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# Plasma amino acid concentrations in healthy elderly men and women[1,2]

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**ABSTRACT** Postabsorptive plasma concentrations of the large neutral amino acids (LNAAs) were measured in 74 elderly (age 71 ± 8 y) and 138 young (age 26 ± 5 y) healthy subjects. Plasma concentrations of valine, leucine, and isoleucine were significantly lower in young females than in young males. This gender-related difference was not observed among elderly subjects because aging was associated with a significant rise in plasma LNAAs in females but not in males. Multiple-regression analysis of plasma amino acid concentrations from female subjects revealed a significant and positive effect of age on plasma valine, leucine, isoleucine, phenylalanine, and tyrosine but not on plasma tryptophan or methionine. Tryptophan was the only amino acid to exhibit a significant response to age in males, consisting of a 14% decline in the elderly subjects. Percentile ranges are presented for young and elderly females and males for each of the amino acids. *Am J Clin Nutr* 1991;53: 1249–52.

**KEY WORDS** Plasma amino acids, aging, normal values

## Introduction

Plasma concentrations of the large neutral amino acids (LNAAs) are not tightly regulated and can fluctuate over a wide range in response to food consumption and consequent hormone secretion (1–4). Plasma amino acid concentrations are also altered in liver and renal disease (5, 6), in diabetes (7), in obesity (8), in response to stress (9, 10), and after administration of amino acid mixtures or amino acid–containing food additives to normal individuals (11, 12).

Two important physiological determinants of plasma LNAAs in fasting, nonexercising normal subjects are age and gender. Significant differences were described between the plasma concentration of several amino acids in young adult males and females (13). Similarly, data from small numbers of elderly individuals suggest that the age-related decline in insulin responsiveness may cause an elevation in the plasma branched-chain amino acid concentration via a mechanism similar to that observed in obese subjects (14–17).

Characterization of the normal ranges for plasma amino acids is important for clinical and diagnostic purposes and for interpreting results from dietary and metabolic experiments in which changes from a baseline plasma amino acid profile are expected. The present study describes age- and gender-related differences in plasma LNAA concentrations observed in healthy elderly and young subjects of both sexes.

## Subjects and methods

The population studied consisted of 212 adult subjects aged 20–90 y. They were divided into two groups: elderly (age ≥ 55 y, $n$ = 74) and young (age ≤ 40 y, $n$ = 138). All subjects underwent a physical examination, blood tests (complete cell count and hemoglobin, glucose, and blood urea nitrogen concentrations), and urinalysis. Exclusion criteria included abnormal results in those tests; a history of kidney, liver, or metabolic disease; or changes in body weight of ≥ 5% during the 2 mo preceding the study. All subjects were free living, and none was receiving special diets or formulas. Twelve subjects in the elderly group were receiving digitalis, antihypertensive drugs, or nonsteroid anti-inflammatory drugs. Their plasma LNAA concentrations did not differ significantly from those of the same age and gender not receiving these medications.

Venous blood samples were obtained in heparin-treated tubes early in the morning, after an overnight fast. Plasma samples were centrifuged and frozen at −30 °C until analysis. Plasma LNAA concentrations (excluding tryptophan) were determined by using cation-exchange columns in an HPLC system (model 334, Beckman Instruments, Carlsbad, CA), with postcolumn *o*-phtalaldehyde derivatization and fluorimetric detection. Duplicate plasma samples were deproteinized with 5% sulfosalicylic acid containing 120 μmol norleucine/L as an internal standard. A sample of pooled human plasma was included in each run as an interassay control. Coefficient of variability across the study was < 6%. Plasma tryptophan was determined fluorimetrically by the method of Denckla and Dewey (18).

Data distribution was examined by using Levene's test of normality. Comparisons of group means between young and elderly subjects and between females and males were performed by two-way analysis of variance. Tukey's studentized range test was used to determine significance of differences between groups. Confidence intervals and quartile ranges were calculated for each group. Data management was done in the *CLINFO* system (BBN Software, Cambridge, MA) and statistical analysis used the *SAS*

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(SAS Institute, Cary, NC) and *BMDP* (BMDP Software, Los Angeles) software packages.

The relative influences of age and body weight on plasma LNAA concentrations were explored by using multiple-regression analysis with standardized regression coefficients. Standardized coefficients are estimates obtained when all variables in the model are standardized to zero mean and unit variance. The advantage of this approach is that the comparisons are not affected by the scale or range of the different variables in the model because the regression coefficients are in SD units (19). This is particularly important when comparing variables such as age, weight, and the individual amino acids.

The obesity index was calculated by dividing the actual body weight by the desirable body weight for medium-frame individuals of the corresponding sex, taken from the 1959 Metropolitan Life Insurance tables (20).

## Results

The characteristics of the groups studied are presented in **Table 1.** Mean (± SEM) age was 25 ± 5 y in the young group and 72 ± 8 y in the elderly group. Within each age group, there were no significant differences between the mean ages of the male and female groups. The obesity index showed a significant increase with age in both males and females.

