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Biochemical responses of healthy subjects during dietary supplementation with L-arginine

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Abstract

Dietary supplements of L-arginine, a substrate for nitric oxide synthases, may promote formation of nitric oxide and thus may be of clinical benefit. However, the optimal level of L-arginine supplementation is unclear. The objective of this study was to evaluate the response of healthy individuals to increasing doses of L-arginine (as free acid). Twelve healthy subjects were recruited and instructed to take L-arginine for 1-week periods at daily doses of 3, 9, 21, and 30 g. At baseline and at the end of each week, 24-hour urine and fasting blood samples were collected, and weight, diastolic blood pressure, and systolic blood pressure were recorded. Samples were analyzed for L-arginine, L-citrulline, glycine, L-lysine, L-ornithine, asymmetric dimethy L-arginine, symmetric dimethy L-arginine, glucose, insulin (serum), creatinine, cGMP (urine), and total nitrates (serum and urine). Ten subjects reported adverse side effects at initial L-arginine doses of 21 g/day (five subjects) or 30 g/day (five subjects), respectively. Blood pressure and weight did not change during the supplementation period. Of the individual biochemical measures, only L-arginine, glycine, and L-ornithine concentrations changed significantly. The mean concentration of L-arginine reached a peak during supplementation at 9 g/d; however, individuals differed markedly in their response. Availability of L-arginine, relative to that of asymmetric dimethy L-arginine, increased significantly at both 9 g/day and 21 g/day. Mean values indicate that supplementation with 9 g/day of L-arginine, a dose associated with minimal adverse side effects, is sufficient to increase circulating L-arginine concentrations. However, subjects varied widely in their responses, indicating that L-arginine supplementation needs to be tailored to individuals.

Keywords: L-arginine; Asymmetric dimethy L-arginine; Symmetric dimethy L-arginine; Total nitrates; Supplements; Human

1. Introduction

Nitric oxide synthases (NOS) convert L-arginine and oxygen into nitric oxide (NO) and L-citrulline. NO has a broad range of autocrine and paracrine effects that have an impact on atherosclerosis. NO promotes vasodilation, regulates platelet activation, inhibits smooth muscle cell proliferation, reduces leukocyte and monocyte adhesion, and modulates the expression of redox-regulated genes. In high concentrations NO is cytotoxic [1–3].

L-arginine is widely distributed in dietary protein and the average person in the western world consumes about 5 g of L-arginine per day [4]. L-arginine is continually synthesized in the liver within the urea cycle, but very little of this L-arginine is available for extrahepatic tissues [5,6]. In adults, enterocytes synthesize L-citrulline from L-glutamate, L-glutamine (both enteral and arterial), and L-proline (enteral), and this L-citrulline is removed by other tissues, predominantly the kidney, for the synthesis of L-arginine [5,7]. Nevertheless, L-arginine is generally considered a conditionally dispensable amino acid: it may become essential under certain circumstances including during periods of rapid growth, during pregnancy, and after injury [2,4,8,9].

Many studies involving animals and humans have shown that exogenous L-arginine, either infused or as a dietary

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Table 1
Subject characteristics

Subject	Sex	Weight (kg)*	SBP (mm Hg)*	DBP (mm Hg)*	L-arginine (μmol/L)*	ADMA (nmol/L)*	SDMA (nmol/L)*	Side Effects†
1	F‡	105	132	74	104	1053	511	30g
2	M§	73	104	60	87	1020	588	21g
3	F	55	70	50	120	1005	797	30g
4	F	73	100	62	119	1301	684	21g
5	F	66	130	80	93	803	445	9g
6	F	60	108	74	108	757	496	30g
7	M‡	112	128	90	92	679	492	30g
8	M‡	110	122	92	98	675	471	30g
9	M	99	110	62	125	1043	460	—
10	M‡	168	130	88	98	1001	374	21g
11	F‡	85	114	62	80	965	600	21g
12	F§	70	118	74	89	759	532	21g

