Original Article

Plasma amino acids by age and gender in Hangzhou, Eastern China

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Background and Objectives: Amino acids (AAs) are crucial nutrients and fundamental building blocks of organisms that can be utilized to assess nutritional status and detect diseases. However, insufficient information has been reported on plasma AA in the Eastern Chinese population. **Methods and Study Design:** 1859 persons who underwent physical examination in our hospital from January to December 2020 were enrolled. Plasma AA levels were determined by ultra-performance liquid chromatography mass spectrometry (UPLC-MS/MS.), and the effects of age and sex on 19 plasma AA profiles were analyzed. The Python language was used for data analysis and graphic visualization. **Results:** Plasma arginine, proline, threonine, asparagine, phenylalanine, and glycine in males, and plasma lysine, leucine, proline, valine, isoleucine, alanine, tyrosine, phenylalanine, and hydroxyproline levels in females increased with age. The 2-aminobutyric acid and serine levels in both sexes, and isoleucine, valine, leucine, and histidine levels in males decreased with age. Glycine level was higher in females than in males, and other 17 AAs except arginine and aspartate were higher in males than in females. **Conclusions:** Our study indicated that plasma AA levels can reflect the nutritional status and dietary structure of the population, with high obesity rate and high incidence of chronic diseases in eastern China. Age has certain effects on plasma AA levels, especially compared with sex.

Key Words: amino acid, Eastern Chinese, age, gender, nutritional status

INTRODUCTION

Amino acids (AAs) are essential for sustaining life and play important roles in key metabolic pathways and processes, such as growth, development, reproduction, and regulation of homeostasis.[1] In addition to being substrates for protein synthesis, they participate in gene expression,[2] act as nutrient signalling molecules, regulate cell signalling pathways, synthesize hormones,[3] and modulate the immune response by activating T cells, NK cells, and macrophages.[4]

Under normal physiological conditions, the plasma AA levels in healthy adults are relatively stable[5] and dependent on their biosynthesis and metabolism. Abnormal metabolism of AAs can lead to physical dysfunction. Inherited metabolic disorders (IMDs) are congenital defects of AA synthesis or catabolism[6] that are characterized by intellectual decline, psychomotor retardation, decreased hepatic synthesis, and other symptoms. Phenylketonuria is caused by mutations in the phenylalanine hydroxylase (PAH) gene, which prevents the conversion of phenylalanine into tyrosine and leads to the accumulation of phenylalanine.[7] Maple syrup urine disease (MSUD) is a congenital defect in the branched-chain α-ketoacid decarboxylase complex, resulting in elevated plasma branched-chain amino acids (BCAA) levels and progressive neuropathy.[8] Tyrosinaemia is the accumulation of tyrosine, leading to liver, kidney, bone, and multiple organ damage.[9] The diagnosis and management of IMDs rely on analysis of AAs levels in the blood.

Under stressful conditions (such as disease, trauma, infection, and pregnancy), plasma AA levels are altered.[10] Blood levels of branched-chain AAs (BCAAs) of leucine, isoleucine, and valine are positively correlated with insulin resistance, obesity, and diabetes. Dietary restriction of BCAAs increases glucose tolerance and significantly improves hepatic insulin sensitivity in mice.[11] Histidine

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Manuscript received 23 February 2023. Initial review completed 30 March 2023. Revision accepted 17 April 2023.
doi: 10.6133/apjcn.202306_32(2).0009

level was decreased, and levels of proline, serine, and glycine were increased significantly in patients with non-small-cell lung cancer.[12] The role of glycine in folate metabolism is particularly important in colon cancer.[13] BCAAs were significantly decreased, whereas the aromatic AAs (AAAs) phenylalanine and tyrosine were significantly increased in cirrhotic patients. Elevated Fischer ratios (BCAAs/AAAs) indicate a high risk for hepatic encephalopathy.[14] In addition to metabolic diseases,[15] tumours,[16] and liver diseases,[14] AAs are implicated in neurodegenerative diseases.[16] kidney diseases,[17] sepsis,[18] and infectious diseases.[19] Therefore, AA profiles have potential applications in nutritional status assessment, early diagnosis, treatment, and prognosis assessment.

Previous studies have focused on the relationship between blood AA profiles in patients and healthy individuals. However, there is insufficient information on the plasma AA levels in the general population. We collected fasting plasma and measured the levels of 19 AAs. Our findings will facilitate further investigations of nutritional status, disease diagnosis, differential diagnosis, treatment, and prognosis of eastern Chinese populations.

METHODS

Study population and design

The study was approved by the Regional Ethics Committee of The Second Affiliated Hospital of Zhejiang University School of Medicine (Decision No. I2022351), and all participants gave their informed consent.

