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Dietary amino acids intake and all-cause and cause-specific mortality: results from the Golestan Cohort Study

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Abstract

Background Less is known whether the amino acid composition of dietary protein sources effects on long-term health outcomes. We aimed to evaluate the association between dietary amino acid composition and all-cause and cause-specific mortality.

Methods This study used data from the Golestan Cohort Study, which was performed in the Golestan Province of Iran from January 2004 to June 2008. Mortality, which was the primary outcome, was ascertained through September 2022. The Cox proportional hazards regression models were used to determine the adjusted hazard ratios (HR) and 95% confidence intervals (CI) for mortality according to the quintiles of amino acid consumption, taking the third quintile as the reference.

Results A total of 47,337 participants (27,293 [57.7%] women) with a mean (standard deviation) age of 51.9 (8.9) years were included. During a median follow-up of 15 years, 9,231 deaths were documented. Regarding essential amino acid intakes, the HRs of all-cause mortality were 1.16 (95% CI, 1.07–1.26) in the first quintile, compared with the reference group (P for non-linear trend < 0.001). Similarly, non-linear associations were observed between risk of all-cause mortality and intake of branched-chain, aromatic, sulfur-containing, or non-essential amino acids (P for non-linear trend < 0.001 for all comparisons), with higher HRs for participants in the first quintiles. There was an age interaction for the associations between dietary amino acids and mortality (P for interaction ˂0.05). While high amino acid diets were detrimental in middle-aged adults (< 65 years), increased hazards of mortality were observed among older adults (≥ 65 years) with low amino acid intake.

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Conclusions This study showed the non-linear trend between amino acids intake and risk of mortality in the middle-aged and older Iranian population. Overall, our findings suggest that diets lower in amino acids were associated with increased hazards of mortality, particularly among older adults.

Keywords Dietary amino acids intake, All-cause and cause-specific mortality, Protein, Golestan cohort study

Introduction

Dietary choices are important preventable risk factors for mortality [1, 2]. Several dietary recommendations and guidelines have been developed [3, 4] and commonly recommend rich dietary diversity and reduced consumption of particular components known to increase the risk of various diseases [5]. These recommendations include reducing the intake of specific types of fat and carbohydrate like trans fatty acids, saturated fatty acids, added sugars, and refined carbohydrates [5, 6]. Although it has been generally accepted that the types of dietary fat and carbohydrate are significant indicators of diet quality, protein and its constituent amino acids are often neglected and most studies on human nutrition considered protein as a single variable [7].

However, each specific amino acid has unique functions and metabolism [8]. Limited epidemiologic evidence, with inconsistent findings, has demonstrated the contribution of dietary amino acid composition to all-cause and cause-specific mortality. For instance, a cohort analysis reported that higher intakes of proline and glutamic acid are negatively associated with cardiovascular events and that amino acid intake pattern with higher loads of sulfur-containing amino acids (SAAs) is associated with decreased cardiovascular events [9]. In contrast, another study reported an increased risk of cardiometabolic diseases with high SAA dietary patterns [10].

These findings motivate and inspire an important question; whether individual amino acid or amino acid category is associated with the risk of mortality.

Given this context, we aimed to prospectively examine the associations between dietary intake of amino acids considering essential amino acids, non-essential amino acids, branched-chain amino acids (BCAAs), aromatic amino acids (AAAs), and SAAs with total and cause-specific mortality using data from a large-scale population-based cohort study in Iran.

Methods

Study population

We analyzed data from the Golestan Cohort Study, which was performed in the Golestan Province of Iran from January 2004 to June 2008. Details of the Golestan Cohort Study have been published previously [11]. The cohort recruited 50,045 individuals, aged 40–75 years, from Gonbad city and 326 nearby villages.

Participants were interviewed by trained nutritionists and general physicians in order to record data on baseline demographic characteristics and dietary intakes. Two structured questionnaires, including a lifestyle questionnaire and a semiquantitative food frequency questionnaire (FFQ), were employed. The lifestyle questionnaire contained questions on demographics, socio-economic status, education, and history of diabetes or hypertension, smoking, alcohol, and opium use. For all recruited participants, anthropometric indices were also measured at the beginning of the cohort. Participants with incomplete or missing data, extreme energy intakes (<500 kcal/day or >5000 kcal/day), or with an implausible body mass index (BMI) (<15 or >50 kg/m^2) were removed from the analysis. Written informed consent was obtained from all participants. Ethical approval for the Golestan Cohort Study was obtained from the Institutional Review Boards of the Digestive Disease Research Center (DDRC) at Tehran University of Medical Sciences, the US National Cancer Institute (NCI), and the World Health Organization International Agency for Research on Cancer (IARC).

Assessment of diet

Dietary data were collected using a validated 116-item FFQ, which has been designed for the Golestan Cohort Study to indicate nutritional intakes [12]. Participants were asked to indicate the frequency and quantity of the consumed food over the last preceding year on a monthly, weekly, or daily basis and then converted into daily consumption values using household measures [13, 14]. Dietary amino acids contents were analyzed using Nutritionist IV (First Databank, Hearst Corp, San Bruno, CA, USA). The contents of amino acids were expressed as grams per day. Measured intake of amino acids were grouped as essential amino acids (tryptophan, lysine, threonine, isoleucine, leucine, methionine, histidine, phenylalanine, and valine), non-essential amino acids (cysteine, tyrosine, proline, arginine, aspartic acid, alanine, glutamic acid, glycine, and serine), BCAAs (valine, leucine, and isoleucine), AAAs (phenylalanine, tyrosine, and tryptophan), and SAAs (methionine and cysteine).

Assessment of covariates

Information on age, sex, education level, cigarette smoking, alcohol and opium consumption, socio-economic status, physical activity, and history of hypertension or diabetes mellitus were collected using lifestyle questionnaires. Education level was categorized into five groups including illiterate, ≤5 years, 6–8 years, 9–12 years, and college or university level. If someone smoked cigarettes,

used opiates, or drank alcohol at least once a week for a period of six months or more defined as ever in the corresponding variable, otherwise, defined as never. Physical activity was defined by calculating the metabolic equivalent of task per minute per week considering the duration, intensity, and frequency of the physical activity and then grouped into low, medium, and high according to tertiles [15]. Wealth index was defined based on house and appliance ownership as described previously [16], and categorized as low, medium, and high according to the tertiles. Measurements of weight and height were performed following a standardized protocol. BMI was calculated by dividing weight in kilograms by height in meters square. Total energy, lipid, and protein intake were calculated using data from FFQ.

Follow-up and outcomes

Follow-up phone calls or home visits were made annually. During each phone call/home visit a case review questionnaire was completed and vital status of participants was recorded. Additionally, monthly inquiries were made on the vital status of participants by local healthcare providers in rural areas. At the time of analysis, participants were followed-up for a median of 15 years. Each participant's person-years were calculated from the recruitment date to either the end of follow-up or the date of death, whatever occurred first. The primary outcome of the present study was mortality from all causes, cardiovascular disease (CVD), cancer, or other causes, during the first visit until September 2022. In the case of death, two internists completed a verbal autopsy questionnaire [17] and independently investigated all relevant medical records to determine the cause of death. In case of different results, a third, more experienced internist studied the records to make the final diagnosis.

Statistical analysis

All participants were categorized according to dietary quintiles of essential amino acids, non-essential amino acids, BCAAs, AAAs, and SAAs. Baseline data were compared between the lowest (quintile 1) and highest (quintile 5) consumption by using the chi-squared or linear regression test, as appropriate. The Cox proportional hazards regression model was used to determine the adjusted hazard ratio (HRs) with 95% confidence intervals (CIs) for all-cause, CVD, cancer, or other causes of mortality during the follow-up period according to the quintiles of amino acid consumption, taking the third quintile as the reference group. The reference value for estimating HRs and 95% CIs was chosen as 1.

Model 1 (minimally adjusted model) was adjusted for age, sex, and energy intake. Model 2 (fully adjusted model) was additionally adjusted for BMI, cigarette smoking, opium use, diabetes, hypertension, alcohol use, wealth index, education, physical activity, total lipids intake, and total protein intake. The non-linear associations of amino acid intake and cause-specific or all-cause mortality were all plotted and investigated by the restricted cubic spline function using four knots.