* Baseline characteristics.
† Daily dose of L-arginine at which side effects were initially noted.
‡ Subject took only some of the 10 g L-arginine doses
§ Subject did not take any of the 10 g L-arginine doses
Abbreviations as in text.

supplement, can promote NO formation and elicit physiological changes. It is not clear whether these effects can be sustained or will be clinically beneficial [10]. The results were unexpected as in vitro the $K_m$of the NOS enzymes for L-arginine (2-20 μmol/L) [11] is considerably less than intracellular levels of L-arginine [12] . The apparent discrepancy between observation and expectation for the effects of extracellular L-arginine on endothelial NO synthesis is known as the "arginine paradox" [12,13]. One explanation for the apparent discrepancy may involve effects of the naturally occurring analogs of L-arginine: asymmetric dimethyl L-arginine (ADMA) and N-monomethyl L-arginine (NMA), which are potent endogenous competitive inhibitors of NOS [14].

In this feeding study, L-arginine (free acid) was provided at increasing levels to 12 healthy subjects over 4 weeks and the serum concentrations of L-arginine, L-citrulline, glycine, L-lysine, L-ornithine, ADMA, symmetric dimethy L-arginine (SDMA), glucose, and insulin were measured. In addition, levels of the NO mediator, cGMP, and of the oxidative products of NO (nitrites and nitrates) were analyzed. The subjects were monitored for side effects. It was anticipated that the results would indicate the appropriate amount of L-arginine to give to subjects.

2. Methods and materials

This study was approved by the Institutional Review Board of the University of Pittsburgh, and informed consent was obtained from each subject. Twelve healthy volunteers (seven women, five men) from the University of Pittsburgh were recruited. Their baseline characteristics are shown in Table 1. After the baseline visit they were instructed to continue their regular diet and to take L-arginine for 1-week periods at daily doses of 1 g (×3), 3 g (×3), 7 g (×3), and 10 g (×3). The L-arginine (free acid) was obtained from Ajinomoto USA, Inc., (Raleigh, NC) and was provided in preweighed packets. The L-arginine was taken swirled in juice at breakfast, lunch, and dinner. At the end of each week, the subjects collected 24-hour urines into bottles containing acetic acid and came to the laboratory for the collection of blood (serum) after a minimum 8-hour fast. Serum was separated by centrifugation within 60 minutes. Urine volume, blood pressure (after sitting for 5 minutes), and the weight of the subjects were recorded. Urine and serum samples were stored at −70°C.

L-arginine, L-citrulline, glycine, L-lysine, and L-ornithine were analyzed using a Beckman 6300 amino acid analyzer using in-line, post-column derivatization with o-phthalaldehyde and fluorometric detection. Aminoethylcysteine was added to the samples as an internal standard; samples were precipitated with sulfosalicyclic acid (50 mg/mL final concentration), centrifuged (5000 × g), and filtered through Rainin 0.22-μm Microfilterfuge tubes (Rainin Instruments, Woburn MA) before injection.

ADMA and SDMA were assayed after solid phase extraction, using a slight modification of the extraction method described by Pettersson et al. [15]. Small solid-phase extraction (SPE) columns (IST isolute SCX columns, 3 mL reservoirs, IST C/N 530-0010-B; Argonaut Technologies, Foster City, CA) were prepared by the sequential addition of methanol (1 mL) and 2% trichloroacetic acid (TCA; 2 mL). Aliquots of plasma (0.5 mL), diluted with 1 mL of deionized water, were then poured over the columns. The columns were then washed sequentially with 2% TCA (1 mL), 0.15 mol/L sodium phosphate (3 mL), pH 8.0, and methanol (1 mL), and the dimethyl arginines were then eluted with a solution of methanol: water: triethylamine (2 mL), prepared daily in the following proportions: 6.8 mL methanol, 3 mL deionized water, 0.2 mL triethylamine (Sigma Chemical Co., St Louis, MO). The eluates were then