From January to December 2020, 2035 community members had physical examination at the Health Management Center of The Second Affiliated Hospital of Zhejiang University School of Medicine (Figure S1). Of these, 63 had cancer, 34 had kidney disease, 1 had haematological disease, 80 had hepatitis B or tuberculosis, 1 was < 20 years of age, and 2 were > 80 years of age; all of these were excluded. Ultimately, 1859 individuals met the inclusion criteria and provided informed consent. The study population included 1261 male individuals (average age 49.4± 10.2 years) and 598 female individuals (average age 50.6 ± 9.89 years). Their characteristics are shown in Table 1. There were 344 participants with abnormal glucose metabolism, 412 participants with hyperuricemia, 1115 participants with dyslipidaemia, 593 participants with elevated blood pressure, and 1014 participants overweight or obese. They were divided into 12 groups according to age ($20 \leq 30$, $30 \leq 40$, $40 \leq 50$, $50 \leq 60$, $60 \leq 70$, and $70 \leq 80$ years) and sex (male and female).

Data collection

Fasting blood samples were taken from the ante-cubital vein using EDTAK tubes, centrifuged at 3500 rpm for 10 min, and then plasma samples were stored at -80℃. Plasma samples (50 μ L) were analysed for the free 2-aminobutyric acid, phenylalanine, alanine, glycine, methionine, arginine, lysine, tyrosine, leucine, proline, hydroxyproline, tryptophan, serine, threonine, aspartate, asparagine, valine, isoleucine, and histidine levels by ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Amino acid standard product was vortexed for 1 min as Marker 7. 0.15 L Marker 7 was added to 0.15 L methyl alcohol and vortexed for 1 min as Marker 6, and Marker 5-1 were prepared sequentially as described above. Markers 1-7, amino acid high and low quality control products were processed in the same way as plasma samples. MassLynx software was used to process data and compute results.

Statistical analysis

Data were analysed using the Python language and add-on packages. Statistical analysis with Scipy, Numpy, Pandas, and Statsmodels and visualization with Streamlit, Plotly, Seaborn, and Matplotlib were used. Data with a normal distribution are described as means ± SDs. Differences among ages were subjected to one-way analysis of variance. Two-independent-samples t-test was used to compare the male and female groups. The significant level was set at $p < 0.05$.

RESULTS

Age-related changes in plasma AAs

The fasting plasma levels of 19 free AAs in males and females are listed in Table 2 and Table 3, respectively. Methionine, tyrosine, and aspartate concentrations in men were similar by age group ($p > 0.05$) (Figure 1a-c). Arginine, tryptophan, threonine, asparagine, and histidine in women were not significantly different within age groups ($p > 0.05$) (Figure 1d-h).

In men, the highest plasma arginine, tryptophan, threonine, and asparagine were in 60–70, 60–70, 50–60, and 70–80 group, (Figure 1d-g). The levels of leucine and histidine in the 60–70 group were significantly lower than younger age groups ($p < 0.05$) (Table 2 and Figure 1h). The highest plasma 2-aminobutyric acid, serine, isoleucine, and valine were in 30–40, 20–30, 20–30, and 30–40 group (Figure 2a-b and Table 2). Plasma phenylalanine and glycine were significantly higher in the 70–80 group than other groups (Figure 2c-d and Figure 3a-b), the plasma alanine in the 20–30 group was significantly lower than other groups ($p < 0.05$, Figure 2e-f).

In women, the highest plasma lysine, leucine, proline, valine, and isoleucine were in 50–60, 50–60, 50–60, 50–60, and 70–80 group (Table 3). The oldest age group had highest plasma tyrosine, phenylalanine, alanine, and hydroxyproline (Figure 1b & Figure 2c-f, and Table 3), which were significantly higher than in other groups ($p < 0.05$). The plasma 2-aminobutyric acid and serine in the 70–80 group were significantly lower than other groups ($p < 0.05$) (Figure 2a-b and Table 3).

Sex-related changes in plasma AAs

Women had higher glycine (Figure 3a-b) than men. Plasma glycine in women aged 40–70 was significantly higher than that in men ($p < 0.05$). Plasma arginine (Figure 1d) and aspartate (Figure 1c) were not significantly different between men and women ($p > 0.05$) in any age group. The other 17 AAs were higher in men than in women in each age group ($p < 0.05$) (Figure 1-3 and Table 2-3).

Table 1. Characteristics of the study population

Sample size	Standard	Number of people			Percentage (%)
		Male	Female	Total	
Age (y)		49.4±10.2y (1261)	50.6±9.89y (598)	(1859)	-
Abnormal glucose metabolism	History of diabetes, or glucose > 6.11mmol/L, or postprandial glucose > 7.78mmol/L, or glycosylated hemoglobin > 6.30%	274	70	344	18.5%
Hyperuricemia	History of gout or hyperuricemia, or uric acid (male) > 428umol/L, uric acid (female) > 357umol/L	345	67	412	22.2%
Dyslipidemia	History of hyperlipidemia, or LDL > 3.42mmol/L, or triglyceride > 1.69mmol/L	814	301	1115	60.0%
Elevated blood pressure	History of hypertension, SP ≥ 140mmHg or DP ≥ 90mmHg	437	156	593	31.9%
Overweight or obesity	BMI≥24	819	195	1014	54.6%

SP: systolic pressure, DP: diastolic pressure, BMI: body mass index.