The median and range values for the 5th, 25th, 75th, and 95th percentiles of all measured amino acids in each age group are presented in **Table 2.** Young females exhibited significantly lower plasma concentrations of valine, leucine, and isoleucine than those found in the other three groups. The concentration of these amino acids increased significantly in elderly females, canceling the difference between sexes observed for young subjects. In addition, the female group exhibited a significant positive correlation between age and the plasma concentration of all measured amino acids except tryptophan ($P < 0.0001$).

Multiple-regression analysis with standardized coefficients (**Table 3**) revealed significant positive effects of age on the plasma concentrations of leucine, isoleucine, valine, phenylalanine, and tyrosine (but not tryptophan or methionine) for female subjects. At the 50th percentile these concentrations were 22–24% higher for the branched-chain amino acids and 7–13% higher for phenylalanine and tyrosine for the elderly group. In males the only significant age-related difference was a 14% reduction in plasma tryptophan concentrations in the elderly subjects.

TABLE 1
Characteristics of the groups studied*

| | Young group | | Elderly group | |
|---|---|---|---|---|
| | Male ($n$ = 68) | Female ($n$ = 72) | Male ($n$ = 32) | Female ($n$ = 42) |
| Age (y) | 26 ± 4 | 25 ± 5[a] | 71 ± 7[b] | 72 ± 8[b] |
| Weight (kg) | 70.5 ± 10.3[a] | 61.4 ± 9.2[b] | 72.4 ± 9.3[a] | 62.4 ± 8.6[b] |
| BMI† | 24.5 ± 2.5 | 26.1 ± 3.1 | 26.2 ± 2.1 | 28.1 ± 3.0 |
| OI‡ | 1.06 ± 0.12 | 1.05 ± 0.14 | 1.12 ± 0.1 | 1.16 ± 0.14 |

* $\bar{x}$ ± SEM. Groups with different superscript letters are significantly different, $P < 0.05$ (Tukey's studentized-range test).

† Body mass index, expressed as weight (kg)/[height (m)]$^2$.

‡ Obesity index, expressed as actual weight/desirable body weight. (Desirable body weight from ref 20.)

TABLE 2
Percentiles of plasma amino acid concentrations by age group and sex

| Amino acid | 5 | 25 | 50 | 75 | 95 |
|---|---|---|---|---|---|
| | | | *μmol/L* | | |
| Valine | | | | | |
| Young group | | | | | |
| Male | 172 | 210 | 238 | 265 | 338 |
| Female | 144 | 171 | 192 | 212 | 289 |
| Elderly group | | | | | |
| Male | 154 | 211 | 231 | 267 | 294 |
| Female | 174 | 201 | 231 | 263 | 291 |
| Leucine | | | | | |
| Young group | | | | | |
| Male | 90 | 120 | 142 | 156 | 218 |
| Female | 81 | 95 | 109 | 120 | 150 |
| Elderly group | | | | | |
| Male | 75 | 126 | 140 | 157 | 190 |
| Female | 80 | 110 | 133 | 148 | 180 |
| Isoleucine | | | | | |
| Young group | | | | | |
| Male | 45 | 62 | 71 | 84 | 111 |
| Female | 39 | 48 | 54 | 63 | 80 |
| Elderly group | | | | | |
| Male | 42 | 64 | 74 | 81 | 108 |
| Female | 37 | 53 | 68 | 77 | 110 |
| Phenylalanine | | | | | |
| Young group | | | | | |
| Male | 41 | 53 | 58 | 65 | 78 |
| Female | 38 | 48 | 54 | 58 | 72 |
| Elderly group | | | | | |
| Male | 42 | 55 | 61 | 66 | 95 |
| Female | 35 | 50 | 58 | 64 | 108 |
| Tyrosine | | | | | |
| Young group | | | | | |
| Male | 38 | 54 | 61 | 70 | 87 |
| Female | 31 | 47 | 52 | 61 | 72 |
| Elderly group | | | | | |
| Male | 35 | 55 | 63 | 69 | 90 |
| Female | 40 | 50 | 59 | 75 | 88 |
| Tryptophan | | | | | |
| Young group | | | | | |
| Male | 46 | 56 | 63 | 68 | 80 |
| Female | 37 | 46 | 52 | 59 | 69 |
| Elderly group | | | | | |
| Male | 46 | 52 | 54 | 59 | 72 |
| Female | 37 | 46 | 52 | 59 | 69 |
| All branched chain | | | | | |
| Young group | | | | | |
| Male | 318 | 399 | 450 | 508 | 630 |
| Female | 273 | 318 | 347 | 394 | 506 |
| Elderly group | | | | | |
| Male | 289 | 420 | 455 | 492 | 580 |
| Female | 292 | 370 | 442 | 473 | 545 |