dried under a stream of nitrogen at 60°C, reconstituted in 100 μL of starting buffer, and 50 μL injected into a Beckman 6300 Amino Acid Analyzer with post-column, o-phthaladehyde derivatization and fluorometric detection. Using the standard lithium buffer method for amino acids, ADMA and SDMA eluted just after histidine and well before L-arginine, with retention times of about 132 and 136 minutes, respectively (flow rate was 16 mL/h). The peaks were baseline separated. The recoveries of ADMA and SDMA were 77% and 81%, respectively; reported values are corrected for recovery. The intra- and interassay coefficients of variation (CV) were respectively 5.9% and 16.5% for ADMA and 5.3% and 16.9% for SDMA.

Total nitrates (NOx; nitrates plus nitrites) in serum and urine samples were measured using a nitric oxide analyzer (NOA 280i Sievers Instruments, Inc., Boulder, CO). Samples (2 μL) were injected into a purge vessel containing VCl_3 and hydrochloric acid. Nitrates and nitrites were reduced to NO, which was purged into the NOA with argon gas. The NO reacted with ozone to create light that was detected by the NOA. The inter-assay CV was <5%.

Urinary cGMP was analyzed using EIA reagents purchased from Amersham (Piscataway, NJ). Urinary creatinine was measured kinetically using the Jaffe reaction according to the procedure of Heinegard and Tiderstrom [16]. Glucose was determined using an enzymatic method as described by Bondar and Mead [17]. Insulin was measured using an RIA procedure developed by Linco Research, Inc. (St. Charles, MO).

In the statistical analysis, means were compared using a paired *t* test and repeated measures analysis of variance. A value of $p < 0.05$ was considered significant.

3. Results

The characteristics of the subjects at baseline are shown in Table 1. The circulating concentrations of L-arginine at baseline ranged from 80 μmol/L to 125 μmol/L and are in the range expected for fasting values. Table 1 also shows that five subjects experienced initial side effects during the 21 g/day supplementation period and an additional five individuals experienced adverse effects during the 30 g/day supplementation period. At 21 g/day, of 12 participants, four (33%) experienced diarrhea (in addition, one complained of trembling), one vomited (8%), and one had a nosebleed. At 30 g/day, of 10 participants (two did not take any of the 30g/day dose), nine (90%) experienced diarrhea and, in addition, one complained of a headache and one of dry mouth. At the lower doses, the only event noted was that one participant suffered a nosebleed at 9 g/day. Only one of the 12 participants did not report any side effects. All the subjects reported consuming all of the L-arginine doses up to and including the 21 g/day level but only five took all of the 30 g/day doses. No significant changes in the weight or blood pressures of the participants occurred during the study.

The results for the biochemical and physiological measures during the supplementation period are shown in Table 2. Data are not shown for week 4 (30 g/day), as seven participants had stopped taking the L-arginine supplement.

The results indicate that during the supplementation period significant changes were observed in circulating levels of L-arginine, glycine, and L-ornithine. This resulted in significant changes in the ratio of L-arginine:(L-ornithine + L-lysine + ADMA + SDMA). All the other biochemical measures, as well as DBP and SBP, were unchanged. Mean glycine concentration was 288 μmol/L at baseline but had decreased to 247 μmol/L after 3 weeks of L-arginine supplementation. A paired *t* test analysis indicated that the mean glycine concentration after three weeks of supplementation was significantly lower than the baseline and 2-week (276 μmol/L) values ($P = 0.010$ and $P = 0.008$, respectively). Mean L-ornithine concentration was 64.5 μmol/L at baseline and had significantly increased after supplementation with 9 g/day and 21 g/day of L-arginine to 85.0 μmol/L ($P = 0.006$) and 79.4 μmol/L ($P = 0.001$), respectively.