Table 2. Plasma 19 AAs concentration in male of different ages (mean ± SD) (μmol/L)

Amino acid	Male					
	20~ (n=54)	30~ (n=186)	40~ (n=431)	50~ (n=426)	60~ (n=147)	70~80 (n=17)
2-aminobutyric acid	22.8 ± 6.18	23.7 ± 6.83	23.5 ± 6.62	23.4 ± 6.56	22.1 ± 6.82	20.2 ± 9.04
Phenylalanine	62.8 ± 10.42	63.3 ± 10.1	62.8 ± 9.00	63.7 ± 9.35	63.8 ± 9.24	69.6 ± 15.5
Alanine	329 ± 83.3	360 ± 80.1	354 ± 83.3	363 ± 94.0	360 ± 84.1	387 ± 127
Glycine	212 ± 47.5	204 ± 40.7	199± 39.7	206± 43.1	205 ± 39.1	232 ± 69.0
Methionine	26.6 ± 4.99	26.6 ± 4.80	26.7 ± 4.64	26.6 ± 4.75	26.9 ± 5.09	27.9 ± 6.73
Arginine	39.2 ± 15.2	37.9 ± 16.6	39.1 ± 15.1	40.4 ± 15.9	42.3 ± 15.8	45.7 ± 19.5
Lysine	155 ± 27.1	169 ± 45.6	169± 45.5	169 ± 52.6	168± 47.8	154 ± 58.4
Tyrosine	63.8± 13.9	64.8 ± 12.0	64.2 ± 11.6	65.4 ± 12.1	64.6 ± 11.5	69.0 ± 17.3
Leucine	154 ± 26.6	156 ± 28.0	151± 25.0	149± 25.4	144 ± 22.0	146 ± 47.2
Proline	175 ± 41.7	181 ± 49.7	178 ± 43.2	184 ± 48.9	190 ± 50.5	208 ± 124
Hydroxyproline	19.4 ± 12.3	17.1 ± 9.70	15.8 ± 9.17	15.7± 9.16	17.0 ± 8.95	18.2 ± 11.4
Tryptophan	69.5 ± 25.9	66.4 ± 21.8	62.3 ± 20.6	62.7± 21.1	62.4 ± 20.7	69.8 ± 43.6
Serine	118 ± 22.0	113 ± 21.1	111± 19.1	115± 20.9	111 ± 21.2	118 ± 29.4
Threonine	139 ± 31.1	145 ± 36.9	149 ± 38.6	155 ± 39.7	153.29 ± 36.6	153 ± 40.0
Aspartate	13.0 ± 33.5	13.6 ± 31.3	14.4 ± 34.9	15.1 ± 34.2	12.6 ± 29.28	22.7 ± 52.8
Asparagine	50.4 ± 8.32	49.9 ± 9.19	49.8 ± 8.65	51.4 ± 9.56	51.3 ± 9.14	55.7 ±14.4
Valine	251 ± 44.9	255 ± 42.0	248 ± 40.4	249 ± 42.7	241 ± 34.8	247 ± 74.6
Isoleucine	96.3 ± 25.0	91.1 ± 23.8	88.9 ± 26.6	94.8± 101	86.3 ± 21.3	86.5 ± 39.8
Histidine	106 ± 24.3	113 ± 23.0	111 ± 23.9	110 ± 24.9	105 ± 22.8	113 ± 45.6

Values were shown as means±SD.

Table 3. Plasma 19 AAs concentration in female of different ages (mean ± SD) (μmol/L)

