Effects of Arginine Supplementation on Amino Acid Profiles in Blood and Tissues in Fed and Overnight-Fasted Rats

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Abstract: Chronic arginine intake is believed to have favorable effects on the body. However, it might be hypothesized that excessive consumption of an individual amino acid exerts adverse effects on distribution and metabolism of other amino acids. We evaluated the effect of chronic intake of arginine on amino acid concentrations in blood plasma, liver, kidneys, and soleus and extensor digitorum longus muscles. Rats were fed a standard diet or a high-arginine diet (HAD) for two months. Half of the animals in each group were sacrificed in the fed state, and the other half after fasting overnight. HAD increased blood plasma concentrations of urea, creatinine, arginine, and ornithine and decreased most other amino acids. Arginine and ornithine also increased in muscles and kidneys; an increase of lysine was observed in both muscle types. Methionine, phenylalanine, threonine, asparagine, glycine, serine, and taurine decreased in most tissues of HAD fed animals. Most of the effects of HAD disappeared after overnight fasting. It is concluded that (i) enhanced dietary arginine intake alters distribution of almost all amino acids; and (ii) to attain a better assessment of the effects of various nutritional interventions, an appropriate number of biochemical measurements must be performed in both postprandial and postabsorptive states.

Keywords: nutritional supplements; arginine; amino acids; starvation; nutrition

1. Introduction

L-Arginine is a basic amino acid that is required for synthesis of proteins and serves as a precursor for synthesis of creatine, agmatine, urea, polyamines, proline, glutamate, and nitric oxide (Figure 1). L-arginine is classified as a conditionally essential amino acid because its endogenous synthesis may not be sufficient to meet metabolic demands in preterm infants and some cases of critical illness [1].

Current interest in l-arginine is focused mainly on its role in biosynthesis of nitric oxide and its stimulatory role in the secretion of insulin and growth hormone. Considerable literature exists from human and animal studies attesting to the fact that l-arginine may lower blood pressure, reduce blood clots and strokes, lower cholesterol and triglycerides, and improve diabetes and sexual functions via its role as a precursor for endothelium-derived nitric oxide [1–3]. There is no standard dose of arginine. A common dosage is 2 to 3 g three times a day; lower and higher doses have also been reported [4]. Of the available human studies, doses up to 20 g/day have been generally well tolerated. Minimal side effects such as nausea or diarrhea have been reported at higher doses [4,5].
Figure 1. Overview of the main pathways of arginine metabolism. 1, arginase; 2, nitric oxide synthase; P-5-C, pyroline-5-carboxylate; α-KG, alpha ketoglutarate.

Despite the many human and animal studies on arginine efficacy, there have been few studies investigating the specific effects of arginine supplementation on the distribution of amino acids in body fluids that may primarily result from alterations in arginine metabolism and amino acid transport across cell membranes. This may impair availability of some amino acids in a number of biochemical pathways and cellular functions, resulting in unexpected responses to various physiological and pathological conditions, such as starvation, exercise, trauma, infection, and cancer development.

The principle aim of the present study was to evaluate the effect of chronic intake of an arginine-supplemented diet on concentrations of free amino acids in selected tissues of white rats. The effect has been examined in two nutritionally different conditions—fed and overnight-fasted animals. In fed (postprandial) state, concentrations of nutrients in body fluids are closely related to the composition of the food and anabolic response of the body, mediated by enhanced secretion of insulin and activity of the parasympathetic system. After overnight fasting (postabsorptive state) food composition has a smaller effect on concentration of nutrients in extracellular fluid, and the main role it plays is the gradual decrease in insulin/glucagon ratio and enhanced catabolism of glycogen, lipids, and proteins. In this state, metabolic alterations are examined routinely in clinical practice.

2. Materials and Methods

2.1. Animals and Materials

Male Wistar rats (BioTest, Konarovice, Czech Republic) were housed in standardized cages in temperature-controlled quarters with a 12-h light-dark cycle. All rats received the standard laboratory diet (SLD) (Velas, Czech Republic) and drinking water ad libitum. The Animal Care and Use Committee of Charles University in Prague, Faculty of Medicine in Hradec Kralove specifically approved this study on 10 February 2010 (identification code: 24774/2006-11020). Chemicals were obtained from Sigma Chemical (St. Louis, MO, USA), Lachema (Brno, Czech Republic), Waters (Milford, MA, USA), Biomol (Hamburg, Germany), and Merck (Darmstadt, Germany).