A significant increase in mean L-arginine level was seen after supplementation with 9 g/day of L-arginine, with no further increase during the 21 g/day supplementation period. A paired *t* test analysis indicated that the L-arginine concentrations at 9 g/day (169 μmol/L) were significantly higher than the baseline (101 μmol/L; $P = 0.009$) and 3 g/day (110 μmol/L; $P = 0.036$) values. Similarly, the L-arginine levels at 21 g/day (164 μmol/L) were significantly higher than the baseline ($P = 0.002$) and 3 g/day ($P = 0.020$) measures. However, individuals varied considerably in their responses (Table 3). L-arginine concentrations showed little change in one individual (subject #2), whereas for two subjects (#4, 11) the levels tended to increase throughout the supplementation period. Circulating L-arginine levels peaked or reached a plateau after doses of 3 g/day, 9 g/day, and 21 g/day for one (#12), four (#1, 7, 9, 10) and four (#3, 5, 6, 8) subjects, respectively. Although the numbers are limited, the changes in L-arginine concentrations did not appear to be related to the sex or weight of the individuals.

Increases in L-arginine concentrations, rather than decreases in levels of L-ornithine, L-lysine, ADMA, or SDMA, were responsible for raising the ratio of L-arginine: (L-ornithine + L-lysine + ADMA + SDMA) from 0.424 at baseline to 0.644 and 0.688 at 9 g/day and 21 g/day, respectively.

4. Discussion

The increases in mean L-arginine concentrations from 101 μmol/L at baseline to 169 μmol/L and 164 μmol/L at 9 g/day and 21 g/day, respectively, are in the anticipated range. Pharmacokinetic data [18] indicate that after an oral

Table 2
Biochemical and physiological measures during L-arginine supplementation

Measurement*	Week (g/day L-arginine)				$P^{\dagger}$
	Baseline (0)	1 (3)	2 (9)	3 (21)	
L-arginine (μmol/L)	101 ± 4.1	110 ± 8.0	169 ± 22.1[‡]	164 ± 16.5[§]	0.002
L-citrulline (μmol/L)	33.9 ± 2.5	32.9 ± 2.2	31.9 ± 3.2	31.6 ± 2.7	0.538
L-ornithine (μmol/L)	64.5 ± 5.1	68.7 ± 4.5	85.0 ± 8.1[¶]	79.4 ± 4.6[‖]	0.001
ADMA (nmol/L)	922 ± 54.2	982 ± 40.5	1030 ± 52.4	976 ± 47.3	0.098
SDMA (nmol/L)	536 ± 33.2	531 ± 35.1	535 ± 38.8	525 ± 28.5	0.962
Total creatinine (urine, millimoles)	13.1 ± 1.79	12.6 ± 1.50	11.3 ± 1.46	11.0 ± 1.57	0.431
NOx (serum) (μmol/L)	31.1 ± 4.9	26.0 ± 2.4	31.2 ± 5.0	28.9 ± 2.8	0.433
NOx (urine) (mmol/L)	1.33 ± 0.17	1.54 ± 0.27	1.47 ± 0.22	1.40 ± 0.23	0.897
Total NOx (urine) (millimoles)	1.56 ± 0.14	2.15 ± 0.36	2.09 ± 0.31	2.23 ± 0.31	0.220
NOx/Creatinine (millimole/millimole)	0.120 ± 0.017	0.138 ± 0.033	0.140 ± 0.019	0.620	
cGMP (urine) (nmol/L)	504 ± 72.2	485 ± 44.6	577 ± 89.5	419 ± 34.8	0.212
Total cGMP (urine) (nanomoles)	612 ± 58.6	711 ± 78.2	796 ± 98.3	677 ± 63.8	0.236
cGMP/creatinine (nanomole/millimole)	43.7 ± 5.3	42.2 ± 4.8	51.7 ± 4.7	44.1 ± 4.7	0.149
Glycine (μmol/L)	288 ± 22.6	259 ± 18.4	276 ± 20.3	247 ± 15.9**	0.029
L-lysine (μmol/L)	178 ± 10.7	175 ± 11.2	172 ± 12.3	159 ± 9.6	0.340
L-arginine/(L-ornithine + L-lysine + ADMA + SDMA) (μmole: μmole)	0.424 ± 0.026	0.467 ± 0.042	0.644 ± 0.068[††]	0.688 ± 0.059[‡‡]	0.001
Glucose (mmol/L)	6.00 ± 0.22	5.87 ± 0.25	6.02 ± 0.12	6.27 ± 0.17	0.176
ADMA/(L-arginine + L-lysine + L-ornithine + SDMA) nanomole; μmole)	2.73 ± 0.19	2.83 ± 0.16	2.55 ± 0.20	2.48 ± 0.15	0.188
Insulin (pmol/L)	137 ± 33	176 ± 44	127 ± 32	127 ± 32	0.090
DBP (mm Hg)	73.7 ± 3.7	69.0 ± 4.0	71.5 ± 3.8	73.8 ± 2.9	0.120
SBP (mm Hg)	115.5 ± 4.7	111.0 ± 4.8	113.3 ± 4.4	115.2 ± 4.3	0.237