Amino acid	Female					
	20~ (n=13)	30~ (n=71)	40~ (n=211)	50~ (n=213)	60~ (n=75)	70~80 (n=15)
2-aminobutyric acid	19.7± 3.89	21.1 ± 7.32	22.1 ± 6.52	21.2 ± 5.90	20.6 ± 6.30	18.4 ± 4.20
Phenylalanine	55.4 ± 9.57	56.9 ± 12.3	56.7 ± 7.98	57.7 ± 7.99	62.7 ± 12.9	59.5 ± 9.96
Alanine	298 ± 75.0	300 ± 104	308 ± 85.8	338 ± 84.8	362 ± 95.1	362± 92.9
Glycine	212 ± 38.6	223 ± 57.8	228 ± 62.7	244 ± 78.3	239 ± 65.2	224 ± 63.8
Methionine	23.3 ± 4.44	23.7 ± 6.94	23.9 ± 4.46	23.5 ± 4.02	24.8 ± 4.34	24.5 ± 4.56
Arginine	46.7 ± 17.5	40.4 ± 14.2	40.0 ± 17.0	40.7 ± 15.2	42.7 ± 16.6	43.9 ± 13.4
Lysine	136 ± 44.4	134 ± 38.6	142 ± 36.5	150 ± 45.3	150 ± 41.8	164 ± 44.6
Tyrosine	54.8 ± 10.8	54.4 ± 11.9	56.1 ± 10.4	60.0 ± 11.2	64.7 ± 13.7	66.2 ± 10.2
Leucine	118 ± 26.4	116 ± 19.6	119 ± 20.5	122 ± 22.0	124 ± 20.8	118 ± 21.8
Proline	155 ± 34.0	150 ± 44.1	150 ± 45.2	165 ± 50.0	166 ± 50.0	185 ± 52.1
Hydroxyproline	15.8 ± 10.3	14.4 ± 9.94	13.4 ± 10.5	14.2 ± 12.9	13.2 ± 7.43	20.0 ± 9.81
Tryptophan	48.9 ± 9.04	56.0 ± 21.0	54.9 ± 16.2	55.5 ± 18.0	53.9 ± 18.5	59.5 ± 19.6
Serine	121 ± 16.2	116 ± 22.6	115 ± 25.6	112 ± 23.0	110 ± 20.2	107 ± 19.7
Threonine	129 ± 31.6	146 ± 48.0	144 ± 43.7	142 ± 35.8	142 ± 40.4	151 ± 31.9
Aspartate	26.2± 68.4	10.8 ± 18.3	14.2 ± 32.7	12.8 ± 30.3	17.3 ± 45.5	8.14 ± 2.03
Asparagine	44.9 ± 9.18	48.3 ± 10.9	47.3 ± 9.39	47.7 ± 8.18	46.4 ± 7.41	45.9 ± 6.63
Valine	208 ± 38.6	203 ± 43.8	208 ± 38.4	218 ± 43.0	220 ± 35.8	217 ± 34.3
Isoleucine	61.7 ± 22.5	66.6 ± 20.5	69.5 ± 20.6	72.2 ± 22.0	74.9 ± 21.0	82.3 ± 26.9
Histidine	103 ± 17.3	101 ± 20.2	105 ± 22.3	103 ± 20.3	107 ± 20.9	101 ± 15.7

