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Serum amino acid concentrations in aging men and women

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Summary. The age and gender related differences in serum amino acid concentrations have been assessed in 72 (23–92 years) medically screened healthy men and women who were divided into three male and three female groups according to age. Free-time physical activity and food intake were analysed from the 5-day diaries. The subjects were instructed to eat according to their normal dietary habits and to avoid any clinical complementary nutritional products or other products that could increase protein or energy intake. The blood samples (5 ml) taken from the antecubital vein after an overnight fast were analysed for their amino acid contents by chromatography. In total nutrient intake of energy ($P < 0.001$), protein ($P < 0.001$), alcohol ($P < 0.05$), water ($P < 0.01$), sodium ($P < 0.001$) and fiber $P < 0.001$) decreased significantly with age. The concentration of total amino acids ($P < 0.01$), essential amino acids ($P < 0.001$), non-essential amino acids ($P < 0.05$) and branched-chain amino acids ($P < 0.05$) decreased, whereas citrulline ($P < 0.001$) and cysteine ($P < 0.001$) were the only amino acids, which increased with aging. In addition, men had significantly higher concentrations than women of essential amino acids ($P < 0.001$), branched-chain amino acids ($P < 0.001$), and 10 of the 22 individual amino acids assayed ($P < 0.01$). Women had significantly higher concentrations of aspartate ($P < 0.05$), glycine ($P < 0.01$), serine ($P < 0.001$) and taurine ($P < 0.01$) than men. It is concluded that the decrease in serum total amino acid concentration is associated with decreased energy and protein intake with aging and men have higher essential amino acid concentration in serum than women.

Keywords: Amino acids – Non-athletes – Age – Gender – Nutrition

Introduction

Aging is associated with physical inactivity, low energy intake (Dutta and Hadley, 1995; Hallfrish et al., 1990; Rising et al., 1994) and a progressive loss of muscle performance and fat-free mass (most of which is skeletal muscle protein), characterized by a decline in muscle strength and increased muscle fatigability (Evans, 1995; Hurley, 1995). Furthermore, it has been suggested that the contribution of skeletal muscles to the protein turnover in the whole body declines with aging (Fukagawa and Young, 1987) and the loss of skeletal muscle mass diminishes the resistance to stress (Young et al., 1989). All proteins in the body are involved in a continuous remodelling process, which includes synthesis and breakdown (turnover) and contributes to the continuous repair of proteins, the maintenance of their quality and the production of essential proteins (Rooyackers et al., 1996). A loss of proteins results in an imbalance between synthesis and degradation. In the elderly muscle protein synthesis is altered. A decrease in protein synthesis may cause a loss of muscle proteins with aging (Balagopal et al., 1997). Several possible mechanisms have been suggested to lead to decrease in muscle mass and strength (sarcopenia): the loss of alpha-motor neurons in the spinal column, decreased production of growth hormone, androgen and estrogen, reduced physical activity and inadequate protein intake (Holloszy, 1995). Volpi et al. (1998a) concluded that although the muscle mass is decreased in the elderly, the muscle protein anabolism can be stimulated by an increased availability of amino acids and the muscle mass is better maintained upon the high protein intake. Previously, Henry et al. (1974) published reference values for 22 amino acid concentrations for adults in plasma, urine and spinal fluid. Recently, the total amino acid concentrations have been demonstrated to be higher in the elderly (80–100

years) (Bancel et al., 1994) or unchanged, except glutamate and ornithine, which increase and aspartate, which decreases with aging (Volpi et al., 1999).

Gender is an important determinant of the body composition and energy expenditure in humans (Volpi et al., 1998b). The fat-free mass and body cell mass are observed to be smaller in women than in men (Gallagher et al., 1996). This difference may affect the lower energy expenditure that has been reported in women (Ravussin et al., 1986). The lower metabolic rate in women (Arciero et al., 1993; Ferraro et al., 1992) may result from different utilization of energy fuels rather than body composition (Volpi et al., 1998b). Since the gonadotrophic hormones (follicle-stimulating hormone and luteinizing hormone) are known to influence fuel metabolism (Jensen et al., 1994), the gender-based differences in protein metabolism are of great interest. Previously, Volpi et al. (1998b) reported that protein oxidation is lower in women than in men in the basal postprandial state. However, there are discrepancies between study results concerning gender based differences in basal levels of leucine oxidation, but there is evidence that during exercise leucine oxidation is higher in men than in women (Tipton, 2001). Furthermore, the plasma concentrations of valine, leucine, isoleucine, proline, glutamine, glutamate and phenylalanine have been demonstrated to be higher in men (Bancel et al., 1994).