To avoid potential surveillance bias, the analyses were also repeated after excluding patients who died, lost to follow-up, or were diagnosed with cancer within the first two years of follow-up (i.e. 2-year lag analysis).

We further stratified our analyses by baseline characteristics of the participants including sex (male or female), age (<65 or ≥65 years), BMI (15-24.9, 25-29.9, 30–50), smoking status (never, ever), history of diabetes (yes or no), and hypertension (yes or no) for potential effect modification. The interaction was tested using multivariate Cox regression models and adjusted for all Model 2 variables, except for the respective stratifying factor. All statistical analyses were performed in STATA software (version 12; StataCorp, College Station, TX) and R software. P-values < 0.05 were considered significant.

Results

Participant's characteristics

This cohort study included data from 47,337 individuals (Fig. 1), including 27,293 (57.7%) women and 20,044 (42.3%) men. Their overall mean (standard deviation) age was 51.9 (8.9) years. During a median follow-up of 15 years, 9,231 deaths were recorded, including 4,215 deaths from CVD, 1,887 deaths from cancer, and 3,373 deaths from other causes. The baseline characteristics of participants, stratified by extreme quintiles of different categories of amino acid consumption, are shown in Table 1.

Compared to individuals in the lowest quintile, those in the highest quintile of all categories of amino acids consumption were more likely to be male, have a higher BMI, and more likely to be alcohol drinkers or cigarette smokers. They were more often highly educated, physically active, and had a higher wealth index. Those with higher dietary amino acid consumption were more likely to have diabetes mellitus, have higher energy intake, total lipid and protein intakes, and were less likely to have hypertension.

Essential amino acids and mortality

In the age-, sex-, and calorie intake-adjusted model (minimally adjusted model), a non-linear association was observed between dietary intake of essential amino acids and hazard of all-cause mortality (P for non-linear trend < 0.001) (Table 2). After fully adjusted model (model 2) was applied, the HRs of all-cause mortality were 1.16 (95%CI, 1.07–1.26) in the first quintile, and 1.08 (95%CI, 1.00-1.16) in the second quintile (P for non-linear trend < 0.001), when compared with the reference group (the third quintile). Figure 2A shows dose-response

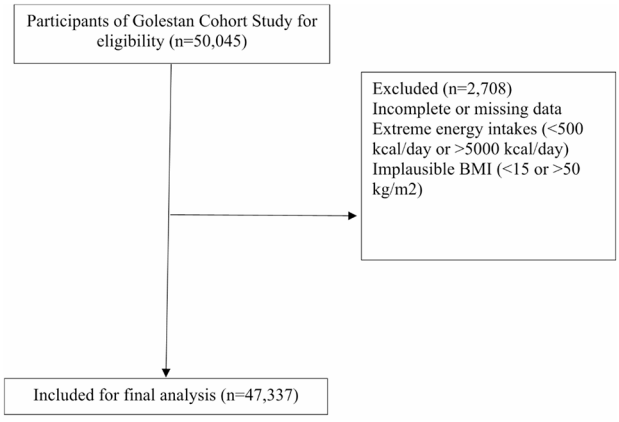

Fig. 1 Flowchart illustrating the selection process of participants

associations between essential amino acid consumption and all-cause mortality in the fully adjusted model, which is slightly U-shaped.

Participants in the first, fourth, and fifth quintiles of essential amino acid intake were at about 11–32% higher risk of CVD mortality, in the minimally adjusted model (P for non-linear trend < 0.001). In the fully adjusted model, compared with the reference group, the HR of CVD mortality was 1.23 (95% CI, 1.09–1.38) in the first quintile (P for non-linear trend = 0.003) (Fig. 2B).

After multivariable adjustment, no significant associations were observed between essential amino acid intake and cancer-related or other causes of mortality.

Non-essential amino acids and mortality

As shown in Table 3, a non-linear trend was found between non-essential amino acid intake and hazard of all-cause, CVD, and other mortality in minimally and fully adjusted models (P for non-linear trend < 0.05) (Fig. 2C-E). Higher HRs of all-cause (Q5 vs. Q3, 1.20; 95% CI, 1.12–1.30), CVD (Q5 vs. Q3, 1.34; 95% CI, 1.20–1.50), and other mortality (Q5 vs. Q3, 1.27; 95% CI, 1.12–1.45) were found in the upper quintile of non-essential amino acid intake in the minimally adjusted model. These associations were attenuated in the fully adjusted model. Moreover, in the fully adjusted model, participants in the lowest quintile of non-essential amino acid intake (median intake of 29.07 g/day) were at higher risk of all-cause mortality (Q1 vs. Q3, 1.16; 95% CI, 1.06–1.26), CVD mortality (Q1 vs. Q3, 1.14; 95% CI, 1.01–1.29), and other mortality (Q1 vs. Q3, 1.16; 95% CI, 1.01–1.33). No significant association was observed between hazard of cancer-related mortality and non-essential amino acid intake.

Branched-chain amino acids and mortality

In the minimally adjusted model, participants in the highest quintile of BCAAs intake were at higher risks of all-cause (HR for Q5 vs. Q3, 1.17; 95%CI, 1.08–1.26), CVD (HR for Q5 vs. Q3, 1.30; 95%CI, 1.17–1.45), and other mortality (HR for Q5 vs. Q3, 1.17; 95%CI, 1.03–1.32). However, when the fully adjusted model was applied, the significance of the associations was lost (Table 4).

In the fully adjusted model, the first quintile was associated with all-cause mortality risk (P for non-linear trend < 0.001). Participants with a median BCAAs intake of 7.87 g/day were at about 15% higher risk of all-cause mortality in the fully adjusted model (HR for Q1 vs. Q3, 1.5; 95%CI, 1.06–1.25) (Fig. 3A).

Furthermore, a non-linear association was noted between BCAAs intake and the risk of CVD mortality, as shown in Fig. 3B. BCAAs intakes within the first quintile were associated with about 19% higher risk of CVD mortality in the fully adjusted model (HR for Q1 vs. Q3, 1.19; 95%CI, 1.05–1.34; P for non-linear trend = 0.005).

Aromatic amino acids and mortality

As shown in Fig. 3C, a non-linear association was observed between dietary aromatic amino acids intake and risk of all-cause mortality (P for non-linear trend < 0.001), with the highest HRs for participants in the first quintile (fully adjusted HR for Q1 vs. Q3, 1.19; 95% CI, 1.09–1.29). A similar trend was found for HRs of mortality from CVD (P for non-linear trend = 0.004) (Fig. 3D). Aromatic amino acids intake within the first quintile (median intake of 4.24 g/day), was significantly associated with increased hazards of CVD mortality (HR for Q1 vs. Q3, 1.21; 95% CI, 1.07–1.36) and cancer mortality (HR for Q1 vs. Q3, 1.28; 95% CI, 1.07–1.54) in the fully adjusted model (Table 5).

Sulfur-containing amino acids and mortality

SAAs intake appeared to be associated with all-cause, CVD, cancer, and other mortality in both minimally and

Table 1 Characteristics of the study population, according to the extreme quintiles of amino acid intake