## Discussion

These data show that fasting plasma LNAA concentrations are significantly lower in young women than in young men. Further, the data indicate that those concentrations rise significantly with age in women but not in men. Thus, the sex-related differences in plasma amino acid concentrations observed in young subjects disappear in the elderly population. Elderly men,

TABLE 3
Effects of aging on plasma amino acid concentrations in males and females*

| Dependent variable | Standardized coefficient | P |
|---|---|---|
| Valine | | |
| Males | −0.0392 | 0.7089 |
| Females | 0.3636 | 0.0001 |
| Leucine | | |
| Males | 0.0098 | 0.9262 |
| Females | 0.4604 | 0.0001 |
| Isoleucine | | |
| Males | 0.1149 | 0.2746 |
| Females | 0.3328 | 0.0003 |
| Phenylalanine | | |
| Males | 0.1475 | 0.1676 |
| Females | 0.3081 | 0.0008 |
| Tyrosine | | |
| Males | 0.0046 | 0.9647 |
| Females | 0.2519 | 0.0085 |
| Tryptophan | | |
| Males | −0.2864 | 0.0050 |
| Females | −0.0222 | 0.8129 |

* Multiple-regression analysis with age as the independent variable and BMI as a fixed factor. Standardized regression coefficients adjust all variables to zero mean and unit variance, and they represent change in the dependent variable (in SD units), relative to changes of the independent variable (in SD units).

on the other hand, exhibit a consistent decline in plasma tryptophan concentration relative to the young group (Table 2).

Plasma concentrations of most of the physiologically relevant amino acids in healthy adults have been reported before, but many studies pooled values for male and female subjects or did not include a separate elderly group. Studies comparing plasma amino acid concentrations in men and women reported significant sex-related differences in plasma LNAA concentrations for adults, most notably a lower concentration of branched-chain amino acids in females (13, 21). These gender-related differences appear to develop after adolescence, because studies in preadolescent children found no major differences in plasma amino acid concentrations between males and females (13, 22). Similarly, our study found significantly lower levels of the branched-chain amino acids, phenylalanine, and tyrosine in young females.

Several factors may play a role in determining the lower plasma LNAA concentrations in females relative to males. One possible factor could be differences in insulin sensitivity. Young females have higher glucose-mediated insulin output and lower glucose disposal rate per unit of plasma insulin than do men during clamp studies (23). Such increased insulin output could tend to lower the plasma concentrations of the most-insulin-responsive amino acids, the branched-chain amino acids, as described for uremia (6). Another factor possibly affecting plasma LNAA concentrations is body composition. We (24) and others (25) reported significant correlations between the summed plasma concentration of the branched-chain amino acids and lean body mass. Such association would favor a lower plasma concentration of branched-chain amino acids in females relative to males.

Our study found a marked rise in the plasma amino acid concentrations in elderly females whereas no significant changes were observed in elderly males (Table 3). This age-related increase in females affected all LNAAs except for tryptophan, and as a consequence the significant sex-related differences in plasma amino acid concentrations found in young subjects disappeared in the elderly subjects. Galante et al (15) also reported increased plasma concentrations branched-chain amino acids in a group of elderly women, but the group included people with > 200% ideal body weight, which in itself may be associated with hyperaminoacidemia. It is well known that aging per se causes a decrease in insulin sensitivity in normal people (17) and that it potentiates the insulin resistance associated with obesity (26). For amino acids such as the branched chain, which are cleared from the circulation under insulin action in a dose-dependent fashion (27), the age-related decrease in insulin responsiveness could conceivably cause a progressive rise in their plasma concentrations. If this biological decrease in insulin sensitivity is more pronounced (or enhanced by other factors) in females, it could explain in part the larger rise in branched-chain amino acids in elderly women.

All subjects in our study were well-nourished and noninstitutionalized. A recent study in 21 men aged 65–85 y found significantly lower plasma concentrations of the amino acids valine, leucine, isoleucine, phenylalanine, and threonine compared with young control subjects (28) but, as pointed out by the authors, the possibility of a marginal protein intake in the population studied could not be ruled out.

The decline in plasma tryptophan concentrations in elderly males found in our study is consistent with earlier reports. In a group of six elderly men, Fukagawa et al (16) reported that mean tryptophan concentrations was 14.7% lower than in young control subjects, a figure very similar to our own finding of a 14% age-related decline in tryptophan. A similar decline in plasma tryptophan concentrations was found in insulin-resistant obese individuals (8, 29). The opposite age-related changes in plasma tryptophan and branched-chain amino acids causes a significant decline in the tryptophan-LNAA ratio, which correlates with brain tryptophan availability (30). Whether this decrease affects serotonin-dependent mood and behavior in elderly people remains to be explored.

In summary, our study provided normative data on plasma LNAA concentrations in healthy, well-nourished elderly men and women. The gender- and age-related differences reported should be considered when evaluating physiological or experimentally induced changes in plasma amino acid concentrations in groups of elderly people.

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