In spite of abundant research on amino acid metabolism, the data are scanty on the changes in amino acid concentrations in blood with aging. In addition, the available information is mostly focused on men. The purpose of this study was therefore to determine if gender or aging per se affects serum amino acid concentrations in sedentary people.

Material and methods

Subjects

Seventy-two healthy non-athletic volunteers (23–92 years old men and women) served as subjects. All were informed of the benefits and risks of the investigation and they gave their written informed consent in accordance with the rules of the Institutional Review Board of the Central Hospital of the local area. Demographic data (age, medication, illnesses, smoking) were gathered with a self-administered questionnaire. All participants had no history or evidence on physical examination of coronary diseases, hypertension, pulmonary diseases, diabetes or back pain and they were not gravid. Each subject was screened to avoid problems that might compromise his or her participation in the study. The subjects were divided into six groups; low-age males 33.3 ± 3.8 (mean ± SD) years (MALE 20–39; n = 12), low-age females 32.6 ± 4.4 years (FEMALE 20–39; n = 12), middle-age males 48.4 ± 6.0 years (MALE 40–59; n = 12), middle-age females 48.0 ± 4.3 years (FEMALE 40–59; n = 12), high-age males 75.9 ± 9.3 years (MALE >60; n = 12) and high-age females 74.7 ± 10.0 years (FEMALE >60; n = 12).

Testing protocol

All the subjects were tested at the Rehabilitation Center (Kankaanpää, Finland). They were familiarized with the test program during 5 days before the measurements. Each subject completed a 5-day dietary record and fasted from 8:30–10:30 pm to 06:30–08:30 am (at least 10 hours) before the blood samples were taken (Fig. 1). The same researcher performed all anthropometrical measurements (height, body weight and skin fold measurements just before the tapping of blood samples (Fig. 1). Body mass was measured to the nearest 0.1 kg by using an electronic scale and standing height was determined with a wall-mounted stadiometer. Body mass index (BMI) was calculated from the ratio of mass (kg)/height (m^2). Fat-percentage was calculated from the bilateral skin fold measurements carried out with the John Bull Skin Fold Caliper (British Indicators, LTD, England). Skin fold readings were taken from four skin fold points (m. subscapularis 2.5 cm below the nipple line at the axillary level, m. triceps brachii at the maximal circumference (MC), m. biceps brachii at the MC and crista iliaca 2.5 cm above the nipple line at the axillary level) from the upper body (Durnin and Rahaman, 1967). These anthropometrical data are given in Table 1.

Physical activity

The subjects were instructed to maintain their normal physical activity throughout the testing period. They reported in diaries their free-time physical activity, as minutes of training, walking and/or run and strength exercise times.

Diet

The food intake was controlled in two consecutive periods: the 5-day familiarization and the 5-day experiment. During both phases the subjects were instructed to eat according to their normal dietary habits and not to eat any clinical complementary nutritional

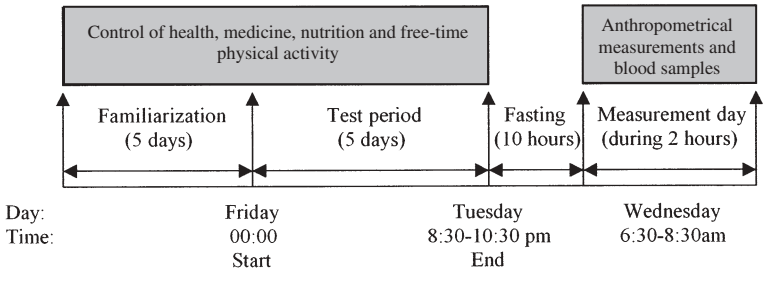

Fig. 1. Schematic diagram of investigation

Table 1. Descriptive characteristics of the subject groups and statistical significances of the main effects of gender (G) and age (A) and their interactions