2.2. Experimental Design

A total of 40 male Wistar rats at 7 weeks of age and weighing approximately 200 g each were randomly divided into two groups and fed an SLD or a high-arginine diet (HAD) for 2 months. HAD was prepared by mixing SLD (contains 24% of nitrogenous substances) with L-arginine (Sigma Chemical, St. Louis, MO, USA) in a ratio of 19:1. This resembles a high-dose supplementation of approximately 25 g of arginine per day in human subject.
At the end of the study the rats were sacrificed by exsanguination via the abdominal aorta and soleus (SOL) and extensor digitorum longus (EDL) muscles, liver, and kidneys were quickly removed and weighed. Half of the animals in each group were sacrificed in the fed state, the other half were sacrificed after an overnight fast. Small samples (of approximately 100 mg) of these tissues were immediately homogenized in 6% (v/v) perchloric acid, and the precipitated proteins were collected via centrifugation for 5 min at 12,000 × g.

2.3. Amino Acid Concentrations in Blood Plasma and Tissues

Amino acid concentrations were identified in the supernatants of deproteinized blood plasma and tissue samples using high-performance liquid chromatography (Aliance 2695, Waters, Milford, MA, USA) after derivatization with 6-aminquinolyl-N-hydroxysuccinimidyl carbamate. The intracellular concentration of each amino acid was calculated by subtracting the free extracellular portion from the total amount, assuming the plasma concentration to be equal to the concentration in the interstitial fluid as described by Bergström et al. [6]. Total tissue water was measured from the tissue weight obtained after drying for 24 h at 90 °C. The determination of extra- and intracellular water was based on the chloride method according to Graham et al. [7].

2.4. Other Techniques

Plasma levels of urea, creatinine, ALT, AST, glucose, triglycerides, and cholesterol were measured using commercial tests (Boehringer, Mannheim, Germany; Elitech, Sées, France and Lachema, Brno, Czech Republic). Na⁺, K⁺, and Cl⁻ were determined with the help of ion-selective electrodes on AVL 983-S (Block Scientific, Englewood, NJ, USA).

2.5. Statistical Analyses

Results are expressed as means ± SE. Analysis of variance (ANOVA) followed by Bonferroni multiple comparison post hoc analysis was used to detect differences between multiple independent groups. NCSS 2001 statistical software (Kaysville, UT, USA) was used for analyses. Differences were considered significant at p < 0.05.

3. Results

3.1. Alterations in Food Intake, Body Weight Gain, and Weight and Protein Content of Tissues

We did not find significant differences in food intake, body weight gain, and weight and protein content of liver, EDL, and SOL between SLD and HAD fed animals. Significantly higher weight and protein content values were observed in the kidneys of animals fed by HAD. The effect was more pronounced in a fed state than after overnight starvation (Tables 1 and 2).

Table 1. Changes in body weight and food intake in animals fed by SLD or HAD.

|                      | SLD (n = 20) | HAD (n = 20) |
|----------------------|--------------|--------------|
| Body weight (g)      |              |              |
| initial              | 198 ± 4      | 200 ± 5      |
| 1 week               | 257 ± 6      | 247 ± 6      |
| 6 weeks              | 450 ± 10     | 438 ± 10     |
| 70 days              | 525 ± 12     | 498 ± 12     |
| Food intake (g/kg b.w./day) |           |              |
| 1st week             | 116 ± 4      | 122 ± 4      |
| 6th week             | 74 ± 1       | 73 ± 1       |
| last week            | 64 ± 2       | 66 ± 1       |

Means ± SE. SLD, rats fed by standard laboratory diet; HAD, rats fed by arginine-enriched diet.
Table 2. Effect of chronic intake of HAD on tissue weights, protein content, and protein concentration in fed and overnight-starved animals.