Variables		Total (*n* = 47,337)	Essential amino acids		Non-essential amino acids		Branched-chain amino acids		Aromatic amino acids		Sulfur-containing amino acids	
			Q1 (*n* = 9,459) < 19.98 g/day	Q5 (*n* = 9,395) > 33.93 g/day	Q1 (*n* = 9,456) < 34.44 g/day	Q5 (*n* = 9,394) > 55.95 g/day	Q1 (*n* = 9,459) < 1.01 g/day	Q5 (*n* = 9,394) > 15.68 g/day	Q1 (*n* = 9,458) < 5.02 g/day	Q5 (*n* = 9,395) > 8.27 g/day	Q1 (*n* = 9,458) < 2.07 g/day	Q5 (*n* = 9,396) > 3.45 g/day
Age (years)		51.9 (8.9)	52.8 (9.1)	52.2 (9.0)	53.1 (9.2)	51.9 (8.9)*	52.8 (9.1)	52.1 (9.0)*	52.9 (9.2)	52.0 (8.9)*	53.0 (9.2)	52.0 (8.9)*
Sex (%)	Female	57.7	25.4	14.7*	25.9	13.8*	25.5	14.4*	25.8	14.1*	25.8	14.4*
	Male	42.3	12.6	26.9*	11.8	28.1*	12.4	27.3*	12.1	27.7*	12.1	27.3*
BMI (kg/m^2)		26.6 (5.4)	25.8 (5.6)	27.1 (5.2)*	25.8 (5.6)	27.1 (5.2)*	25.7 (5.6)	27.2 (5.2)*	25.7 (5.6)	27.1 (5.2)*	25.8 (5.6)	27.1 (5.2)*
Cigarette smoking (%)	Never	82.8	21.1	18.6*	21.2	18.5*	21.2	18.6*	21.2	18.5*	21.2	18.6*
	Ever	17.2	14.4	25.7*	14.0	26.5*	14.2	25.9*	14.3	26.3*	14.1	26.0*
Education (%)	Illiterate	69.8	23.9	16.9*	23.6	17.1*	23.9	16.8*	23.8	17.0*	23.6	17.2*
	≤ 5 years	17.1	13.7	22.5*	13.9	22.7*	13.6	22.5*	13.8	22.6*	13.9	22.8*
	6–8 yeas	4.5	9.0	29.2*	9.6	28.3*	9.1	29.3*	9.2	28.9*	10.2	18.6*
	9–12 years	6.4	7.1	31.9*	8.8	30.8*	7.1	32.5*	7.6	31.6*	8.6	29.6*
	University	2.2	4.3	37.3*	6.0	35.3*	5.6	37.6*	5.0	37.0*	6.1	33.9*
Physical activity (%)	Low	34.2	24.1	17.7*	24.8	16.8*	24.2	17.4*	24.6	17.1*	24.5	17.3*
	Medium	32.5	19.5	19.2*	19.7	18.6*	19.5	19.1*	19.5	18.9*	19.8	18.8*
	High	33.3	15.4	23.0*	14.3	24.4*	15.3	23.3*	14.7	23.9*	14.6	23.7*
Opiate (%)	Never	83.2	19.6	19.6	19.5	19.5	19.5	19.6	19.5	19.6	19.5	19.5
	Ever	16.8	22.1	21.0	22.1	21.5	22.2	21.1	22.3	21.3	22.1	21.3
Alcohol (%)	Never	96.5	20.4	19.3*	20.4	19.3*	20.4	19.3*	20.4	19.3*	20.3	19.4*
	Ever	3.5	8.9	33.7*	9.4	33.5*	8.8	33.9*	9.1	33.7*	9.8	32.5*
Wealth index (%)	Low	35.1	28.7	15.3*	26.4	16.5*	28.5	15.3*	27.7	15.8*	26.8	16.3*
	Medium	31.2	19.3	18.8*	19.6	19.3*	19.3	18.9*	19.5	19.1*	19.5	19.3*
	High	33.6	11.5	25.5*	13.6	23.8*	11.7	25.5*	12.4	24.7*	13.3	24.1*

Table 1 (continued)

Variables		Total (*n* = 47,337)	Essential amino acids		Non-essential amino acids		Branched-chain amino acids		Aromatic amino acids		Sulfur-containing amino acids	
			Q1 (*n* = 9,459) < 19.98 g/day	Q5 (*n* = 9,395) > 33.93 g/day	Q1 (*n* = 9,456) < 34.44 g/day	Q5 (*n* = 9,394) > 55.95 g/day	Q1 (*n* = 9,459) < 1.01 g/day	Q5 (*n* = 9,394) > 15.68 g/day	Q1 (*n* = 9,458) < 5.02 g/day	Q5 (*n* = 9,395) > 8.27 g/day	Q1 (*n* = 9,458) < 2.07 g/day	Q5 (*n* = 9,396) > 3.45 g/day
Diabetes mellitus (%)	No	93.3	20.2	19.4*	20.1	19.4*	20.2	19.4*	20.1	19.4*	20.2	19.4*
	Yes	6.7	16.5	26.6*	17.9	25.8*	16.9	26.5*	17.7	25.9*	17.2	16.3*
Hypertension (%)	No	80.5	19.3	20.0*	19.1	20.2*	19.2	20.0*	19.1	20.1*	19.1	20.1*
	Yes	19.4	22.8	19.3*	23.6	18.3*	23.0	19.1*	23.5	18.8*	23.3	18.8*
Total energy intake (kcal/day)		2129.0 (1778.8–2498.0)	1539.7 (1282.0-1773.0)	2746.4 (2429.6-3137.1)*	1493.6 (1254.7-1698.8)	2814.6 (2532.3-3172.1)*	1528 (1274.4-1752.9)	2767.6 (2457.5-3149.3)*	1509.6 (1262.9-1721.8)	2795.1 (2500.7-3163.6)*	1520.8 (1267.7–1743.0)	2772.2 (2459.7-3154.2)*
Total lipid intake (g/day)		73.8 (60.1–88.5)	53.8 (43.0–64.0)	96.5 (83.5-111.3)*	54.7 (43.4–65.6)	96.3 (83.5-111.2)*	53.8 (42.9–64.0)	96.7 (83.9-111.4)*	54.1 (43.1–64.4)	96.7 (84.0-111.5)*	54.9 (43.7–66.1)	95.4 (82.4-110.4)*
Total protein intake (g/day)		73.2 (59.6–88.4)	47.8 (40.6–52.9)	106.3 (98.2-120.1)*	47.8 (40.6–52.6)	106.3 (98.4-120.1)*	47.8 (40.6–52.8)	106.3 (98.4-120.1)*	47.8 (40.6–52.6)	106.3 (98.4-120.1)*	47.8 (40.6–52.6)	106.3 (98.4-120.1)*

Variables expressed as mean (SD), median (25th percentile – 75th percentile), or percentage

P-trend was tested with linear regression (continuous variables) or with Chi-square test (categorical variables)

*P-value < 0.05 for trend across quintiles

Abbreviations: BMI, body mass index; Q, quintile

Table 2 Multivariate Cox regression derived HRs and 95% CIs for all-cause mortality and cause-specific mortality by essential amino acid intakes

Essential amino acids	Total (*n* = 47,337)	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for non-linearity
Median intake (g/day)	26.30	16.90	22.27	26.31	30.86	39.05	
All-cause mortality							
Number of deaths	9,231	2,093	1,797	1,634	1,734	1,973	
Model 1		**1.16 (1.08–1.25)**	**1.07 (1.00-1.14)**	Ref	1.03 (0.96–1.10)	**1.18 (1.10–1.27)**	**< 0.001**
Model 2		**1.16 (1.07–1.26)**	**1.08 (1.00-1.16)**	Ref	1.02 (0.95–1.10)	1.06 (0.97–1.17)	**< 0.001**
Cardiovascular mortality							
Number of deaths	4,215	978	792	718	808	919	
Model 1		**1.15 (1.03–1.28)**	1.04 (0.94–1.15)	Ref	**1.11 (1.00-1.23)**	**1.32 (1.18–1.46)**	**< 0.001**
Model 2		**1.23 (1.09–1.38)**	1.10 (0.99–1.23)	Ref	1.09 (0.95–1.21)	1.09 (0.95–1.25)	**0.003**
Cancer mortality							
Number of deaths	1,887	398	401	342	357	389	
Model 1		**1.30 (1.11–1.53)**	**1.23 (1.06–1.43)**	Ref	0.95 (0.82–1.11)	0.93 (0.79–1.09)	0.241
Model 2		1.17 (0.98–1.41)	1.14 (0.98–1.33)	Ref	0.97 (0.83–1.14)	1.03 (0.83–1.27)	0.174
Other mortality							
Number of deaths	3,373	772	662	614	614	711	
Model 1		1.10 (0.98–1.24)	1.04 (0.93–1.16)	Ref	0.98 (0.87–1.10)	**1.17 (1.04–1.32)**	**< 0.001**
Model 2		1.06 (0.93–1.21)	1.02 (0.91–1.15)	Ref	0.99 (0.87–1.11)	1.08 (0.92–1.26)	0.083