* Measurements are means ± SEM of 12 values.
† Repeated-measures ANOVA.
‡ Significantly different from baseline ($P = 0.009$) and 3 g/day ($P = 0.036$).
§ Significantly different from baseline ($P = 0.002$) and 3 g/day ($P = 0.020$).
¶ Significantly different from baseline ($P = 0.006$) and 3 g/day ($P = 0.030$).
‖ Significantly different from baseline ($P = 0.001$) and 3 g/day ($P = 0.017$).
**Significantly different from baseline ($P = 0.091$) and 9 g/day ($P = 0.008$).
†† Significantly different from baseline ($P = 0.006$) and 3 g/day ($P = 0.016$).
‡‡ Significantly different from baseline ($P = 0.001$) and 3 g/day ($P = 0.002$).

dose, L-arginine levels peak at about 60 minutes and are still elevated at 8 hours. Thus our fasting blood values should reflect the last dose of L-arginine consumed. Nevertheless, it would be anticipated that in the immediate postabsorptive state supplementation with 21 g/day or 30 g/day of L-arginine would raise the serum L-arginine levels above those observed at 9 g/day. There is, however, considerable variation among subjects in the bioavailability of L-arginine, possibly reflecting differences in the extent of L-arginine absorption in the jejunum and catabolism by enterocytes [19].

One surprising aspect of this study is the number of subjects who complained of side effects at the higher arginine dosages. Five of the 12 participants noted adverse effects at 21 g/day of L-arginine and an additional five at 30 g/day. Studies in the literature frequently use 20–30 g/day of L-arginine and the rates of side effects reported are much lower: none of eight healthy subjects at 30 g/day [20], one of 12 healthy subjects at 24.8 g/day [21], and three of 27 hypercholesterolemic subjects at 21 g/day [22]. However, Chin-Dusting et al. [23] reported that three of nine patients with heart failure experienced diarrhea during supplementation with 20 g/day of L-arginine. The studies differ in the spacing of the doses over a day and in the volume of juice in which the L-arginine was dissolved. More importantly, of the reports noted above only that of Clarkson et al. [22], as in this study, used L-arginine (free acid) as the supplement. The other studies used L-arginine-HCl, a neutral molecule, as the supplement. A large bolus of the positively charged free acid may disturb the acid–base balance of the stomach and GI tract and thus give rise to gastrointestinal symptoms.

It should also be noted that the number of subjects studied is limited and, in this study, represented both men and women with a wide range of body weights. Nevertheless, during the supplement period, there was no significant change in their body weight, consistent with the absence of any self-reported changes in food consumption.