|                  | Fed Animals (n = 10) | Overnight-Starved Animals (n = 10) |
|------------------|----------------------|-------------------------------------|
|                  | SLD                  | HAD                  | SLD + S                  | HAD + S                  |
| Liver            |                      |                      |                        |                        |
| weight (g/kg b.w.) | 32.42 ± 0.63         | 33.34 ± 1.16         | 23.13 ± 0.42           | 23.66 ± 0.44           |
| protein (mg/g wet t.w) | 160 ± 9          | 147 ± 4              | 168 ± 4               | 156 ± 3               |
| protein (g/kg b.w.) | 5.17 ± 0.29         | 4.88 ± 0.18          | 3.88 ± 0.11           | 3.70 ± 0.10           |
| Kidney           |                      |                      |                        |                        |
| weight (g/kg b.w.) | 2.91 ± 0.06         | 3.45 ± 0.07 *        | 2.85 ± 0.09           | 3.22 ± 0.08 *         |
| protein (mg/g wet t.w) | 128 ± 2          | 125 ± 4              | 126 ± 3              | 115 ± 3              |
| protein (g/kg b.w.) | 0.37 ± 0.01         | 0.43 ± 0.01 *        | 0.36 ± 0.01           | 0.37 ± 0.01 *         |
| EDL              |                      |                      |                        |                        |
| weight (g/kg b.w.) | 0.43 ± 0.01         | 0.41 ± 0.01          | 0.43 ± 0.01           | 0.45 ± 0.01           |
| protein (mg/g wet t.w) | 150 ± 3          | 146 ± 3              | 150 ± 3              | 145 ± 4              |
| protein (g/kg b.w.) | 0.06 ± 0.00         | 0.06 ± 0.00          | 0.06 ± 0.00           | 0.07 ± 0.00           |
| SOL              |                      |                      |                        |                        |
| weight (g/kg b.w.) | 0.46 ± 0.01         | 0.46 ± 0.01          | 0.48 ± 0.01           | 0.50 ± 0.01           |
| protein (mg/g wet t.w) | 127 ± 4          | 128 ± 4              | 129 ± 3              | 118 ± 2              |
| protein (g/kg b.w.) | 0.06 ± 0.00         | 0.06 ± 0.00          | 0.06 ± 0.00           | 0.06 ± 0.00           |

Means ± SE, p < 0.05. * effect of arginine (HAD vs. SLD or HAD + S vs. SLD + S); # effect of starvation (SLD + S vs. SLD or HAD + S vs. HAD). SLD, rats fed by standard laboratory diet; HAD, rats fed by arginine-enriched diet.

3.2. Alterations in Blood Plasma

Standard blood biochemistry assays have shown that HAD increased concentrations of urea and creatinine and decreased potassium, triglycerides, and atherogenicity index (Table 3). Consumption of HAD increased blood plasma concentrations of arginine and ornithine and decreased a number of both essential (histidine, lysine, methionine, phenylalanine, threonine, and valine) and non-essential (asparagine, aspartate, glycine, proline, serine, taurine, and tyrosine) amino acids (Table 4). Plasma concentrations of isoleucine, leucine, alanine, glutamine, citrulline, and glutamate were unaffected. Most of the differences observed between SLD- and HAD-fed animals disappeared after overnight fasting. The exceptions were lower concentrations of citrulline, glutamine, glycine, and serine in animals fed before starvation by HAD than in animals fed by SLD.

Table 3. Effect of chronic intake of HAD on blood biochemistry in fed and overnight-starved animals.

|                  | Fed Animals (n = 10) | Overnight-Starved Animals (n = 10) |
|------------------|----------------------|-------------------------------------|
|                  | SLD                  | HAD                  | SLD + S                  | HAD + S                  |
| Glucose (mmol/L) | 10.7 ± 0.2           | 10.2 ± 0.2           | 9.1 ± 0.3 #              | 9.9 ± 0.1 *              |
| Urea (mmol/L)   | 7.1 ± 0.2            | 10.4 ± 0.4 *         | 6.6 ± 0.2               | 7.1 ± 0.2 #              |
| Creatinine (μmol/L) | 27.6 ± 0.8         | 33.1 ± 0.9 *         | 31.8 ± 1.5 #            | 30.2 ± 1.1              |
| Sodium (mmol/L) | 142.3 ± 0.5          | 141.6 ± 0.4          | 143.1 ± 0.3            | 142.5 ± 0.2            |
| Potassium (mmol/L) | 4.4 ± 0.1           | 3.8 ± 0.1 *          | 3.8 ± 0.1 #            | 3.8 ± 0.1               |
| Chloride (mmol/L) | 100.9 ± 0.6         | 102.1 ± 0.5          | 103.2 ± 0.3 #           | 102.3 ± 0.5            |
| ALT (μkat/L)    | 0.9 ± 0.0            | 0.9 ± 0.0            | 0.6 ± 0.1 #            | 0.7 ± 0.0              |
| AST (μkat/L)    | 1.3 ± 0.1            | 1.5 ± 0.1            | 1.2 ± 0.0 #            | 1.2 ± 0.0 #            |
| Cholesterol (mmol/L) | 1.8 ± 0.1           | 1.8 ± 0.1            | 1.5 ± 0.1              | 1.4 ± 0.1              |
| HDL cholesterol (mmol/L) | 1.1 ± 0.1        | 1.2 ± 0.1            | 1.1 ± 0.1              | 1.2 ± 0.0              |
| LDL cholesterol (mmol/L) | 0.3 ± 0.0         | 0.4 ± 0.0            | 0.3 ± 0.0              | 0.2 ± 0.0 #            |
| Atherogenicity index | 0.7 ± 0.0         | 0.5 ± 0.0 *          | 0.3 ± 0.0 #            | 0.3 ± 0.0 #            |
| Triglycerides (mmol/L) | 1.6 ± 0.2        | 1.2 ± 0.1 *          | 1.0 ± 0.2 #            | 0.8 ± 0.1 #            |
| Total protein (g/L) | 63.7 ± 0.8          | 62.6 ± 0.5           | 61.1 ± 0.5 #           | 62.5 ± 0.6             |
| Albumin (g/L)   | 38.6 ± 0.9           | 40.1 ± 0.7           | 39.5 ± 0.5            | 40.4 ± 0.3             |