Significant values are in bold

Model 1: Adjusted for age, sex and intake of energy

Model 2: Adjusted for age, sex, intake of energy, BMI, cigarette smoking, opium use, diabetes, hypertension, alcohol use, wealth index, education, physical activity, total lipid intake, and total protein intake

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; Ref, reference

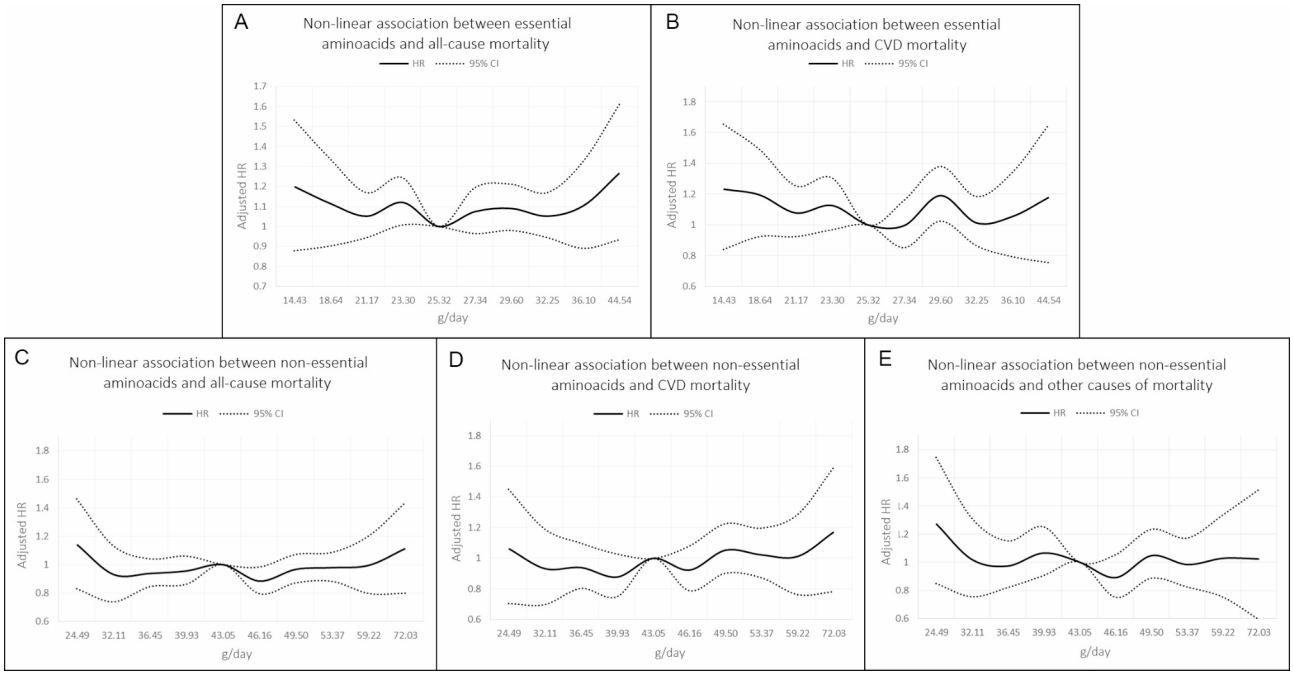

Fig. 2 Multivariable adjusted cubic spline models for the association between essential amino acids intake and HRs for all-cause mortality (**A)** and CVD mortality (**B**); and for the association between non-essential amino acids intake and hazard ratios for all-cause mortality (**C**), CVD mortality (**D**), and other cause of mortality (**D**). Solid lines demonstrate estimates of HRs, while dashed lines demonstrate 95% confidence intervals. Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio

Table 3 Multivariate Cox regression derived HRs and 95% CIs for all-cause mortality and cause specific mortality by non-essential amino acids intake

Non-essential amino acids	Total (*n* = 47,337)	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for Non-linearity
Median intake (g/day)	44.56	29.07	38.21	44.58	51.29	63.54	
All-cause mortality							
Number of deaths	9,231	2,144	1,745	1,674	1,703	1,965	
Model 1		**1.12 (1.04–1.21)**	1.01 (0.94–1.08)	Ref	1.04 (0.97–1.11)	**1.20 (1.12–1.30)**	**< 0.001**
Model 2		**1.16 (1.06–1.26)**	1.03 (0.95–1.10)	Ref	1.02 (0.95–1.09)	1.06 (0.96–1.16)	**< 0.001**
Cardiovascular mortality							
Number of deaths	4,215	981	771	762	795	906	
Model 1		1.01 (0.90–1.13)	0.94 (0.85–1.04)	Ref	1.10 (0.99–1.22)	**1.34 (1.20–1.50)**	**< 0.001**
Model 2		**1.14 (1.01–1.29)**	0.99 (0.89–1.11)	Ref	1.05 (0.94–1.17)	1.05 (0.91–1.21)	**0.016**
Cancer mortality							
Number of deaths	1,887	412	376	371	329	399	
Model 1		**1.28 (1.09–1.51)**	1.08 (0.94–1.26)	Ref	**0.82 (0.71–0.96)**	0.85 (0.71-1.00)	0.126
Model 2		1.18 (0.98–1.42)	1.08 (0.92–1.26)	Ref	0.86 (0.73–1.01)	0.93 (0.75–1.15)	0.185
Other mortality							
Number of deaths	3,373	808	653	582	628	702	
Model 1		**1.19 (1.05–1.34)**	1.07 (0.96–1.21)	Ref	1.11 (0.99–1.24)	**1.27 (1.12–1.45)**	**< 0.001**
Model 2		**1.16 (1.01–1.33)**	1.06 (0.94–1.19)	Ref	1.09 (0.96–1.23)	1.15 (0.97–1.34)	**0.005**

Significant values are in bold

Model 1: Adjusted for age, sex and intake of energy

Model 2: Adjusted for age, sex, intake of energy, BMI, cigarette smoking, opium use, diabetes, hypertension, alcohol use, wealth index, education, physical activity, total lipid intake, and total protein intake

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; Ref, reference

Table 4 Multivariate Cox regression derived HRs and 95% CIs for all-cause mortality and cause specific mortality by BCAAs intake

BCAAs	Total (*n* = 47,337)	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for Non-linearity
Median intake (g/day)	12.21	7.87	10.37	12.22	14.27	17.97	
All-cause mortality							
Number of deaths	9,231	2,110	1,771	1,647	1,737	1,966	
Model 1		**1.16 (1.07–1.24)**	1.05 (0.98–1.12)	Ref	1.03 (0.96–1.10)	**1.17 (1.08–1.26)**	**< 0.001**
Model 2		**1.15 (1.06–1.25)**	1.04 (0.97–1.12)	Ref	1.02 (0.95–1.10)	1.05 (0.95–1.15)	**< 0.001**
Cardiovascular mortality							
Number of deaths	4,215	979	784	736	795	921	
Model 1		1.10 (0.99–1.23)	1.00 (0.91–1.11)	Ref	1.08 (0.97–1.19)	**1.30 (1.17–1.45)**	**< 0.001**
Model 2		**1.19 (1.05–1.34)**	1.05 (0.94–1.17)	Ref	1.06 (0.95–1.18)	1.07 (0.93–1.23)	**0.005**
Cancer mortality							
Number of deaths	1,887	407	390	347	352	391	
Model 1		**1.33 (1.13–1.56)**	**1.19 (1.03–1.38)**	Ref	0.92 (0.76–1.06)	0.90 (0.76–1.06)	0.159
Model 2		1.19 (0.99–1.42)	1.09 (0.93–1.28)	Ref	0.93 (0.79–1.09)	0.98 (0.80–1.21)	0.150
Other mortality							
Number of deaths	3,373	782	650	605	639	698	
Model 1		**1.13 (1.00-1.27)**	1.04 (0.92–1.16)	Ref	1.04 (0.93–1.16)	**1.17 (1.03–1.32)**	**< 0.001**
Model 2		1.08 (0.95–1.24)	1.01 (0.90–1.14)	Ref	1.04 (0.92–1.17)	1.06 (0.91–1.24)	0.112

Significant values are in bold

Model 1: Adjusted for age, sex and intake of energy

Model 2: Adjusted for age, sex, intake of energy, BMI, cigarette smoking, opium use, diabetes, hypertension, alcohol use, wealth index, education, physical activity, total lipid intake, and total protein intake