	Male 20–39		Male 40–59		Male >60		Female 20–39		Female 40–59		Female >60		Effect of G	Effect of A	Effect of G*A
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Antropometry															
Height (m)	1.77	0.07	1.80	0.04	1.72	0.05	1.66	0.04	1.66	0.04	1.58	0.06	$P < 0.001$	$P < 0.001$	NS
Weigth (kg)	78.7	11.7	85.6	14.4	77.0	10.9	66.4	5.9	67.1	11.9	64.0	10.0	$P < 0.001$	NS	NS
BMI[a]	24.9	2.7	26.5	4.0	25.9	3.4	24.1	2.3	24.4	3.6	25.7	2.7	NS	NS	NS
Fat (%)	16.5	2.9	23.0	4.3	25.3	4.3	26.5	4.5	33.1	4.3	35.2	3.9	$P < 0.001$	$P < 0.001$	NS
Free-time physical activity															
Total training time (min)	37.2	36.4	35.0	22.3	34.1	29.2	38.2	32.3	39.3	21.8	34.6	30.3	NS	NS	NS
Total walk/Jogging (min)	29.3	27.0	26.3	22.8	23.0	21.1	25.9	21.8	30.0	12.3	23.9	22.2	NS	NS	NS
Total strength exercise (min)	8.0	11.4	8.9	11.8	11.1	10.3	12.2	16.0	9.1	14.0	11.2	9.4	NS	NS	NS

Data are mean ± SD. [a] *BMI*, body mass index. *NS*, non-significant

products or other products that could increase protein or energy intake. A dietician provided to each of them a food scale and taught the subjects in small groups how to complete the dietary record. The diets were recorded on 3 weekdays (Friday, Monday, Tuesday) and 2 weekend days (Jula et al., 1999). The dietician checked the food records and asked supplemental questions if necessary and then analysed the food records using the Micro Nutrica software (version 3.0, Social Insurance Institution, Finland).

Blood collection and analysis

The blood samples (5 ml) were drawn at 06:30–08:30 Wednesday morning from an antecubital vein (Fig. 1). The samples were centrifuged for 10 min at 3500 rpm to separate cells from serum. The serum samples were immediately frozen and stored at −20°C until analyzed within 2–4 weeks. The samples were thawed on the day of assays, mixed with 50% (v/v) sulfosalicylic acid (final concentration: 5%) and the proteins precipitated by a 10-min centrifugation at 25,000 g. Then, 300 μl of clear supernatant was admixed with 25 μl saturated LiOH and 175 μl 0.2 M lithium citrate buffer (pH 2.20) and subjected to liquid chromatography. Chromatography was by a Shimadzu (Japan) LC-10Avp, equipped with automatic SIL-10Advp sampler, 10 cm precolumn of Ultropac 4 resin (Pharmacia, Sweden), 20 cm separation column of Ultropac 8 resin, CTO 10 Avp oven, SCL 10Avp system controller, and RF-10AXL fluorescence detector. The order of lithium buffers applied was 0.20 M (pH 2.80), 0.30 M (pH 3.00), 0.50 M (pH 3.15), 0.90 M (pH 3.50) and 1.60 M (pH 3.30), and the stepwise increments in the oven temperature from 34°C to 75°C. After separation, the amino acids were detected by post-column derivatization with 0.2% (w/v) o-phthaldialdehyde in 1 M borate buffer (pH 10.4) in the presence of 1% (v/v) ethanol and 0.1% (v/v) mercaptoethanol. The quantification was done with the aid of amino acid solutions of known composition using DL-2,4-diaminobutyric acid as an internal standard.

All samples were analysed in duplicate and the samples from one individual were run in the same assay to minimize interassay variability. Intrassay variation ranged from 1.7% to 2.8% for single amino acids.

Statistical methods

The effect of gender, age and their interaction were tested statistically by analysis of variance based on the model (n = 12 per age group; males 20–39, 40–59 and >60 years and females 20–39, 40–59 and >60 years), in which all these effects were perceived as between subject effects. In addition, if an effect of age was detected, but not an interaction between gender and age, pairwise comparisons were made between different age groups (n = 24 per age group; males and females combined to one age group) using Tukey HSD. If an interaction between gender and age was found, the effect of age was separately estimated for males (n = 12 per age group) and females (n = 12 per age group) using Tukey HSD.