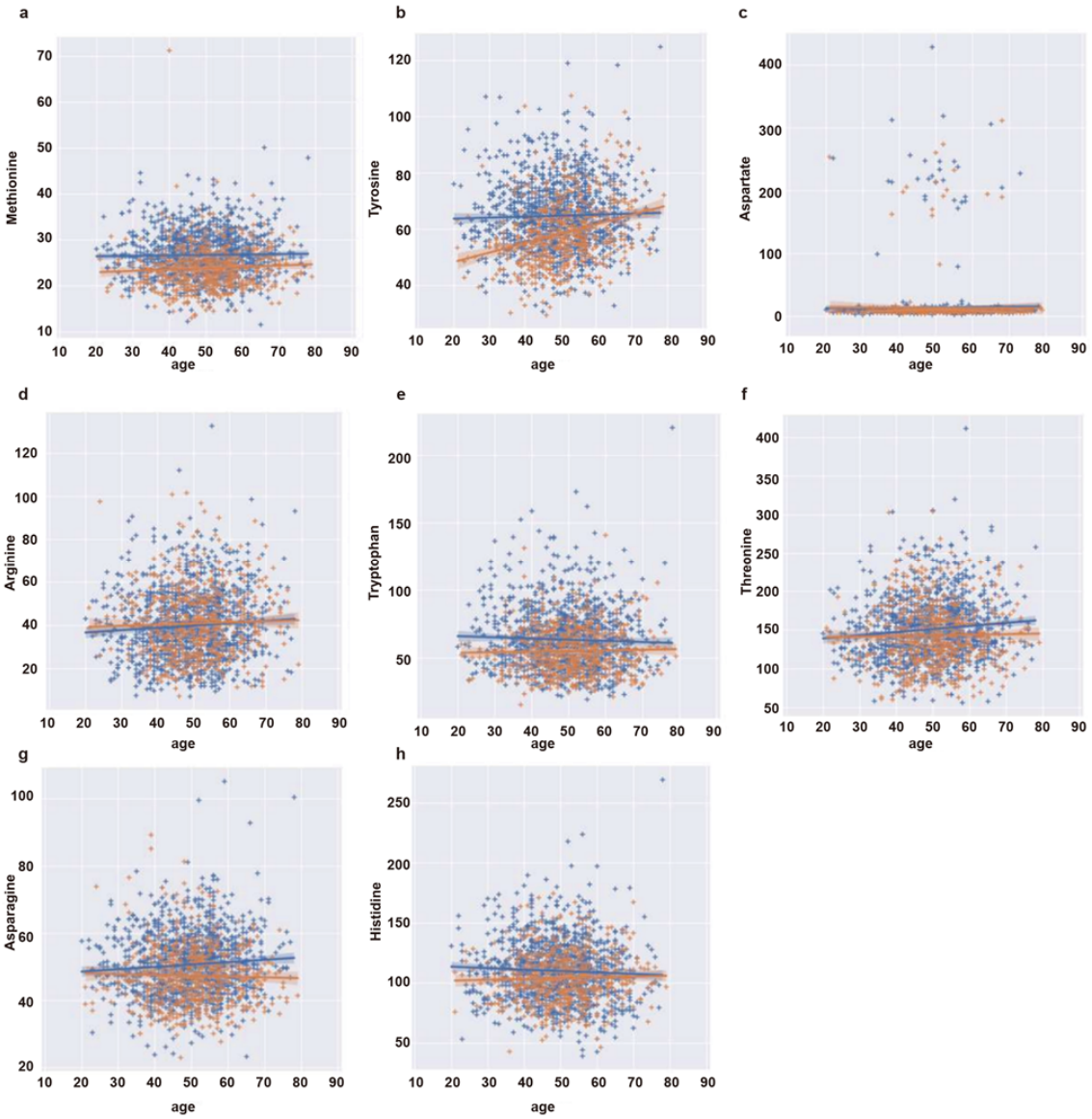

Figure 1. Trends in plasma amino acids concentrations with age. (Blue line is male and orange line is female. a: Correlations between age and methionine, b: Correlations between age and tyrosine, c: Correlations between age and aspartate, d: Correlations between age and arginine, e: Correlations between age and tryptophan, f: Correlations between age and threonine, g: Correlations between age and asparagine, h: Correlations between age and histidine.

DISCUSSION

We used the Python language to assess plasma AA. It is a widely used, interpreted, efficient, and versatile programming language that supports dynamic type validation and provides efficient dynamic data types. Unlike previous reports, this study used data visualisation to present plasma AA changes and trends.

Age, sex, ethnicity, and residence affect plasma AA. High-performance liquid chromatography mass spectrometry has showed that plasma AA profiles of Japanese, South Koreans, and Chinese living in Japan differ by gender rather than by subpopulation.[20] Lawton et al.[21] measured plasma metabolites in healthy Europeans, African Americans, and Hispanics and found little variation in AA profiles by ethnicity. Pedersen et al.[22] revealed that AA varied by place of residence, not ethnicity or diet for Greenlanders and Europe and Danes. However, Tan and Gajra[23] reported different non-fasting plasma AA concentrations between multi-ethnic Singaporean and Caucasian populations. Differences in AA profiles between ethnic population races are much smaller than those observed for gender, age, and place of residence. Therefore, the AA profiles in the present study are likely to represent those in eastern China. Thus, the AA concentrations found in eastern Chinese can be considered similar to those of Japanese, Singaporeans, and Caucasians, although with a wider range (Table 4).[20, 23, 24]

Eastern China has a relatively developed economy, sufficient water resources, and a biodiverse dietary pattern. Na,[25] Shanrong,[26] and Erdai et al. [27] have found that the intake of meat, salt, and oil in eastern China exceeds national recommendations, while the intake of fruits, milk, and dairy products is insufficient. Compared with other regions, eastern China has a higher obesity prevalence and a higher incidence of chronic diseases. In this study, 18.5 % of our population had abnormal glucose metabolism, 31.9 % had elevated blood pressure, and 54.6% were overweight or obese, reflecting the prevalent obesity