Abbreviations: BCAAs, branched-chain amino acids; BMI, body mass index; CI, confidence interval; HR, hazard ratio; Ref, reference

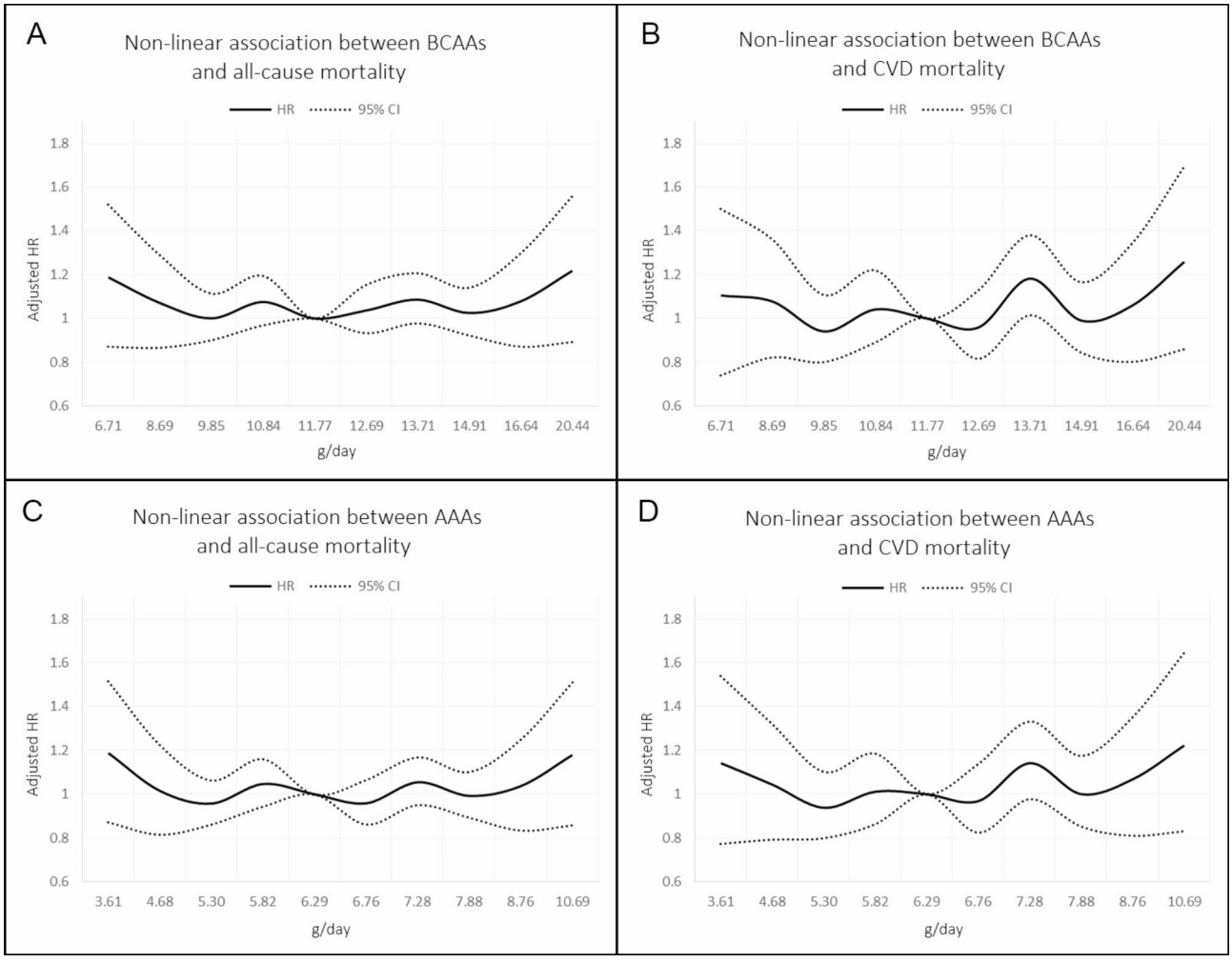

Fig. 3 Multivariable adjusted cubic spline models for the association between BCAAs intake and HRs for all-cause mortality (**A**) and CVD mortality **(B)**; and for the association between AAAs intake and hazard ratios for all-cause mortality (**C**) and CVD mortality (**D**). Solid lines demonstrate estimates of HRs, while dashed lines demonstrate 95% confidence intervals. Abbreviations: AAAs, aromatic amino acids; BCAAs, branched chain amino acids; CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio

fully adjusted models (P for non-linear trend < 0.05), as demonstrated in Fig. 4A-D.

Those who were in the highest quintile of SAAs intake (3.96 g/day) had increased hazards of all-cause, CVD, and other mortality in the minimally adjusted models (P for non-linear trend < 0.001). However, this trend did not remain significant in the fully adjusted model.

Compared with those in the reference group, higher HRs for all-cause (HR for Q1 vs. Q3, 1.18; 95% CI, 1.09–1.28), CVD (HR for Q1 vs. Q3, 1.17; 95% CI, 1.03–1.32), cancer (HR for Q1 vs. Q3, 1.21; 95% CI, 1.01–1.45), and other (HR for Q1 vs. Q3, 1.18; 95% CI, 1.03–1.35) mortality were observed in the fully adjusted model for participants in the first quintile, with a median SAAs intake of 1.75 g/day (Table 6).

Each specific amino acids and mortality

We further investigated the associations between dietary intake of each amino acid and all-cause and cause-specific mortality. Minimally and fully adjusted HRs are presented in Supplementary Tables 1–18.

In the fully adjusted model, lower intakes of amino acids (first quintile) compared with third quintile were associated with about 12–18% increased hazards of all-cause mortality, for all amino acids except lysine (P for non-linear trend < 0.05 for all comparisons). Furthermore, high intake of proline was associated with significantly increased hazards of all-cause mortality (HR for Q5 vs. Q3, 1.11; 95% CI, 1.01–1.22).

In the fully adjusted model, participants with lower consumption of amino acids (first quintile) had about 13–22% higher hazards of CVD mortality, compared with the reference group (P for non-linear trend < 0.05 for all comparisons). However, such association was not

Table 5 Multivariate Cox regression derived HRs and 95% CIs for all-cause mortality and cause specific mortality by AAAs intake

AAAs	Total (*n* = 47,337)	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for Non-linearity
Median intake (g/day)	6.52	4.24	5.56	6.52	7.56	9.44	
All-cause mortality							
Number of deaths	9,231	2,147	1,745	1,647	1,733	1,959	
Model 1		**1.18 (1.10–1.27)**	1.05 (0.98–1.12)	Ref	1.05 (0.98–1.12)	**1.19 (1.10–1.28)**	**< 0.001**
Model 2		**1.19 (1.09–1.29)**	1.05 (0.97–1.13)	Ref	1.03 (0.96–1.11)	1.05 (0.95–1.15)	**< 0.001**
Cardiovascular mortality							
Number of deaths	4,215	992	777	745	787	914	
Model 1		1.10 (0.99–1.23)	1.00 (0.90–1.10)	Ref	1.08 (0.98–1.20)	**1.32 (1.18–1.47)**	**< 0.001**
Model 2		**1.21 (1.07–1.36)**	1.04 (0.94-1.16)	Ref	1.05 (0.94–1.17)	1.05 (0.91–1.21)	**0.004**
Cancer mortality							
Number of deaths	1,887	422	372	347	350	396	
Model 1		**1.42 (1.21–1.67)**	**1.16 (1.00-1.35)**	Ref	0.92 (0.79–1.07)	0.90 (0.76–1.06)	**0.026**
Model 2		**1.28 (1.07–1.54)**	1.10 (0.94–1.29)	Ref	0.95 (0.81–1.11)	0.97 (0.78–1.19)	0.052
Other mortality							
Number of deaths	3,373	790	655	594	640	694	
Model 1		**1.17 (1.03–1.32)**	1.08 (0.96–1.21)	Ref	1.09 (0.97–1.22)	**1.21 (1.07–1.37)**	**< 0.001**
Model 2		1.11 (0.96–1.27)	1.05 (0.93–1.18)	Ref	1.06 (0.94–1.20)	1.1 (0.94–1.28)	0.058

Significant values are in bold

Model 1: Adjusted for age, sex and intake of energy

Model 2: Adjusted for age, sex, intake of energy, BMI, cigarette smoking, opium use, diabetes, hypertension, alcohol use, wealth index, education, physical activity, total lipid intake, and total protein intake

Abbreviations: AAAs, aromatic amino acids; BMI, body mass index; CI, confidence interval; HR, hazard ratio; Ref, reference

evident for cysteine, glutamic acid, and glycine. Moreover, participants with high intake of glycine had significantly higher hazards of CVD mortality (HR for Q5 vs. Q3, 1.13; 95% CI, 1.00-1.29).