Means ± SE, p < 0.05. * Effect of arginine (HAD vs. SLD or HAD + S vs. SLD + S); # effect of starvation (SLD + S vs. SLD or HAD + S vs. HAD). Atherogenicity index was calculated as: (cholesterol—HDL cholesterol)/HDL cholesterol. SLD, rats fed by standard laboratory diet; HAD, rats fed by arginine-enriched diet.
After overnight fasting, the differences in amino acid concentrations between animals fed by SLD and HAD were mostly insignificant (Tables 5–8).

A unique effect observed in skeletal muscle was an increase of lysine. Consumption of HAD increased arginine and ornithine and decreased intracellular concentration of a number of amino acids (particularly of methionine, phenylalanine, threonine, asparagine, glycine, serine, and taurine) in most of the examined tissues. The exception was the liver, in which arginine concentration was unchanged. A unique effect observed in skeletal muscle was an increase of lysine. After overnight fasting, the differences in amino acid concentrations between animals fed by SLD and HAD were mostly insignificant (Tables 5–8).

### 3.3. Alterations in Tissues

Consumption of HAD increased arginine and ornithine and decreased intracellular concentration of a number of amino acids (particularly of methionine, phenylalanine, threonine, asparagine, glycine, serine, and taurine) in most of the examined tissues. The exception was the liver, in which arginine concentration was unchanged. A unique effect observed in skeletal muscle was an increase of lysine. After overnight fasting, the differences in amino acid concentrations between animals fed by SLD and HAD were mostly insignificant (Tables 5–8).

### Table 4. Effect of chronic intake of HAD on amino acid concentrations in blood plasma (μmol/L) in fed and overnight-starved animals.

|                     | Fed Animals | Overnight-Starved Animals |
|---------------------|-------------|---------------------------|
|                     | Plasma      | SLD (n = 10) | HAD (n = 10) | SLD + S (n = 10) | HAD + S (n = 10) |
| **Essential amino acids** |             |              |             |              |               |
| Histidine           | 63 ± 1      | 54 ± 2 #     | 54 ± 2 #    | 55 ± 1       |
| Isoleucine          | 84 ± 2      | 81 ± 3       | 93 ± 2 #    | 89 ± 3       |
| Leucine             | 148 ± 5     | 141 ± 5      | 149 ± 6     | 152 ± 4      |
| Lysine              | 293 ± 8     | 257 ± 7 *    | 308 ± 8     | 289 ± 10 #   |
| Methionine          | 53 ± 1      | 41 ± 1 *     | 48 ± 1 #    | 48 ± 2 #     |
| Phenylalanine       | 66 ± 2      | 55 ± 2 *     | 65 ± 2      | 65 ± 1 #     |
| Threonine           | 248 ± 7     | 123 ± 5 *    | 234 ± 8     | 216 ± 7 #    |
| Valine              | 189 ± 5     | 166 ± 5 *    | 179 ± 5     | 182 ± 5      |
| ∑ EAA               | 1143 ± 21   | 917 ± 26 *   | 1130 ± 24   | 1097 ± 27    |
| **Non-essential amino acids** |         |              |             |               |
| Alanine             | 507 ± 13    | 495 ± 23     | 387 ± 16 #  | 377 ± 16 #   |
| Asparagine          | 59 ± 2      | 45 ± 3 *     | 58 ± 1      | 55 ± 2 #     |
| Aspartate           | 28 ± 21     | 17 ± 1 *     | 15 ± 1 #    | 12 ± 1 #     |
| Glutamine           | 671 ± 16    | 624 ± 16     | 620 ± 15    | 544 ± 13 *   |
| Glycine             | 297 ± 7     | 161 ± 7 *    | 364 ± 13 #  | 244 ± 7 #    |
| Serine              | 252 ± 9     | 155 ± 5 *    | 230 ± 6     | 198 ± 5 #    |
| Taurine             | 570 ± 40    | 181 ± 15 *   | 241 ± 15 #  | 192 ± 8      |
| Tyrosine            | 85 ± 2      | 57 ± 3 *     | 81 ± 3      | 64 ± 3       |
| **Arginine and its metabolites** |         |              |             |               |
| Arginine            | 173 ± 5     | 387 ± 27 *   | 151 ± 7     | 132 ± 5 #    |
| Citrulline          | 71 ± 3      | 73 ± 2       | 70 ± 2      | 54 ± 2 #     |
| Glutamate           | 123 ± 8     | 112 ± 7      | 104 ± 5     | 96 ± 5       |
| Ornithine           | 53 ± 3      | 132 ± 25 *   | 41 ± 1      | 30 ± 1 #     |
| Proline             | 229 ± 15    | 180 ± 15 *   | 133 ± 3 #   | 117 ± 4 #    |
| ∑ NEAA-Arg          | 2863 ± 78   | 2223 ± 62 *  | 2345 ± 39 # | 1984 ± 46 *  |
| ∑ AA-Arg            | 4026 ± 95   | 3150 ± 78 *  | 3475 ± 58 # | 3081 ± 69 *  |