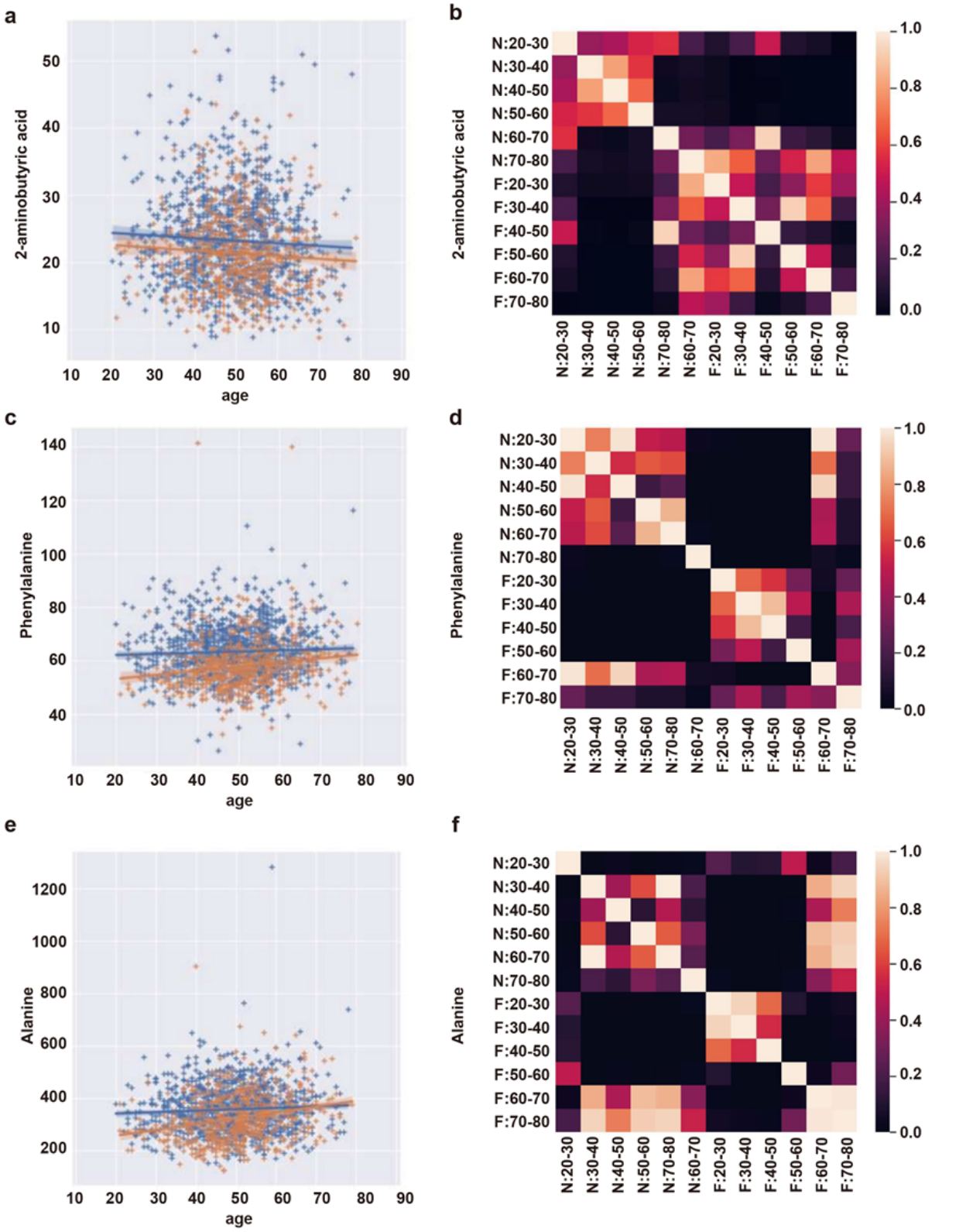

Figure 2. Gender- and age- correlation of amino acids concentrations. (Blue line is male and orange line is female. In the block diagram of heatmap, the darker the color is, the stronger the correlation is. Black block represents $p<0.05$. a: Correlations between age and 2-aminobutyric acid. b. Heatmap of 2-aminobutyric acid between age groups. c: Correlations between age and phenylalanine. d. Heatmap of phenylalanine between age groups. e: Correlations between age and alanine. f. Heatmap of alanine between age groups.

and high incidence of chronic diseases in the eastern Chinese population. The AA profiles of residents of eastern China were similar to those in Japan, Singapore, and the United States (Table 4). By contrast, eastern Chinese had lower plasma arginine and higher valine, leucine, isoleucine and histidine (Table 4). Arginine, a semi-essential AA, is a precursor to nitric oxide (NO) involved in endothelium-dependent vasodilation in the cardiovascular.28 Insufficient in vivo arginine may decrease the NO to increase the risk of cardiovascular and cerebrovascular

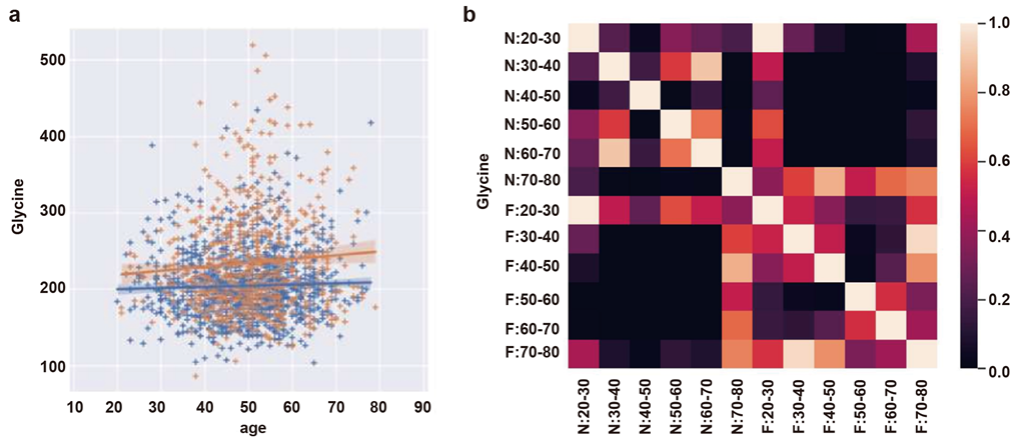

Figure 3. Age and gender correlation of glycine. (Blue line is male and orange line is female. In the block diagram of heatmap, the darker the color is, the stronger the correlation is. Black block represents $p<0.05$.) a. Correlations between age and glycine. B. Heatmap of glycine between age groups.