Increased adjusted HRs of cancer-related mortality were found among those who consumed low amount of tryptophan (Q1 vs. Q3, 1.22; 95%CIs, 1.02–1.47), threonine (Q1 vs. Q3, 1.21; 95%CIs, 1.00-1.45), phenylalanine (Q1 vs. Q3, 1.24; 95%CIs, 1.03–1.50), tyrosine (Q1 vs. Q3, 1.21; 95%CIs, 1.01–1.45), valine (Q1 vs. Q3, 1.20; 95%CIs, 1.00-1.44), arginine (Q1 vs. Q3, 1.24; 95%CIs, 1.04–1.48), glycine (Q1 vs. Q3, 1.21; 95%CIs, 1.02–1.44), and serine (Q1 vs. Q3, 1.25 (1.04–1.50).

Adjusted HRs of other causes of mortality were increased among participants within the first quintile of cysteine (Q1 vs. Q3, 1.23; 95%CIs, 1.07–1.42), arginine (Q1 vs. Q3, 1.16, 95%CIs, 1.01–1.32), glycine (Q1 vs. Q3, 1.14; 95%CIs, 1.00-1.30), and proline (Q1 vs. Q3, 1.15; 95%CIs, 1.00-1.32).

Stratified and sensitivity analyses

As a sensitivity analysis, the robustness of data was further examined by excluding individuals who lost to follow-up, died or were diagnosed with cancer within the first two years of the study. In the two-year lag analysis, the risk of all-cause and cause-specific mortality did not differ significantly among the extreme quintiles of amino acids consumption (data not shown).

We conducted stratified analyses to investigate whether the associations between categories of amino acids and mortality were modified by baseline characteristics. Stratification by age, revealed significant interactions between age and intake of essential amino acids (P for interaction = 0.025), BCAAs (P for interaction = 0.010), AAAs (P for interaction = 0.011), and SAAs (P for interaction = 0.038), in relation to hazards of all-cause mortality. Among those younger than 65 years, increased hazards of mortality in higher intakes were observed. However, in older adults (≥65 years), lower intakes were associated with increased hazards of mortality (Fig. 5). After stratification by smoking status, there was a significant interaction between smoking status and BCAAs and AAAs intake in relation to the risk of all-cause mortality (P for interaction ˂0.05). In general, the higher risk of all-cause mortality in lower amino acid consumption appeared to be more pronounced in ever-smoker participants in comparison to never-smoker participants. For non-essential amino acids, the associations appeared to be greater among diabetic participants (P for interaction 0.026). In SAAs, in addition to age, significant interaction with sex was also observed for all-cause mortality (P for interaction 0.027). No significant interactions have been found across the stratum for BMI and history of hypertension (Supplementary Figs. 1–5).

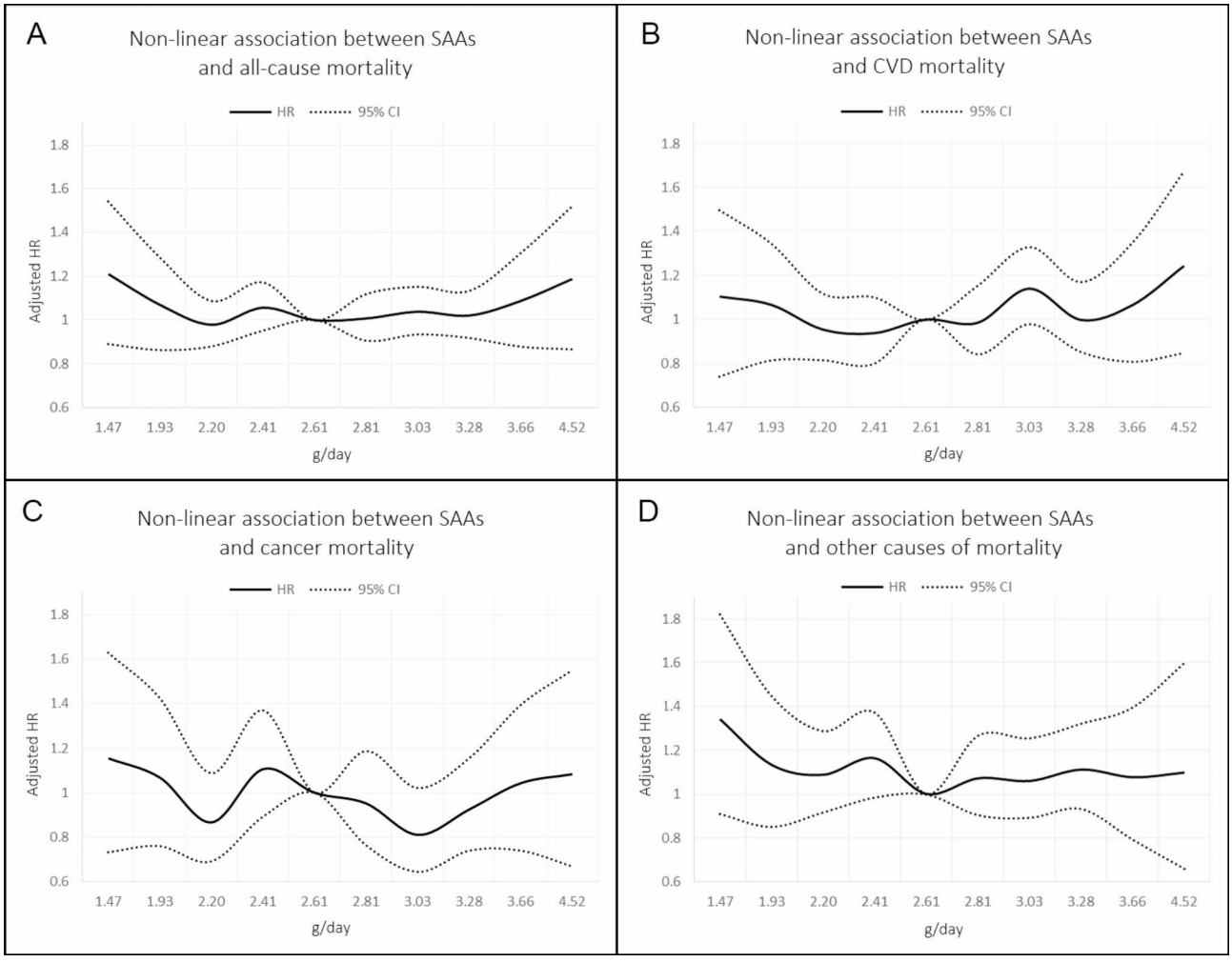

Fig. 4 Multivariable adjusted cubic spline models for the association between SAAs intake and HRs for all-cause mortality (**A**), CVD mortality (**B**), cancer mortality (**C**) and other causes of mortality (**D**). Solid lines demonstrate estimates of HRs, while dashed lines demonstrate 95% confidence intervals. Abbreviations: CI, confidence interval; CVD, cardiovascular disease; HR, hazard ratio; SAAs, sulfur-containing amino acids

Discussion

In this large Iranian prospective study, we found evidence for the association of dietary patterns of amino acids with mortality from all causes, CVD, cancer, or other causes. These findings tended to follow non-linear associations. Lower amino acid intake within the first quintiles compared to the median quintile, was either associated with increased mortality risk or had no added benefit. One important finding of our study was the age interaction for the associations between dietary amino acids and mortality. While a high amino acid diet, irrespective of the amino acid group, was detrimental in middle-aged adults, the opposite effect was found in older adults and increased hazards of mortality were evident among those with low amino acid intake. Accordingly, it would be of great significance for older adults to consume sufficient dietary amino acids, from the view of longevity. Previous studies carried out in older adults also found an inverse association between protein intake and mortality [18–21]. Inadequate protein intake in older adults contributes to frailty, sarcopenia, osteoporosis, and impaired immune responses [22–24]. The responsiveness to the anabolic stimulus of amino acid intake decreases by the increasing age [25]. Several factors may cause decreased sensitivity of muscles in response to dietary protein intakes in older individuals including: impaired protein digestion and amino acid absorption, increased splanchnic amino acids retention and subsequent decreased circulatory amino acid levels, decreased physical activity, decreased amino acid uptake by muscles, and impaired intracellular anabolic signaling [26].