Means ± SE, p < 0.05. * effect of arginine (HAD vs. SLD or HAD + S vs. SLD + S); # effect of starvation (SLD + S vs. SLD or HAD + S vs. HAD). SLD, rats fed by standard laboratory diet; HAD, rats fed by arginine-enriched diet.

### Table 5. Effect of chronic intake of HAD on amino acid concentrations in liver (μmol/L of intracellular water) in fed and overnight-starved animals.

|                     | Fed Animals | Overnight-Starved Animals |
|---------------------|-------------|---------------------------|
|                     | Liver       | SLD (n = 10) | HAD (n = 10) | SLD + S (n = 10) | HAD + S (n = 10) |
| **Essential amino acids** |             |              |             |              |               |
| Histidine           | 1688 ± 41   | 1486 ± 29 * | 1424 ± 43 # | 1390 ± 20    |
| Isoleucine          | 379 ± 14    | 342 ± 19     | 386 ± 12    | 359 ± 16     |
| Leucine             | 652 ± 42    | 754 ± 109    | 619 ± 21    | 646 ± 28     |
| Lysine              | 853 ± 45    | 885 ± 50     | 1107 ± 40 # | 1132 ± 53 #  |
| Methionine          | 103 ± 5     | 92 ± 6       | 97 ± 8      | 83 ± 3       |
| Phenylalanine       | 237 ± 9     | 214 ± 15     | 238 ± 10    | 227 ± 11     |
| Threonine           | 894 ± 63    | 472 ± 23 *   | 970 ± 73    | 775 ± 47 #   |
| Valine              | 614 ± 38    | 500 ± 27 *   | 561 ± 19    | 561 ± 26     |
| ∑ EAA               | 5420 ± 178  | 4746 ± 238 * | 5401 ± 136  | 5173 ± 157   |
### Table 5. Cont.

|                  | Fed Animals | Overnight-Starved Animals | Non-essential amino acids | Overnight-Starved Animals |
|------------------|-------------|---------------------------|---------------------------|---------------------------|
| Liver            | SLD (n = 10)| HAD (n = 10)              | SLD + S (n = 10)          | HAD + S (n = 10)          |
| Arginine         | 48 ± 4      | 42 ± 6                    | 20 ± 4                    | 46 ± 4                    |
| Citrulline       | 69 ± 5      | 78 ± 5                    | 89 ± 6                    | 71 ± 7                    |
| Glutamine        | 423 ± 18    | 4893 ± 239 *              | 3879 ± 119 *              | 3599 ± 83 *               |
| Ornithine        | 810 ± 58    | 1010 ± 130 *              | 804 ± 33                  | 638 ± 47 *                |
| Proline          | 387 ± 25    | 312 ± 14 *                | 325 ± 10                  | 292 ± 21                  |
| ∑ NEAA-Arg       | 46,441 ± 791| 44,807 ± 1022             | 44,656 ± 818              | 43,568 ± 812              |
| ∑ AA-Arg         | 51,862 ± 909| 49,511 ± 1184             | 50,058 ± 840              | 48,741 ± 859              |

Means ± SE, *p < 0.05. * Effect of arginine (HAD vs. SLD or HAD + S vs. SLD + S); # effect of starvation (SLD + S vs. SLD or HAD + S vs. HAD). SLD, rats fed by standard laboratory diet; HAD, rats fed by arginine-enriched diet.