Before the analysis of variance, the accordance of the data with the assumption of equality of group variances was checked by diagnostic methods and Levene's test. In addition, the normality assumption of errors was assessed by graphic presentations, for example by the steam and leaf display and normal probability plot. The distributions of some variables were skewed and the above assumptions were violated. In these cases (alcohol intake, cysteine) a log(x + 1) – transformation was made before the statistical analysis to render the variable distributions correct for the analysis. All analyses were done using the SPSS 10.1. for Windows software package.

Results

The body height and weight were greater in men and height decreased in both genders with age whereas body fat percentage was lower in men and increased in both genders with age ($P < 0.001$; Table 1). There were no significant differences in free-time physical activity between the age groups (Table 1). Men had greater total energy intake ($P < 0.001$), greater protein intake ($P < 0.001$) and total sodium intake ($P < 0.001$) than women. Nutrient intake of total energy ($P < 0.001$), protein ($P < 0.001$), alcohol ($P < 0.05$), water ($P < 0.01$), sodium ($P < 0.001$) and fiber ($P < 0.001$) decreased significantly with age (Table 2). In addition, the consumption of alcohol, especially in 20–39 and 40–59-year-old males and 40–59 year-old females, was markedly higher, because more than 27% of the subjects reported consuming alcohol as 3.0% or more of the total energy intake.

The concentrations of serum amino acids are shown in Table 3. Compared with women, men had significantly higher concentrations of essential amino acids (EAAs; $P < 0.001$), branched-chain amino acids (BCAAs; $P < 0.001$), and 10 out of 22 individual amino acids ($P < 0.01$). On the other hand, women had significantly higher concentrations of aspartate ($P < 0.05$), glycine ($P < 0.01$), serine ($P < 0.001$) and taurine ($P < 0.01$). The total amino acids (TAAs; $P < 0.01$), EAAs ($P < 0.001$), non-essential amino acids (NEAAs; $P < 0.05$) and BCAAs ($P < 0.05$) decreased with age. Citrulline and cysteine were the only single amino acids to be higher ($P < 0.001$) in the older than in the younger groups. In addition, significant interactions between gender and age were observed in 7 out of 22 single amino acids.

The Tukey HSD post hoc test revealed significant differences in the pairwise comparisons of TAAs, EAAs, NEAAs and BCAAs (Fig. 2) and in 8 out of 22 single amino acids (Fig. 3) between the age groups. In addition, significant differences were obtained in 5 single amino acids in men (Fig. 4) and also in 5 single amino acids in women (Fig. 5).

Discussion

The results of this study indicated that all amino acids, except citrulline and cysteine, decreased with aging. However, the serum amino acid concentrations exceeded the plasma reference values of Henry et al. (1974) in all age groups. Furthermore, despite the

Table 2. Macronutrient intakes of the subject groups and statistical significances of the main effects of gender (G) and age (A) and their interactions

	Male 20–39		Male 40–59		Male >60		Female 20–39		Female 40–59		Female >60		Effect of G	Effect of A	Effect of G * A
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Total energy (kJ)	10,946	1,862	9,565	1,491	6,448	1,085	8,485	1,812	6,860	1,124	5,861	1,450	$P < 0.001$	$P < 0.001$	$P < 0.05$
Protein (g)	116	28	106	21	59	12	91	28	72	14	65	14	$P < 0.001$	$P < 0.001$	$P < 0.05$
Protein (g/kg BW)	1.5	0.4	1.3	0.4	0.8	0.2	1.4	0.5	1.1	0.3	0.9	0.2	NS	$P < 0.001$	NS
Protein (%)	18	3	19	4	16	3	18	3	18	3	18	3	NS	NS	NS
Fat (%)	29	5	33	6	31	6	31	8	28	6	32	4	NS	NS	NS
Carbohydrate (%)	49	8	44	10	51	7	50	7	51	7	50	7	NS	NS	NS
Alcohol (%)	4.3	4.6	4.8	4.5	1.9	3.9	0.8	1.0	3.0	3.6	0.8	2.4	$P < 0.05$	$P < 0.05$	NS
Total water (g)	3,059	839	2,647	332	2,100	522	3,020	896	2,483	967	2,292	703	NS	$P < 0.01$	NS
Total sodium (g)	3,915	864	3,969	832	2,710	567	3,198	782	2,674	592	2,471	396	$P < 0.001$	$P < 0.001$	$P < 0.05$
Total fiber (g)	25	6	26	7	20	6	25	7	22	4	17	5	NS	$P < 0.001$	NS