Nonetheless, this decreased sensitivity in older persons can be overcome by higher amounts of amino acids consumptions. Induction of responses similar to those in younger adults by larger dose of protein provides evidence for favorable effects of higher protein intake in older ages [27]. On the other hand, Levin et al. reported higher risks of all-cause and cancer mortality among

Table 6 Multivariate Cox regression derived HRs and 95% CIs for all-cause mortality and cause specific mortality by SAAs intake

SAAs	Total (*n* = 47,337)	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for Non-linearity
Median intake (g/day)	2.70	1.75	2.30	2.70	3.14	3.96	
All-cause mortality							
Number of deaths	9,231	2,134	1,731	1,678	1,711	1,977	
Model 1		**1.15 (1.07–1.24)**	1.01 (0.94–1.08)	Ref	1.02 (0.94–1.08)	**1.19 (1.10–1.28)**	**< 0.001**
Model 2		**1.18 (1.09–1.28)**	1.02 (0.95–1.10)	Ref	1.00 (0.95–1.08)	1.04 (0.95–1.14)	**< 0.001**
Cardiovascular mortality							
Number of deaths	4,215	977	761	754	800	923	
Model 1		1.06 (0.95–1.18)	0.95 (0.86–1.05)	Ref	1.09 (0.98–1.20)	**1.33 (1.19–1.48)**	**< 0.001**
Model 2		**1.17 (1.03–1.32)**	0.99 (0.89–1.11)	Ref	1.05 (0.94–1.16)	1.07 (0.93–1.22)	**0.003**
Cancer mortality							
Number of deaths	1,887	420	368	366	333	400	
Model 1		**1.32 (1.13–1.55)**	1.07 (0.92–1.24)	Ref	**0.84 (0.72–0.98)**	0.89 (0.76–1.05)	**0.023**
Model 2		**1.21 (1.01–1.45)**	1.04 (0.89–1.21)	Ref	0.87 (0.74–1.02)	0.97 (0.79–1.19)	**0.036**
Other mortality							
Number of deaths	3,373	792	657	598	628	698	
Model 1		**1.17 (1.04–1.32)**	1.07 (0.95–1.19)	Ref	1.05 (0.94–1.18)	**1.20 (1.07–1.36)**	**< 0.001**
Model 2		**1.18 (1.03–1.35)**	1.08 (0.95–1.21)	Ref	1.05 (0.93–1.18)	1.06 (0.91–1.24)	**0.032**

Significant values are in bold

Model 1: Adjusted for age, sex and intake of energy

Model 2: Adjusted for age, sex, intake of energy, BMI, cigarette smoking, opium use, diabetes, hypertension, alcohol use, wealth index, education, physical activity, total lipid intake, and total protein intake

Abbreviations: BMI, body mass index; CI, confidence interval; HR, hazard ratio; Ref, reference; SAAs, sulfur-containing amino acids

middle-aged adults with higher protein intakes, who received 20% or more of their required calories from proteins [21], which was supported by our results.

We found that essential amino acid intakes within the first quintile (median intake of 16.9 g/day) were associated with increased risk of all-cause and CVD mortality. The association of low intake of essential amino with the increased risk of all-cause and CVD mortality in the present study among Iranians is concordant with those reported by Ha et al., in an analysis from a national cohort survey in the U.S., with a median intake of 11.7 g/day [28]. On the other hand, an association of diets with high positive loadings for essential amino acids and CVD mortality was found among adults in Canada and the U.S [29]. Given the paucity of evidence on these associations for essential amino acids, further studies are warranted to provide information on the association and its direction.

We also found increased hazard of all-cause, CVD, and other causes of mortality in low intake of non-essential amino acids (median intake of 29 g/day). Previously, it was shown that diets with high loading on non-essential amino acids particularly arginine, glycine, and asparagine had negative association with risk of CVD mortality [29]. Plant-based proteins contain relatively high non-essential amino acids and low essential amino acids content, when compared with animal-based proteins [30]. Considerable evidence has been accumulated regarding the beneficial role of plant-based protein in long-term health outcomes. When animal proteins such as egg, red and processed meats were replaced by plant-based proteins, lower risk of total, cancer, and CVD mortality has been observed [31–33]. Our data suggest that the beneficial effects of plant-based protein might not be related to their amino acid contents and attract the attention to the other components related to protein-rich foods. Whole grain food contains high bioactive substances, including antioxidants, minerals, vitamins, phenolic compounds, and phytoestrogens [34], associated with reduced CVD risk factors [35]. Additionally, the effects of red meat on human health outcomes might be due to ingredients like sodium, nitrates, nitrites, and heme iron [36, 37].

Regarding BCAAs, our study indicated increased risks of all-cause and CVD mortality in those with lower BCAAs intake (median intake of 7.9 g/day). Previous studies on dietary BCAAs intake patterns and mortality have produced mixed results. High BCAAs intake has shown adverse associations with CVD mortality in adults living in the U.S. and Canada [29]. In our study, such association was evident in the minimally adjusted model, however, this did not remain significant after further adjustment for potential confounders. In a population from the Third National Health and Nutrition Examination Survey (NHANES III) database, BCAAs intake was inversely associated with the risk of all-cause mortality, but was not associated with cancer or CVD mortality [38]. The results from the Health Professionals Follow-up Study (HPFS) and Nurses' Health Study (NHS) were suggestive of an adverse association between high