### Table 6. Effect of chronic intake of HAD on amino acid concentrations in kidney (µmol/L of intracellular water) in fed and overnight-starved animals.

|                  | Fed Animals | Overnight-Starved Animals | Essential amino acids | Overnight-Starved Animals |
|------------------|-------------|---------------------------|-----------------------|---------------------------|
| Kidney           | SLD (n = 10)| HAD (n = 10)              | SLD + S (n = 10)      | HAD + S (n = 10)          |
| Histidine        | 462 ± 22    | 349 ± 12 *                | 391 ± 15 #            | 403 ± 20                  |
| Isoleucine       | 280 ± 14    | 254 ± 10                  | 270 ± 13              | 261 ± 11                  |
| Leucine          | 529 ± 32    | 454 ± 20                  | 493 ± 26              | 451 ± 17                  |
| Lysine           | 765 ± 31    | 636 ± 25 *                | 737 ± 37              | 730 ± 21                  |
| Methionine       | 110 ± 5     | 86 ± 5 *                  | 102 ± 6               | 102 ± 6                   |
| Phenylalanine    | 232 ± 13    | 181 ± 8 *                 | 209 ± 10              | 188 ± 7                   |
| Threonine        | 1395 ± 83   | 799 ± 30 *                | 1222 ± 56             | 1232 ± 37 #               |
| Valine           | 554 ± 28    | 453 ± 22 *                | 474 ± 25              | 481 ± 19                  |
| ∑ EAA            | 4326 ± 200  | 3214 ± 114 *              | 3898 ± 157            | 3849 ± 119 #              |

Means ± SE, *p < 0.05. * effect of arginine (HAD vs. SLD or HAD + S vs. SLD + S); # effect of starvation (SLD + S vs. SLD or HAD + S vs. HAD). SLD, rats fed by standard laboratory diet; HAD, rats fed by arginine-enriched diet.
Table 7. Effect of chronic intake of HAD on amino acid concentrations in extensor digitorum longus muscle (μmol/L of intracellular water) in fed and overnight-starved animals.

| Fed Animals | Overnight-Starved Animals |
|-------------|---------------------------|
| EDL (n = 10) | HAD (n = 10) | SLD + S (n = 10) | HAD + S (n = 10) |
| **Essential amino acids** | | | |
| Histidine 373 ± 17 | 391 ± 11 | 288 ± 11 * | 315 ± 9 * |
| Isoleucine 134 ± 5 | 153 ± 4 * | 162 ± 7 * | 174 ± 5 * |
| Leucine 223 ± 6 | 223 ± 8 | 256 ± 13 * | 281 ± 7 * |
| Lysine 782 ± 61 | 1034 ± 58 * | 635 ± 35 | 657 ± 29 * |
| Methionine 87 ± 3 | 63 ± 2 * | 83 ± 4 | 86 ± 3 * |
| Phenylalanine 123 ± 3 | 108 ± 4 * | 122 ± 4 | 136 ± 3 * * |
| Threonine 953 ± 31 | 602 ± 19 * | 882 ± 34 * | 914 ± 15 * |
| Valine 293 ± 10 | 290 ± 7 | 293 ± 13 | 335 ± 8 * * |
| ∑ EAA 3008 ± 109 | 2865 ± 84 | 2721 ± 102 | 2898 ± 50 |
| **Non-essential amino acids** | | | |
| Alanine 3722 ± 143 | 4580 ± 118 * | 3556 ± 158 | 3915 ± 112 * |
| Asparagine 337 ± 18 | 279 ± 22 * | 367 ± 13 | 402 ± 6 * * |
| Aspartate 524 ± 22 | 680 ± 20 * | 754 ± 30 * | 960 ± 62 * * |
| Cysteine 7538 ± 247 | 7362 ± 303 | 6424 ± 290 * | 5824 ± 221 * * *
| Glycine 4240 ± 300 | 2928 ± 99 * | 4362 ± 346 | 4052 ± 246 * * *
| Serine 1497 ± 76 | 943 ± 31 * | 1217 ± 37 * | 1168 ± 17 * * *
| Taurine 27014 ± 467 | 21951 ± 645 * | 26070 ± 597 | 24280 ± 531 * * *
| Tyrosine 201 ± 6 | 150 ± 8 * | 189 ± 7 | 175 ± 6 * * *

Means ± SE, p < 0.05. * effect of arginine (HAD vs. SLD or HAD + S vs. SLD + S); * * effect of starvation (SLD + S vs. SLD or HAD + S vs. HAD). HAD, rats fed by standard laboratory diet; HAD, rats fed by arginine-enriched diet.

