al. 2017; Kayacelebi et al. 2017)]. None of the variables were adversely affected by dietary supplementation with 15 and 30 g Arg/day for 90 days. The numeric rise in the systolic blood pressure of all groups of male subjects (including the control group), which was

Table 7 Rationale for major clinical and biochemical measurements in human subjects

Parameter	Rationale
1. Clinical examinations	
Body mass index	An indicator of degree of adiposity and skeletal muscle mass
Blood pressure	An indicator of hypertension as well as nitric oxide bioavailability
General physical exam	To ensure that study subjects remain in generally good health
Complete review of medical records	To assess for changes in health and/or side effects due to arginine supplementation
Gastrointestinal discomfort	An indicator of gastrointestinal dysfunction due to an abrupt increase in local NO production; a side effect of high doses of oral arginine
2. Hormones, enzymes, and metabolites in serum	
GH, insulin and TSH	An indicator of effects of arginine on the endocrine status
Lipids	Indicators of fat metabolism; risk factors for cardiovascular disease
Glucose	An indicator of insulin resistance and normality of metabolism
Electrolytes	Indicators of dietary intake of minerals and systemic function
Albumin and total protein	Indicators of hepatic protein synthesis and protein nutrition status
Total bilirubin	An indicator of catabolism of heme (a component of hemoglobin) in the liver, and therefore, liver function
Ala transaminase and Asp transaminase	Abundant enzymes in liver that are released from hepatocytes into blood in response to cell injury; indicators of liver integrity
3. Metabolites in both serum and urine	
Arginine	Verification of the efficacy of dietary arginine supplementation in increasing its circulating levels
Ornithine	An indicator of arginine degradation and dietary intake of arginine
Lysine	An indicator of balance among basic amino acids in the circulation (lysine shares the same transporters with arginine)
Other amino acids	An indicator of the overall metabolism of amino acids
Ammonia	An indicator of the urea cycle activity that depends on arginine; a risk factor for the toxicity of high doses of amino acid intake
Urea	The major product of protein and amino acid catabolism; an indicator of dietary intake of protein and amino acids
Creatinine	An indicator of renal function in clinical medicine

GH growth hormone

not statistically significant, may result from natural metabolic, physiological and hormonal changes in overweight or obese subjects over a 3-month period (Mertens and Van Gaal 2000; Zheng et al. 2016). Increases in the concentrations of homoarginine in the serum and urine of both males and females receiving Arg supplementation (Tables 4, 6), as reported for healthy pigs (Hou et al. 2016b) and for patients with peripheral arterial occlusive disease or coronary artery disease (Schneider et al. 2015), should not be viewed as an undesirable outcome. Rather, homoarginine, which is synthesized from Arg and lysine (Tsikas and Wu 2015), can ameliorate metabolic syndrome in diet-induced obese mice (Stockebrand et al. 2015). Elevated concentrations of homoarginine in serum are associated with improved neurological function in humans (Bernstein et al. 2015) and rats (Sase et al. 2016), as well as renal and cardiovascular functions (Papageorgiou et al. 2015; Pilz et al. 2015). The reductions in the serum concentrations of bicarbonate (likely due to decreased renal reabsorption), glutamine and glycine (likely due to decreased endogenous synthesis) in both males and females and of sodium and chloride (likely due to increased renal excretion) in males after the oral administration of 30 g Arg/day for 90 days (Tables 3, 4) were within the physiological ranges of these metabolites. Likewise, increases in the urinary excretion of lysine and ornithine in males and females ingesting 30 g Arg/day (Table 6) can be explained by decreases in the renal reabsorption of these two amino acids, which share the same transporters with Arg for reentering into blood from the lumen of the kidney tubules (Closs et al. 2004; Liao et al. 2018). However, these changes were only moderate. Thus, no abnormal metabolic patterns were observed in Arg-supplemented adult humans.