Data are mean ± SD. Values are intake per day. *BW*, body weigth. %, percent of total energy. *NS*, non-significant

Table 3. Serum amino acid concentrations (μmol/L) of the subject groups and statistical significances of the main effects of gender (G) and age (A) and their interactions

	Male 20–39		Male 40–59		Male >60		Female 20–39		Female 40–59		Female >60		Effect of G	Effect of A	Effect of G * A
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Total amino acids	3,085	272	3,121	336	2,832	286	3,050	238	2,889	365	2,706	271	NS	$P < 0.01$	NS
Essential amino acids	1,259	139	1,260	130	1,097	168	1,115	112	1,060	134	966	122	$P < 0.001$	$P < 0.001$	NS
Non-essential amino acids	1,826	162	1,861	215	1,736	154	1,935	183	1,829	262	1,740	180	NS	$P < 0.05$	NS
Branched chain amino acids	495	69	492	77	430	92	386	58	383	57	340	62	$P < 0.001$	$P < 0.05$	NS
Alanine	379	65	406	102	418	87	391	60	347	67	359	64	NS	NS	NS
Arginine	131	26	128	22	118	22	110	20	99	12	106	11	$P < 0.001$	NS	NS
Asparagine	60	8	65	10	54	9	68	12	58	10	53	6	NS	$P < 0.01$	$P < 0.05$
Aspartate	21	8	17	7	13	3	29	12	18	6	16	4	$P < 0.05$	$P < 0.001$	NS
Citrulline	39	6	39	5	48	11	35	6	37	7	44	11	NS	$P < 0.001$	NS
Cysteine	9	8	4	2	15	7	2	3	7	3	16	9	NS	$P < 0.001$	$P < 0.05$
Glutamate Acid	48	18	66	26	44	15	67	23	45	19	41	9	NS	$P < 0.05$	$P < 0.01$
Glutamine	611	75	561	68	515	41	579	54	556	58	525	82	NS	$P < 0.01$	NS
Glycine	250	35	262	47	244	44	306	67	315	100	279	107	$P < 0.01$	NS	NS
Histidine	112	15	107	13	88	13	98	12	96	8	81	14	$P < 0.01$	$P < 0.001$	NS
Isoleucine	79	10	74	17	69	14	53	7	58	9	51	11	$P < 0.001$	NS	NS
Leucine	166	22	151	25	130	32	117	14	121	17	103	17	$P < 0.001$	$P < 0.001$	NS
Lysine	211	33	232	24	191	31	187	25	203	33	181	25	$P < 0.01$	$P < 0.01$	NS
Methionine	31	5	33	6	27	4	24	3	26	4	21	3	$P < 0.001$	$P < 0.001$	NS
Ornithine	77	25	80	14	78	13	64	18	74	18	75	14	NS	NS	NS
Phenylalanine	76	10	67	7	61	9	60	8	60	7	58	9	$P < 0.001$	$P < 0.01$	$P < 0.05$
Serine	131	22	132	21	127	18	167	23	155	22	129	21	$P < 0.001$	$P < 0.01$	$P < 0.05$
Taurine	130	33	150	36	108	31	169	39	155	26	137	33	$P < 0.01$	$P < 0.01$	NS
Threonine	137	15	148	26	128	28	200	33	142	29	134	27	$P < 0.01$	$P < 0.001$	$P < 0.001$
Tryptophan	65	11	54	4	55	8	50	8	51	7	46	9	$P < 0.001$	$P < 0.05$	$P < 0.05$
Tyrosine	71	14	80	14	73	16	58	11	62	11	67	16	$P < 0.001$	NS	NS
Valine	250	39	267	39	231	46	216	40	204	33	186	36	$P < 0.001$	$P < 0.05$	NS