Because dietary L-arginine and L-lysine, an essential amino acid, are taken up by the same transport system in the small intestine, there was a possibility that increasing L-arginine intake might competitively inhibit L-lysine uptake, resulting in a partial deficiency of L-lysine. However, the

Table 3
Individual L-arginine concentrations (μmol/L)

Subject	Week				
	0	1	2	3	4
1	104	92	351	146	N/A
2	87	109	103	106	N/A
3	120	99	187	301	227
4	119	126	141	161	189
5	93	105	124	245	189
6	108	112	114	154	152
7	92	96	275	147	N/A
8	98	83	90	125	N/A
9	125	120	190	168	194
10	98	103	189	117	N/A
11	80	87	121	187	N/A
12	89	188	126	117	N/A

N/A indicates that the subject took none or only some of the 30 g/day supplement.

absence of any change in serum L-lysine concentration during the supplementation period indicates that increasing dietary L-arginine did not interfere with uptake of L-lysine in the GI tract under the regimen used during this trial.

In contrast, serum glycine concentration decreased by the end of week 3. As L-arginine and glycine are used in stoichiometric amounts for creatine synthesis, this result suggested that increased L-arginine intake may enhance creatine synthesis and thus reduce circulating glycine levels [5]. Creatine was not measured during this study, but a change in creatine levels could have an impact on muscle activity. However, casting doubt on this interpretation, no changes in urinary creatinine levels were observed. Possibly, any excess guanidinoacetate formed from L-arginine and glycine was excreted rather than converted to creatine and creatinine. Alternatively, L-arginine supplementation, through unknown mechanisms, may enhance purine synthesis, which requires glycine, or the L-arginine may stimulate glycine catabolism via the mitochondrial glycine cleavage system.

Circulating L-ornithine levels increased during the 9 g/day and 21 g/day supplementation periods, most likely reflecting a direct mass-action effect via increased L-arginine availability for catabolism via arginase.

Reported effects of L-arginine supplementation on values for nitrates, cGMP, and blood pressure are variable and depend on the health status of the subjects [24–26]. In agreement with Theilmeier et al. [27], we did not find increased NOx levels with increasing dietary arginine intake. However, as the typical diet contains high levels of nitrites and nitrates, using these metabolites as markers for NO production should only be done when dietary intake of nitrites and nitrates is strictly controlled [28]. Similarly, cGMP is the mediator for many actions and is not useful as a marker of NO activity.

As ADMA and SDMA are produced by proteolysis of proteins containing methylated arginine residues, their levels would not be expected to be altered by L-arginine supplementation [29,30]. Indeed, this is an aspect of the intended utility of L-arginine supplementation: it increases the substrate available to NOS without also raising the level of inhibitors. As L-arginine, L-ornithine, L-lysine, ADMA, and SDMA share the same transport system, the L-arginine:(L-ornithine + L-lysine + ADMA + SDMA) ratio is a more accurate indicator of relative L-arginine availability than is the L-arginine:ADMA ratio. Relative to baseline (0.424), this ratio was significantly higher at supplemental levels of 9 g/day (0.644, $P = 0.006$) and 21 g/day (0.688, $P = 0.001$), indicating that at these levels the availability of L-arginine to the NOS was enhanced. Although the ratio of ADMA:(L-arginine + L-lysine + L-ornithine + SDMA), an indicator of relative ADMA availability, exhibited a trend toward lower values at higher levels of L-arginine supplementation, it did not reach statistical significance, possibly as a consequence of the limited sample size. Any increases in arginine accessibility as the availability of ADMA remains constant or declines slightly will favor NO production. The concentration of ADMA is somewhat higher than that of SDMA, as is expected, and the actual concentrations (≤ 1 μmol/L) are typical of those reported for healthy individuals [31,32]. During disease, particularly in hypercholesterolemia and renal failure, the concentrations of the dimethyl analogs may increase several-fold [33].

We did not observe any effect of L-arginine supplementation on glucose or insulin levels. Previous reports have noted that the oral ingestion of protein [34] or the intravenous administration of amino acids, including arginine [35], stimulated insulin secretion. However, the concentrations of insulin reverted to preadministration levels within a few hours. Thus elevated concentrations of insulin were unlikely to be observed in our study as the analyses were made on fasting (at least 8 hours) samples.