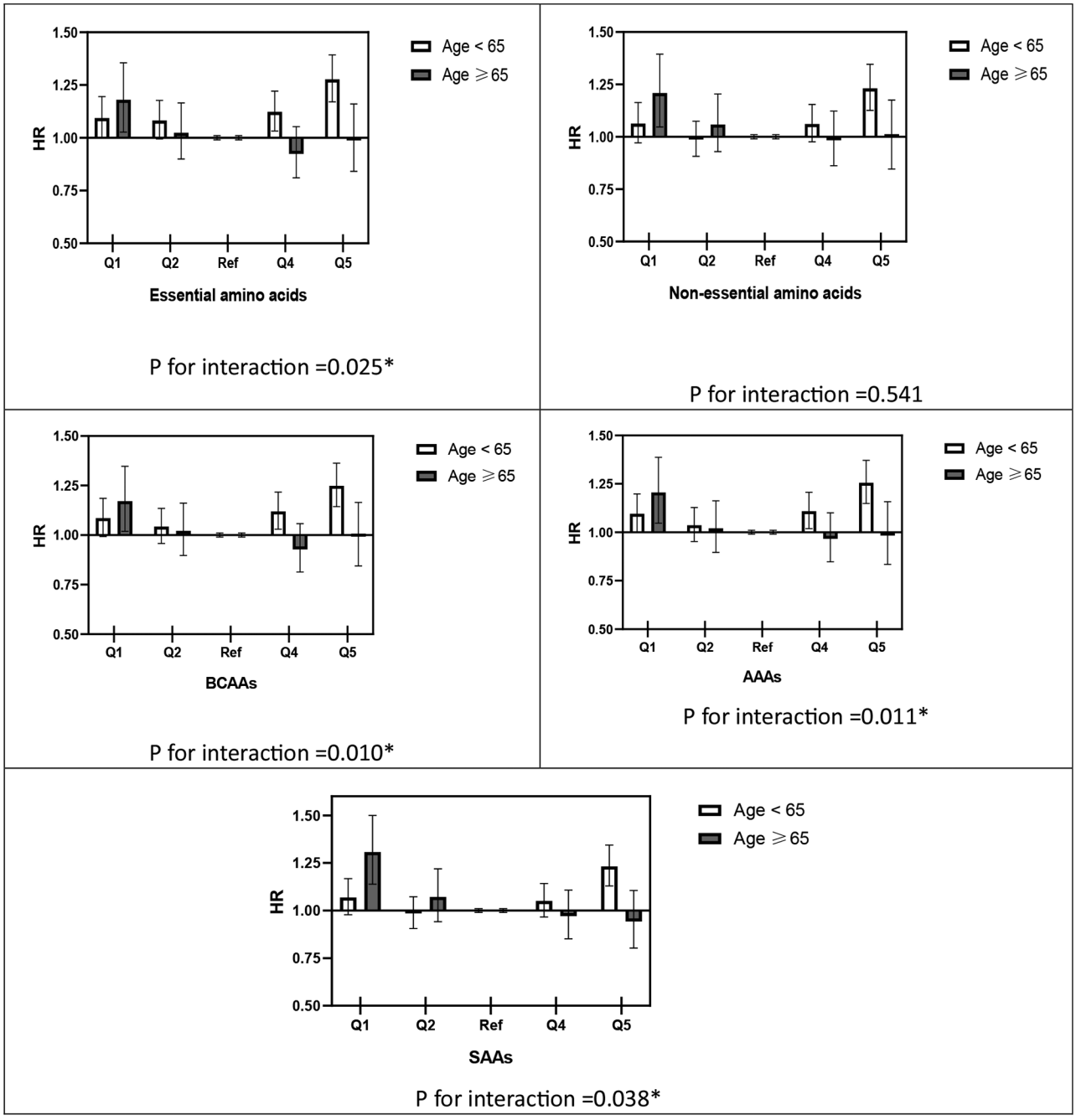

Fig. 5 Multivariate Cox regression derived HRs and 95% CIs for all-cause mortality by amino acids intake which were categorized according to age. Multivariable models were adjusted for sex, intake of energy, BMI, cigarette smoking, opium use, diabetes, hypertension, alcohol use, wealth index, education, physical activity, total lipid intake, and total protein intake.*Indicate significant effect (P-value < 0.05). Abbreviations: BCAA, branched-chain amino acids; AAAs, aromatic amino acids; SAAs, sulfur-containing amino acids; AAAs aromatic amino acids; CI, confidence interval; HR, hazard ratio; Q, quintile; Ref, reference

dietary BCAAs intake (median intake of 16.14 g/day) and all-cause mortality in patients with colorectal cancer [39]. Some other studies have examined the association between serum BCAAs concentrations and mortality. Serum BCAAs concentrations were inversely associated with cardiovascular risk factors and CVD mortality in the elderly [40, 41]. With increasing serum BCAA concentration, the risk of all-cause mortality steadily decreases, whereas, the risk of CVD mortality only decreases up to a certain concentration of BCAAs [40]. In the UK Biobank cohort study, total BCAAs, isoleucine, and valine levels were shown to be independently linked to stroke [42].

Nonetheless, recent investigations found only a weak positive correlation between amino acids consumption and their corresponding serum levels [43].

Our analysis revealed a significant non-linear association between dietary aromatic amino acid and risk of all-cause and CVD mortality. In another recent analysis from NHANES III, higher aromatic amino acid intake (median intake of 6.4 g/day) was associated with decreased risk of CVD mortality [44]. However, excessive dietary aromatic amino acids (median intake of 9.29% of total daily protein intake) may increase the risk of hypertension [45] and might be harmful to human health [44]. Although we did not find decreased risk of mortality in high aromatic amino acids intakes, the increased risk of all-cause and CVD mortality was found in those with low aromatic amino acid intake (median intake of 4.24 g/day). Considering the different median values of amino acids intake across studies, comparison between study results should interpreted with caution.

Based on our results, increased hazards of all-cause, CVD, cancer, and other causes of mortality were found to be associated with low SAAs intake (median intake of 1.7 g/day). Dong et al. in NHANES III study revealed that SAAs intake close to the estimated average requirement (15 mg/kg/day), decreased the risk of cardiometabolic disease [10]. They also found increased risk of diabetes mortality [46] and cardiometabolic diseases [10] in higher SAAs intake. However, increased hazard of mortality in higher intakes was not confirmed in the present study after multivariable adjustment. Another previous epidemiological research on the Iranian population found decreased risks of CVD events in those who consume higher amounts of SAA (median intake of 3.13 g/day) [9]. Some other studies explored each of the SAAs. Decreased methionine intake by postmenopausal women was associated with a lower risk of breast cancer mortality and all-cause mortality [47]. Other studies reported no association between methionine intake and the risk of esophageal, gastric [48], and breast cancer [49]. Serum levels of methionine were also not associated with the risk of lung cancer [50].

Compatible with the results from amino acid groups, increased risks of mortality were observed in lower intakes of each amino acid, but high intakes of glycine and proline amino acids were also found to increase the risk of CVD and all-cause mortality, respectively. Keeping in mind that dietary intake of amino acids might not necessarily correlate with their circulating levels. Genome-wide association studies of glycine metabolism support potential mechanisms and causality of the association between CVD and glycine level, albeit in favor of lower CVD risk in those with higher genetically predicted plasma glycine level [51, 52]. Another epidemiological study reported increased risk of mortality from ischemic stroke in higher glycine intakes [53]. Dietary glycine may directly have an adverse effect on blood pressure [54]. Nonetheless, replication of our results is required in future epidemiological studies.

Probable explanations for discrepancy in the findings among studies noted above include differences in the important study elements like endpoint determination, dietary pattern derivation, population characteristics, adjusted confounders, sample size, and methodology employed.

The strengths of this analysis are its prospective design including a representative sample of the general population with a high length of follow-up. Another strength of our research is the adjustment for several potential confounders, including multiple dietary and lifestyle factors.

We also need to acknowledge several limitations. First, the use of self-reported FFQ is a suboptimal approach to measuring nutrient intake. Second, habitual nutrient intakes were assessed only at baseline and it may have been altered during the follow-up. Third, our data on a number of confounding factors were self-reported by study participants, which may have led to measurement error. Fourth, observed findings could be subject to unmeasured or residual confounding factors like diet quality. Finally, the study results based on an Iranian middle-aged population, cannot be generalized to other ethnicities or very old populations who may have other nutritional requirements [55].

Additional studies in other populations are required to determine optimal ranges and sources of dietary protein and refine global dietary recommendations.

Conclusion

This study found the suggestion of non-linear associations between amino acid intake and risk of mortality in the middle-aged and older Iranian population. Low intake of different amino acid groups was found to be associated with increased mortality. For instance, increasing amount of dietary amino acids from the ranges within the first quintile to the second quintile is accompanied to decreased HRs of CVD mortality. Accordingly, adequate intake of all categories of amino acids is critical to maintain the overall health. The significant age interaction for the association between mortality and amino acids consumption highlights the importance of individualized protein recommendations for each age group. Moreover, our findings highlight the complex and divergent associations of amino acid categories with health outcomes, indicating that it could be misleading to provide advice on protein intake without specifying the components, even though this can be challenging to measure in practice.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12937-024-01044-x.

Supplementary Material 1

Supplementary Material 2

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Author contributions
A.M. Analysis and interpretation of data, Drafting of manuscript, Critical revision, S.M.S.J. Drafting of manuscript, Critical revision. M.M. Drafting of manuscript, Critical revision, P.A. Drafting of manuscript, M.S. Analysis and interpretation of data, S.M. Analysis and interpretation of data, H.P. Acquisition of data, A.P. Acquisition of data, M.H. Critical revision, A.H. Critical revision, R.M. Acquisition of data, Critical revision.

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Data availability
Data described in the manuscript, code book, and analytic code will be made available upon reasonable request from the corresponding author.

Declarations

Ethical approval
Ethical approval for the Golestan Cohort Study was obtained from the Institutional Review Boards of the Digestive Disease Research Center (DDRC) at Tehran University of Medical Sciences, the US National Cancer Institute (NCI), and the World Health Organization International Agency for Research on Cancer (IARC). Written informed consent was obtained from all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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