Table 8. Effect of chronic intake of HAD on amino acid concentrations in soleus muscle (μmol/L of intracellular water) in fed and overnight-starved animals.

| Fed Animals | Overnight-Starved Animals |
|-------------|---------------------------|
| SOL (n = 10) | HAD (n = 10) | SLD + S (n = 10) | HAD + S (n = 10) |
| **Essential amino acids** | | | |
| Histidine 759 ± 40 | 748 ± 28 | 655 ± 30 | 779 ± 43 |
| Isoleucine 106 ± 5 | 120 ± 5 | 122 ± 4 * | 130 ± 4 |
| Leucine 175 ± 8 | 172 ± 7 | 192 ± 6 | 209 ± 6 * * |
| Lysine 1327 ± 73 | 1704 ± 115 * | 1562 ± 99 * | 2012 ± 130 * * |
| Methionine 74 ± 3 | 56 ± 2 * | 68 ± 2 | 68 ± 2 * * |
| Phenylalanine 107 ± 4 | 92 ± 3 * | 104 ± 2 | 113 ± 4 * * |
| Threonine 926 ± 19 | 607 ± 17 * | 918 ± 36 | 1047 ± 34 * * * |
| Valine 228 ± 9 | 214 ± 8 | 222 ± 9 | 230 ± 7 * * |
| ∑ EAA 3701 ± 108 | 3713 ± 136 | 3843 ± 143 | 4608 ± 179 * * *
| **Non-essential amino acids** | | | |
| Alanine 3298 ± 129 | 3964 ± 167 * | 3517 ± 215 | 3887 ± 214 |
| Asparagine 636 ± 30 | 517 ± 36 * | 743 ± 23 | 879 ± 42 * * * |
| Aspartate 2114 ± 213 | 2764 ± 285 | 3797 ± 193 * | 3351 ± 224 |
| Glutamine 11,552 ± 464 | 11,498 ± 378 | 10,861 ± 403 | 11,224 ± 416 |
| Glycine 2764 ± 88 | 2732 ± 50 * | 3400 ± 92 * | 3293 ± 90 * * |
| Serine 2904 ± 127 | 1673 ± 56 * | 2631 ± 104 | 2571 ± 108 * * |
| Taurine 33,561 ± 633 | 31,283 ± 467 | 32,960 ± 844 | 33,547 ± 659 |
| Tyrosine 157 ± 5 | 111 ± 5 * | 148 ± 6 | 132 ± 6 * * |

Means ± SE, p < 0.05. * effect of arginine (HAD vs. SOL or HAD + S vs. SOL + S); * * effect of starvation (SOL + S vs. SOL or HAD + S vs. HAD).
4. Discussion

To the best of our knowledge, this is the first study assessing the specific effects of arginine supplementation on the distribution of amino acids in body fluids in postprandial and postabsorptive states. The data clearly demonstrate that chronically enhanced arginine intake leads to marked alterations in aminoacemia in both the blood and tissues. As differences in food intake and weight gain between animals fed by SLD and HAD have been insignificant, the observed alterations are clearly due to the replacement of 5% of the SLD by L-arginine. The alterations are related to both the specific effects of arginine and effects of enhanced intake of nitrogen.

4.1. Alterations in Arginine Levels

As expected, chronic intake of HAD enhanced arginine concentrations in blood plasma, both types of skeletal muscle, and kidneys. The finding of unaffected arginine concentration in hepatic tissue may be explained by the response of the liver to enhanced arginine availability. Exogenous arginine induces arginase expression [8] and activates N-acetylglutamate synthase that catalyses production of N-acetylglutamate, which is an allosteric cofactor for carbamoyl phosphate synthetase I that acts as the controller of flux through urea cycle [9]. Therefore, unaltered concentrations of arginine in the liver are probably due to its activated catabolism as indicated by increased urea concentrations in blood plasma.

4.2. Alterations in Arginine Metabolites

It is believed that enhanced arginine availability and arginase activities have a role in synthesis of ornithine, glutamate, proline, and citrulline [10]. Markedly increased ornithine concentrations in plasma, muscles, and kidneys indicate that chronic consumption of arginine activates arginase not only in the liver, but also in extrahepatic tissues. However, the effect of HAD on glutamate and citrulline was less pronounced, and even a decrease of proline was observed in blood plasma, liver, and kidneys. These findings indicate that other metabolic pathways or alterations in amino acid transport across plasma membranes have more important influences on glutamate and proline concentrations than enhanced availability of one of their precursors. Limited influence of HAD on citrulline levels is in agreement with the general opinion that the amount of citrulline produced via NO synthase is small, and that the main source of circulating citrulline is its synthesis from glutamine in small intestine.