The mean values noted in this study indicate that supplementation with 9 g/day of L-arginine is sufficient to raise circulating L-arginine concentration. This level is about twice the amount of L-arginine present in a typical western diet and, in this study, was associated with minimal side effects. However, within this small population of 12 subjects there was considerable variation in the responses elicited. This suggests that the level of L-arginine supplementation needs to be tailored to individuals and the circulating L-arginine concentrations monitored. It cannot be ruled out that individuals did not take all of the L-arginine doses supplied. Participants reported that at 7 g (21 g/day) the L-arginine drink assumed an unpleasant taste. It is noteworthy, however, that the individual who did not report any side-effects (subject #9) exhibited large increases in his circulating L-arginine levels. All other participants reported side-effects irrespective of the change in their circulating L-arginine levels. The influence of L-arginine supplementation for a period greater than 1 month on circulating L-arginine levels and on other biochemical and physiolog-

ical measures needs to be further evaluated, as do the relative merits of using L-arginine-HCl versus the free acid.

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References

[1] Moncada S, Palmer RMJ, Higgs EA. Biosynthesis of nitric oxide from L-arginine. Biochem Pharmacology 1989;38:1709–15.
[2] Böger RH, Bode-Böger SM. The clinical pharmacology of L-arginine. Annu Rev Pharmacol Toxicol 2001;42:79–99.
[3] Ignarro LJ. Nitric oxide. Biology and pathobiology. San Diego: Academic Press, 2000.
[4] Visek WJ. Arginine needs, physiological state and usual diets: a reevaluation. J Nutr 1986;116:36–46.
[5] Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. Biochem. J 1998;336:1–17.
[6] Beaumier L, Castillo L, Yu Y-M, Ajami AM, Young VR. Arginine: new and exciting developments for an "old" amino acid. Biomed Environ Sci 1996;9:296–315.
[7] Wu G. Synthesis of citrulline and arginine from proline in enterocytes of postnatal pigs. Am J Physiol 1997;272(Gastrointest Liver Physiol 35):G1382–90.
[8] Barbul A. Arginine: biochemistry, physiology, and therapeutic implications. J Parenter Enter Nutr 1986;10:227–238.
[9] Flynn NE, Meininger CJ, Haynes TE, Wu G. The metabolic basis of arginine nutrition and pharmacotherapy. Biomed Pharmacother 2002; 56:427–38.
[10] Cyonber L. Immune-enhancing diets for stressed patients with special emphasis on arginine content: analysis of the analysis. Curr Opin Clin Nutr Metab Care 2003;6:189–93.
[11] Griffith OW, Stuehr DJ. Nitric oxide synthases: properties and catalytic mechanism. Annu Rev Physiol 1995;57:707–36.
[12] Forstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I, Kleinert H. Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. Hypertension 1994;23:1121–31.
[13] Kurz S, Harrison DG. Insulin and the arginine paradox. J Clin Invest 1997;99:369–70.
[14] Tsikas D, Boger RH, Sandmann J, Bode-Boger SM, Frolich JC. Endogenous nitric oxide synthase inhibitors are responsible for the L-arginine paradox. FEBS Lett 2000;478:1–3.
[15] Pettersson A, Uggla L, Backman V. Determination of dimethylated arginines in human plasma by high-performance liquid chromatography. J Chromatogr 1997;692:257–62.
[16] Heinegard D, Tiderstrom G. Determination of serum creatinine by a direct colorimetric method. Clin Chim Acta 1973;43:305–10.