Data are mean ± SD. *NS*, non-significant

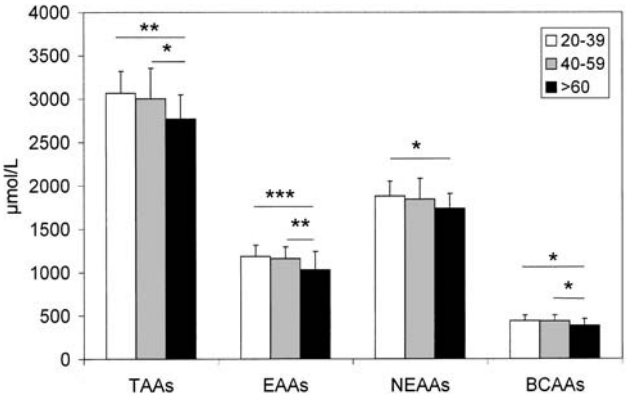

Fig. 2. Comparison in concentrations of TAAs, EAAs, NEAAs and BCAAs between age groups (N = 24 per age groups; 20–39, 40–59 and >60 years). Data are presented as mean ± SD. Tukey HSD test significance; *P < 0.05, **P < 0.01, ***P < 0.001

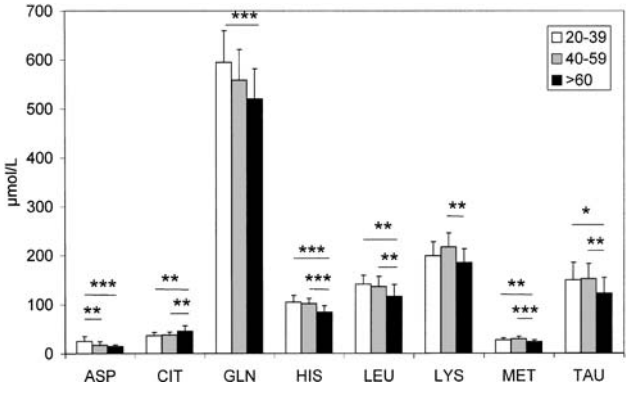

Fig. 3. Comparison in concentrations of aspartate (*ASP*), citrulline (*CIT*), glutamine (*GLN*), histidine (*HIS*), leucine (*LEU*), lysine (*LYS*), methionine (*MET*) and taurine (*TAU*) between age groups (N = 24 per age groups; 20–39, 40–59 and >60 years). Data are presented as mean ± SD. Tukey HSD test significance; *P < 0.05, **P < 0.01, ***P < 0.001

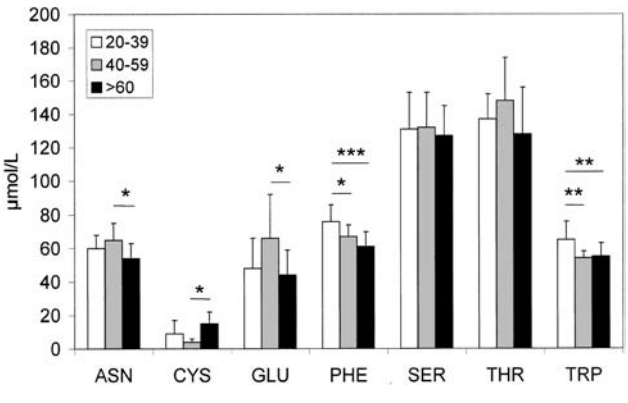

Fig. 4. Comparison in concentrations of asparagine (*ASN*), cysteine (*CYS*), glutamate acid (*GLU*), phenylalanine (*PHE*), serine (*SER*), threonine (*THR*) and tryptophane (*TRP*) between age groups of male (N = 12 per age groups; 20–39, 40–59 and >60 years). Data are presented as mean ± SD. Tukey HSD test significance; *P < 0.05, **P < 0.01, ***P < 0.001