Table 4. Comparison of amino acids profiles among eastern Chinese, Japanese, Singaporeans and Caucasians (μmol/L)[†]

Amino acid	Eastern Chinese		Japanese[20]	Singaporeans[23]	Caucasians[24]
	n=431M Age: 40-50; plasma Mean ± SD	n=211F Age: 40-50; plasma Mean ± SD	n=1890 (901M, 989F) Age: 20-79; plasma P2.5-P97.5	n=60 (24M, 36F) Age: 20-60y; plasma RI (Median)	n=280 (140M, 140F) Age: 17-65y; plasma RI (Mean)
2-aminobutyric acid	23.5 ± 6.62	22.1 ± 6.52	11.2-31.6	7-51 (28)	8-29 (16)
Phenylalanine	62.8 ± 9.00	56.7 ± 7.98	43.0-72.8	48-73 (58)	36-88 (55)
Alanine	354 ± 83.3	308 ± 85.8	211-477	260-585 (396)	191-531 (350)
Glycine	199 ± 39.7	229 ± 62.7	150-370	135-342 (216)	142-297 (211)
Methionine	26.7 ± 4.64	23.9 ± 4.46	17.8-33.3	26-48 (34)	13-43 (25)
Arginine	39.1 ± 15.1	39.9 ± 16.9	54.3-121	61-132 (94)	48-146 (91)
Lysine	169 ± 45.5	142 ± 36.4	125-237	108-234 (173)	112-271 (175)
Tyrosine	64.2 ± 11.5	56.1 ± 10.4	41.9-80.9	48-96 (67)	30-97 (58)
Leucine	152 ± 25.0	119 ± 20.5	76.7-160	96-203 (125)	56-189 (111)
Proline	179 ± 43.2	150 ± 45.2	74.8-222	82-301 (164)	89-361 (194)
Hydroxyproline	15.8 ± 9.17	13.4 ± 10.5	Nd	5-43 (18)	6-32 (14)
Tryptophan	62.3 ± 20.6	54.9 ± 16.2	42.9-74.4	Nd	Nd
Serine	112 ± 19.1	116 ± 25.6	81.5-154	77-167 (120)	71-165 (111)
Threonine	150 ± 38.6	144 ± 43.7	73.7-169	97-221 (134)	69-182 (120)
Aspartic acid	14.4 ± 34.9	14.2 ± 32.7	33.6-59.2	2-18 (6)	2-11 (5)
Asparagine	49.8 ±8.65	47.3 ± 9.39	Nd	39-83 (53)	18-106 (62)
Valine	249 ± 40.4	208 ± 38.4	143-287	169-354 (226)	109-300 (197)
Isoleucine	88.9 ± 26.6	69.5 ± 20.6	Nd	50-111 (68)	26-95 (54)
Histidine	111 ± 23.9	105 ± 22.3	63.8-97.9	56-113 (77)	58-104 (78)

Nd: not discovered; [†]RI: reference intervals were defined as the 2.5th to 97.5th centile of the distribution.

diseases. Dong et al. conducted a meta-analysis with oral L-arginine intervention ranging from 4 to 24 g/d. Compared with placebo, L-arginine intervention significantly lowered systolic BP by 5.39 mmHg and diastolic BP by 2.66 mmHg, indicating that oral L-arginine can protect cardiocerebral vascular system.[29] Increased BCAAs are related to obesity, insulin resistance, and type 2 diabetes.[30, 31] The high BCAAs among eastern Chinese may reflect a higher risk for obesity and diabetes compared to other regions in China. Histamine is a vasodilator, linked to allergic reactions.[32] The high histidine level in eastern Chinese population may relate to the high incidence of allergy in eastern China.

We observed that arginine, proline, threonine, asparagine, phenylalanine, and glycine in men and lysine, leucine, proline, valine, isoleucine, alanine, tyrosine, phenylalanine, and hydroxyproline in women increased with age. Bancel et al.33 found that people aged 80–100 years had higher plasma of total AAs, cysteine, histidine, glutamine, glutamate, lysine, and phenylalanine than younger adults. Lawton et al.[21] proposed that decreased rate of transamination and subsequent oxidation of carbon skeletons in the citric acid cycle result in decreased AA catabolism and increased blood AAs with age. Lee et al.34 observed that the coenzymes isocitrate and malic acid (intermediates of the citric acid cycle) are increased in older adults, supporting Lawton's hypothesis. During aging, renal function, water retention, and urine concentration decrease, leading to water loss.[35, 36]

In our study, 2-aminobutyric acid, serine, isoleucine, valine, leucine, and histidine levels in males, and the 2-aminobutyric acid and serine levels in females decreased with age. Rudman et al.[37] reported that EAA, tryptophan, histidine, and glycine levels significantly decreased, and glutamine significantly increases in the fasting plasma of elderly subjects. This is associated with reduced energy intake, decreased muscle function, loss of adipose tissue (lean body mass),[38] reduced protein synthesis,[39] and changes in hormones that affect protein synthesis and metabolism, such as insulin, growth hormone, glucagon, and thyroid hormone in the elderly.[40] Pitkänen et al.[41] showed that the levels of total AAs, EAAs, NEAAs, and BCAAs significantly decreased with age, which may be related to reduced energy and protein intake in the elderly.