Our findings from the present long-term study are consistent with the previous reports regarding the high tolerance of humans to Arg supplementation (see McNeal et al. 2016 for review). For example, oral administration of Arg (20 g/day for 4 week) to healthy adults has been reported to be safe (Chin-Dusting et al. 1996). There is also evidence that

daily intake of 40 g Arg/day for 6 days has no detectable adverse effects on healthy adults (Beaumier et al. 1995). Furthermore, there are reports that patients with cystic fibrosis can tolerate 42 g Arg/day for 6 weeks (Grasemann et al. 2005). However, due to individual differences, some subjects may experience intolerable GI discomfort after receiving a high dose of Arg (> 15 g/day), such as nausea, GI upset, and diarrhea (Evans et al. 2004; Grimble 2007; Tanghao et al. 1999). Oral intake of Arg at least twice daily could prevent an abrupt increase in NO synthesis by the GI tract and discomfort of the digestive system (Wu and Meininger 2000). Thus, with divided doses, no risk appears to be associated with supplementing 15–30 g Arg/day to adult men and women for 3 months.

Dietary intake of protein can affect the ability of subjects to tolerate supplemental amino acids (Wu 2013). Dietary supplementation with 15 or 30 g Arg/day did not affect intakes of nutrients (including lipids, protein, carbohydrates, vitamins and minerals) by adult males or females (Table 2). On the basis of results from human clinical studies and Arg pharmacokinetics showing a half-life of ~ 1 h for circulating Arg (Boger and Bode-Boger 2001; Tanghao et al. 1999), dietary Arg supplementation at 15 and 30 g/day (equally divided into at least two administrations per day) via drinking water increased serum concentrations of Arg and ornithine in post-absorptive subjects (Table 4). Of note, serum concentrations of lysine and histidine were not affected by dietary supplementation with Arg in males or females (Table 4), indicating the lack of antagonism among basic amino acids. Similar results have been reported for rats and pigs (Wu et al. 2016). For example, healthy adult pigs and rats can tolerate large amounts of supplemental Arg (at least 0.21–0.42 and 2.1–5.7 g/kg BW per day, respectively) (Wu et al. 2007a, b). In addition, Tsubuku et al. (2004) reported that adult rats can tolerate at least 3.6 g/kg BW per day. Most recently, we found that animals fed conventional diets can tolerate large amounts of supplemental Arg (up to 630 mg Arg/kg BW per day in pigs or 3.6 g Arg/kg BW per day in rats) for 91 d (Hou et al. 2016b; Yang et al. 2015), which are equivalent to 573 mg Arg/kg BW per day for humans (Wu et al. 2016). 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 BW per day (or 15–40 g/kg BW per day for a 70-kg subject) (Wu et al. 2007a, b, 2016). The data from animal studies are helpful in predicting a safe upper limit for oral administration of Arg to healthy adults. Results of our human clinical studies provide further high-level confidence in an OSL value of at least 30 g/day for Arg supplementation to adult men and women.

In summary, dietary supplementation with 15 and 30 g Arg/day for 90 days did not affect the intake of energy, protein, carbohydrates, vitamins or minerals by overweight or obese but otherwise healthy adults. Based on many clinical and biochemical variables for safety monitoring (including cardiovascular function, renal function, hepatic function, endocrine status, and serum concentrations of amino acids, glucose, fatty acids, and their metabolites), we conclude that adult men and women can tolerate oral administration of at least 30 g Arg/day. Because Arg is now recognized to have physiological roles in activating key signaling pathways that are beneficial for health, data on the safety of oral administration of Arg to adults are expected to promote the global use of Arg as a beneficial ingredient in dietary supplements, functional foods, and beverages to improve human health. Additionally, our findings will provide much-needed scientific basis for regulatory agencies and policymakers to make sound decisions regarding the use of cost-effective Arg supplements or Arg-containing products by men and women.

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Compliance with ethical standards

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

Ethical statement This study was approved by Baylor Scott & White Hospital and Texas A&M University Institutional Review Boards.

Informed consent Informed consent was obtained from all study subjects.

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