[17] Bondar RJL, Mead DC. Evaluation of glucose-6-phosphate dehydrogenase from Leuconostoc mesenteroides in the hexokinase method for determining glucose in serum. Clin Chem 1974;20:586–590.
[18] Tangphao O, Grossman M, Chalon S, Hoffman BB, Terrence F. Pharmacokinetics of intravenous and oral L-arginine in normal volunteers. Br J Clin Pharmacol 1999;47:261–6.
[19] Reyes AA, Karl IE, Klahr S. Role of arginine in health and in renal disease. Am J Physiol 1994;267:F331–46.
[20] Luiking YC, Weusten BLAM, Portincasa P, Van Der Meer R, Smout AJMP, Akkermans LMA. Effects of long term oral L-arginine on esophageal motility and gallbladder dynamics in healthy humans. Am J Phys 1998;274:G984–91.
[21] Barbul A, Lazarou SA, Efron DT, Wasserkrug HL, Efron G. Arginine enhances wound healing and lymphocyte immune responses in humans. Surgery 1990;108:331–7.
[22] Clarkson P, Adams MR, Powe AJ, Donald AE, McCredle R, Robinson J, McCarthy SN, Keech A, Celermajer DS, Deanfield JE. Oral L-arginine improves endothelium-dependent dilation in hypercholesterolemic young adults. J Clin Invest 1996;97:1989–94.
[23] Chin-Dusting JPF, Kaye DM, Lefkovits J, Wong J, Bergin P, Jennings GL. Dietary supplementation with L-arginine fails to restore endothelial function in forearm resistance arteries of patients with severe heart failure. J Am Coll Cardiol 1996;27:1207–13.
[24] Lerman A, Burnett JC Jr, Higano St, McKinley LJ, Holmes DR Jr. Long term L-arginine supplementation improves small vessel coronary endothelial function in humans. Circulation 1998;97:2123–8.
[25] Herlitz H, Jungersten LU, Wikstrand J, Widgren BR. Effect of L-arginine infusion in normotensive subjects with and without a family history of hypertension. Kidney Int 1999;56:1838–45.
[26] Beaumier L, Castillo L, Ajami AM, Young VR. Urea cycle intermediate kinetics and nitrate excretion at normal and "therapeutic" intakes of arginine in humans. Am J Physiol 1995;269:E884–96.
[27] Theilmeier G, Chan JR, Zalpour C, Anderson B, Wang BY, Wolf A, Tsao PS, Cooke JP. Adhesiveness of mononuclear cells in hypercholesterolemic humans is normalized by dietary L-arginine. Arter Thromb Vasc Biol 1997;17:3557–64.
[28] Baylis C, Vallance P. Measurement of nitrite and nitrate levels in plasma and urine—what does this measure tell us about the activity of the endogenous nitric oxide system? Curr Opin Nephrol Hypertens 1998;7:59–62.
[29] Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, Blaschke TF, Cooke JP. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 1998;98:1842–7.
[30] Bode-Boger SM, Boger RH, Kienke S, Junker W, Frölich JC. Elevated L-arginine/dimethylarginine ratio contributes to enhanced systemic NO production by dietary L-arginine in hypercholesterolemic rabbits. Biochem Biophys Res Comm 1996;219:598–603.
[31] Anderstam B, Katzarski K, Bergström J. Serum levels of N^G, N^G-dimethyl-L-arginine, a potential endogenous nitric oxide inhibitor in dialysis patients. J Am Soc Nephrol 1997;8:1437–42.
[32] Fleck C, Janz A, Schweitzer F, Karge E, Schwertefeger M, Stein G. Serum concentrations of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine in renal failure patients. Kidney Int 2001; 59:S14–8.
[33] Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric synthesis in chronic renal failure. Lancet 1992;339:572–5.
[34] Floyd JC Jr, Fajans SS, Conn JW, Knopf RF, Rull J. Insulin secretion in response to protein ingestion. J Clin Invest 1966;45:1479–86.
[35] Floyd JC Jr, Fajans SS, Conn JLS, Knopf RF, Rull J. Stimulation of insulin secretion by amino acids. J Clin Invest 1966;45:1487–1502.