4.3. Alterations in Other Amino Acids

One remarkable effect of arginine consumption was the decrease in concentration of a number of amino acids (particularly of methionine, phenylalanine, threonine, asparagine, glycine, serine, and taurine) in both blood plasma and tissues. The decrease of aminoacemia in animals fed by HAD is markedly different from the minimal changes observed in animals chronically fed a high-protein diet [11]. A marked decrease (more than 10%) was found in EDL and kidneys. We believe this is a new and important finding, indicating a possible negative influence of abundant consumption of arginine. The lack of amino acids may exert a negative influence on various metabolic pathways, particularly on protein synthesis.

We suppose that the main cause of the decrease in aminoacemia is the effect of a high concentration of arginine on the rate of transport of various amino acids across the cell membranes and not decreased availability of amino acids in the food. This suggestion is supported by unaltered concentrations of branched-chain amino acids, particularly leucine and isoleucine, which use different transporters than arginine [12].

Greater decreases in amino acid concentration in EDL (muscle composed of white, fast-twitch fibers) than in SOL (muscle composed of red, slow-twitch fibers) corresponds with our previous reports demonstrating that white muscles are more sensitive to various physiological or pathological signals than muscles containing mostly red fibers [13–16].
A noteworthy effect of chronic arginine consumption is an increased concentration of lysine in skeletal muscle. It has been shown that this tissue has a very high affinity for free lysine, and it has been suggested that muscle represents a major storage organ for free lysine [17]. Bergstrom et al. [18] have observed relatively greater elevations of lysine in muscle than of other indispensable amino acids after a protein-rich meal. However, the mechanism by which protein-rich meals or HAD increase lysine concentration in muscle is obscure.

4.4. Other Alterations Induced by HAD

Among the results of the effects of HAD obtained by standard blood biochemistry, the decrease of potassium in blood plasma is of special interest. This contradicts reports of its increase in acute studies in which arginine was infused as arginine monohydrochloride [19–21]. It has been suggested that the rise of potassium may be due to the exchange of cellular potassium for the proton from the arginine hydrochloride [20]. The decrease of potassium in our study, in which a pure L-arginine was used as a supplement, may be related to the well-known stimulatory effect of arginine on insulin secretion. The observed decrease in plasma triglycerides and atherogenicity index is in agreement with other studies reporting the triglyceride-lowering effect of L-arginine [22]. Higher weight and protein content of the kidneys in HAD fed animals is probably related to enhanced intake of nitrogen and not to the specific effect of arginine. We did not find an increase in weights of extensor digitorum longus and soleus muscles in animals fed by HAD as reported recently by Yang et al. [23].

4.5. Effect of Overnight Starvation

Most of the differences observed between animals fed by SLD and HAD in a fed state disappeared after overnight fasting. Minimal changes in amino acid concentrations have also been reported in animals fed a high-arginine diet that fasted 5 h before collection of blood samples for amino acid analysis [23]. These findings indicate that the body can maintain aminoacidemia in physiological ranges in conditions of excessive intake of arginine, and that laboratory biochemistry that is routinely performed after overnight fasting should also be performed in postprandial states in some conditions. It is noteworthy that overnight starvation did not decrease concentrations of arginine that were expected due to supposed up-regulation of arginine catabolizing enzymes, particularly arginase.

5. Conclusions

We conclude that enhanced dietary arginine intake has a significant effect upon the tissue distribution of all amino acids. The increase of arginine and ornithine and a decrease in concentration of a number of amino acids in blood plasma and tissues are among the main findings observed in animals sacrificed in postprandial state. The increase of lysine in skeletal muscle and the decrease of potassium, triglycerides, and atherogenicity index in blood plasma should also be noted. Observation that most of the alterations occurring in the fed state disappeared after overnight starvation indicates that some routine biochemical measurements should be performed in the postprandial state to get a clear picture of the effects of various nutritional interventions.

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Abbreviations

The following abbreviations are used in this manuscript:

SLD standard laboratory diet  
HAD high-arginine diet  
EDL extensor digitorum longus muscle  
SOL soleus muscle  
EAA essential amino acids  
NEAA non-essential amino acids

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