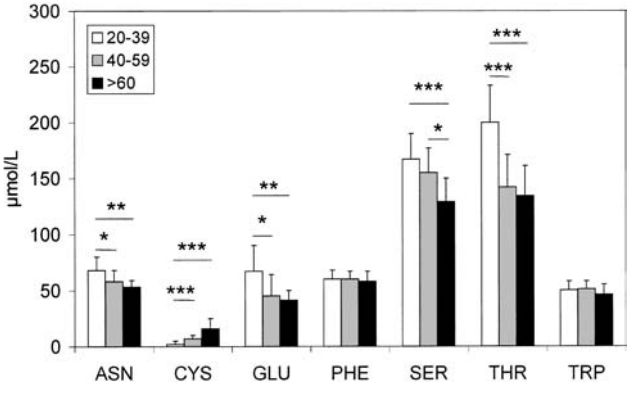

Fig. 5. Comparison in concentrations of asparagine (*ASN*), cysteine (*CYS*), glutamate acid (*GLU*), phenylalanine (*PHE*), serine (*SER*), threonine (*THR*) and tryptophane (*TRP*) between age groups of male (N = 12 per age groups; 20–39, 40–59 and >60 years). Data are presented as mean ± SD. Tukey HSD test significance; *P < 0.05, **P < 0.01, ***P < 0.001

observed decrease in protein intake with aging, all age groups had daily protein intake over recommended dietary allowances (RDA; 0.8 g/kg/day body weight). Compared with women, men had higher concentrations of EAAs, BCAAs and 10 out of 22 single amino acids.

Aging

The strong decrease in the amino acid concentrations in the elderly subjects of this cross-sectional study was associated with the decreased daily intake of energy and protein. The intake of energy varied from 3 to 35% under RDA in males and from 3 to 22% under RDA in females. However, the intake of protein varied from 0 to 87% over RDA in males and from 12 to 75% over RDA in females. Aging is suggested to be associated with the reduced synthesis of muscle protein. This results in a decrease in the muscle mass and protein content (Nair, 1995). However, in this cross-sectional study there were no changes in body mass in either gender with age. The absolute increase in fat percentage was similar in both men and women, which is normal in aging (Proctor et al., 1998). Women appear to lose less total muscle than men with aging, whereas the relative reduction in muscle mass is similar (Going et al., 1995). This process may be due partly to a low energy and protein intake and

consequently to an inadequate supply of amino acids for protein synthesis. The loss of muscle mass and protein with aging is compensated for by the accumulation of body fat (Proctor et al., 1998), which was seen in the present study. Irreversible physiologic changes associated with aging are thought to result from progressive neuromuscular alterations and from decreased levels of anabolic hormones (Lexell et al., 1988; Rudman et al., 1991). Serum total and free testosterone concentrations in healthy men have been shown to decrease (Tenover, 1992) and some changes in body composition may be due to the decrease in growth hormone secretion with aging (Bross, 1999). A decrease in growth hormone and insulin-like growth factor (Tenover, 1992) may also be one explanation for the present reduction in the amino acid concentrations in the elderly.

However, our results are not in agreement with those of Volpi et al. (1999), who found no significant differences in arterial amino acid concentrations between the older subjects (age 71 $\pm$ 2 years) and younger subjects (30 $\pm$ 2 years). Their subjects were also untrained active individuals and the samples were taken in the morning after an overnight fast. In the literature, there are data on blood amino acid concentrations in very old age groups and in the young age groups but there is a lack of data in the middle age groups (e.g. Going et al., 1995; Volpi et al., 1999). The subjects of this study were not either very fit or regularly exercising, and they were instructed to continue with their normal free-time physical activity during the test period. Some of them trained actively and some were totally inactive. However, the free-time physical activity varied only little (under 13% between all groups) between the groups, being thus a minor factor in this study. This study was planned beforehand to be completed under carefully controlled conditions. The food diaries and the physical free-time activity recordings were analysed, since several studies have shown that dietary protein intakes affect both whole body and muscle protein metabolism (e.g. Arnal et al., 1999). In addition, low dietary protein intake may augment the loss of skeletal muscle mass (Starling et al., 1999).