The plasma levels of 17 AAs in men in our study were higher than those in females, mainly at age 30–60 years, particularly 30–40 years. Glycine was significantly higher in women than men, consistent with previous studies. Bancel et al.[33] reported higher plasma valine, leucine, isoleucine, proline, glutamine, glutamate, and phenylalanine in men than women. Pitkänen et al.[41] reported significantly higher EAAs and BCAAs and significantly lower levels of aspartic acid, glycine, serine, and taurine in men than in women. These sex differences are related to sex hormones. Testosterone, an androgen, promotes protein synthesis and increases lean body mass.[42] In contrast, oestrogen inhibits muscle protein synthesis.[43] Compared with men, women have lower fat-free mass, less energy expenditure,[44] and a lower basal metabolic rate,[45] resulting in lower plasma AA. In the present study, the difference was particularly pronounced at 30–40 years, the period of reproductive sex hormones maturity.

Some plasma AA in the eastern Chinese population do change significantly with age, and the plasma AA in the elderly are affected by multiple factors. The association between gender and plasma AA is stronger than age, so reference ranges for plasma AA should be reported by sex.

CONFLICT OF INTEREST AND FUNDING DISCLOSURES

All authors declare that they have no conflicts of interest.

This work was supported by grants obtained from Zhejiang Basic Public Welfare Research Program (LGF20H150004)

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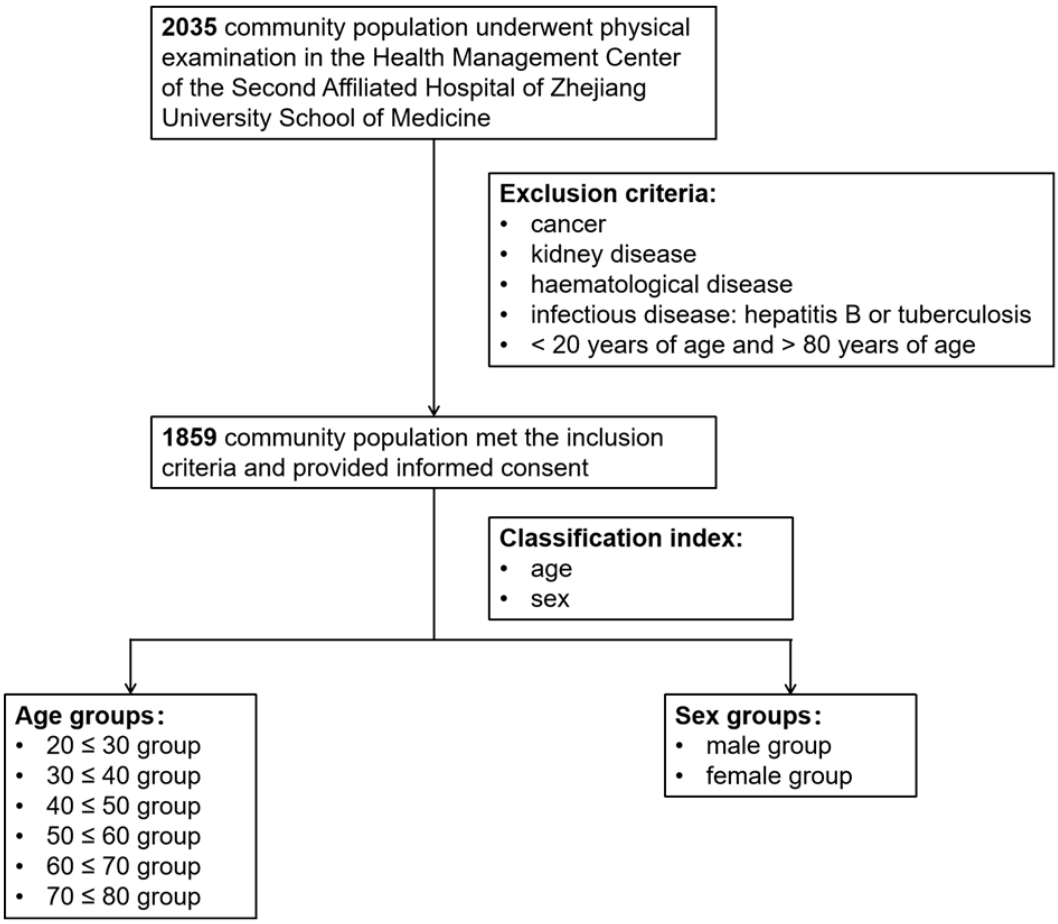

Supplementary figure 1. Participant selection flow chart