The nutritional status, analysed by the dietician, revealed an inverse relationship between age and energy intake that has been previously shown in both cross-sectional and longitudinal studies (McGandy et al., 1966; Elahi et al., 1983). Because Coran et al. (1992) have indicated older individuals under-report their energy intake, we recruited younger helpers (nurses of the service house, where the oldest subjects visited a few hours per day) to access and scale the intake of the oldest participants (>70 years) during the 5-day records. The decrease in the total energy intake was associated with a diminished protein intake. The relationship between the sources of protein and the concentrations of serum amino acids was unknown, because only the total protein intake was calculated. Previously, Flynn et al. (1992) have demonstrated in a longitudinal study significant decreases in the intake of all nutrients as age increases. In the present study the observed age-related increase in fat percentage is partly explicable by a decrease in the intensity and amount of physical activity and/or by changes in the qualitative characteristics of nutrition and living standards upon aging. Bancel et al. (1994) reported that the concentrations of total plasma amino acids, citrulline, cysteine, histidine, glutamine, glutamate, lysine, ornithine and phenylalanine are higher in the blood plasma of very old subjects (80–100 years) than in younger adults (20–25 years). In our present material this finding could only be confirmed with citrulline and cysteine, whereas in the study of Volpi et al. (1999) the only significant increases were seen in ornithine and glutamate. In our study, the concentrations of citrulline varied in all subjects between 35–48μmol/L (reference values in plasma are 12–55μmol/L; Henry et al.), and the concentration of cysteine varied between 4–16μmol/L (no reference values were presented for cysteine in plasma). Small intestine is the major source of circulating citrulline, which is synthesized from proline, and which is further converted to arginine (Dillon et al., 1999). About 11% of the plasma arginine flux originates from plasma citrulline via de novo synthesis (Castillo et al., 1996). Arginine is the physiological precursor of nitric oxide, which is an important free radical (Moncada and Higgs, 1993), furthermore citrulline has a role in the formation of urea (Di Pasquale, 1997). Cysteine is one of the sulfur-containing amino acids, like taurine. Taurine has been shown to be associated with the impairment of muscle function, e.g., following exhausting exercise both the concentration of taurine in the blood plasma and the excretion into urine markedly increase (Bazzarre et al., 1992; Cuisinier et al., 2001). However, we found no increase in taurine with aging. In this study, the concentration of taurine varied in all subjects between 108–169μmol/L (reference value in plasma are 35–140μmol/L; Henry

et al., 1974). The present increase of cysteine in elderly subjects is a finding confirmed by other investigations (Bancel et al., 1994). We speculate that citrulline and cysteine may be the amino acids related to the impaired muscle function in the elderly. In addition, cysteine is an important antioxidant (Johnson and Hammer, 1992), which may be mobilized by the physiological process of aging.

The total water intake of the subjects in this study was over the recommended (RDA; 1,51 water per day in adults) in all age groups, even though it decreased with aging. Our finding is in line with the study of Schoeller (1989), who found a diminution in total body water in elderly and very old individuals. Physiological causes of dehydration are related to the impaired capacity to conserve water or to the inability of kidneys to concentrate urine effectively in aging subjects (Rowe et al., 1976). Biochemical parameters, including renal function and hydration, are shown to be affected with aging (Mitchell et al., 1982). Among the oldest subjects the intake was 31% less than among the youngest subjects in men and 24% in women, respectively. In the case that the intake would be greater in old subjects, the amino acid concentrations would be lower in older subjects than seen in this study, since the amino acids are concentrated in the blood during dehydration. The real decreases in amino acids in elderly could be thus even greater than exhibited by this study.

Gender

Our data revealed gender-related differences in the amino acid concentrations between men and women, while the concentrations of EAAs and BCAAs were higher in men than in women. Hormones play an important role in the gender-based differences (Tipton, 2001). It has been suggested that estrogen would be required for the maintenance of protein balance and fat-free mass in women (Toth et al., 2000) and that the higher estrogen concentration in women may partly contribute to the lower oxidation of amino acids in women compared to men (Tarnopolsky, 2000). Sex hormones contribute to differences in musculature, since higher testosterone levels in males during puberty increase muscle protein synthesis and net muscle protein balance resulting in greater muscle mass than in females (Kanehisa et al., 1995). Data concerning the effects of ovarian hormones in muscle protein metabolism is scant (Tipton, 2001). In addition, to greater musculature, men have a total blood volume about 6–8% larger than women (Guyton, 1986). Thus the amino acid concentration in blood is diluted more in men compared with women resulting in even greater difference between genders than presented in this study. These findings support the previous evidence that adult men and women exhibit clear differences in the muscle mass and protein metabolism (Bancel et al., 1994; Tipton, 2001).

In conclusion, the observed decreases in the energy, protein and water intakes and in the amino acid concentrations in serum confirm earlier findings with aging. Furthermore, the concentration of EAAs and BCAAs is greater in men than in women. This information may be beneficial for the planning of nutritional support, rehabilitation and pharmacological treatment of elderly women and men.

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