Patterns of amino acids intake are strongly associated with cardiovascular mortality, independently of the sources of protein

Marion Tharrey, François Mariotti, Andrew Mashchak, Pierre Barbillon, Maud Delattre, Jean-François Huneau, Gary Fraser

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Author Names: Marion Tharrey[1,2,4 *], Francois Mariotti[2†], Andrew Mashchak[1], Pierre Barbillon[3], Maud Delattre[3], Jean-François Huneau[2], Gary E. Fraser[1†]

Authors Affiliation:

1 Loma Linda University, School of Public Health, 92350, CA, USA (MT, AM, GF);

2 UMR PNCA, AgroParisTech, INRA, Université Paris-Saclay, 75005, Paris, France (MT, FM, JFH);

3 UMR MIA-Paris, AgroParisTech, INRA, Université Paris-Saclay, 75005, Paris, France (PB, MD)

4 MOISA, INRA, CIHEAM-IAMM, CIRAD, Montpellier SupAgro, Univ Montpellier, Montpellier, France (MT)
†those authors are joint senior authors

*** Corresponding author.** INRA, UMR MOISA, Campus Inra-SupAgro de la Gaillarde, 2 place Pierre-Viala - Bât. 26, 34060 Montpellier Cedex 2, France.

Email: marion.tharrey@supagro.fr

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Abstract

Background: The intake of specific amino acids has been associated with cardiovascular health but amino acids are consumed together as dietary protein. Here we investigated the association between identified patterns of amino acid intake and cardiovascular mortality.

Methods: 2216 cardiovascular death from 79 838 men and women from the Adventist Health Study-2, were included in our analysis. Baseline dietary patterns based on the participants' amino acids intakes were derived by factor analysis. Using Cox regression analyses, we estimated multivariate-adjusted hazard ratios (HRs) adjusted for sociodemographic and lifestyle factors and other dietary components.

Results: Three patterns of amino acids were identified. Factor 1 was positively associated with cardiovascular disease (CVD) mortality [$HR_{Q5\text{-}Q1}$: 1.62, 98.75% CI: 1.15 2.28; P-trend < 0.001]; and Factors 2 and 3 were inversely associated with CVD mortality [$HR_{Q5\text{-}Q1}$ Factor 2: 0.74, 98.75% CI: 0.53 1.04, *P*-trend<0.01; $HR_{Q5\text{-}Q1}$ Factor 3: 0.65, 98.75% CI: 0.44 0.95, *P*-trend< 0.05]. The associations with Factor 1 (with high loadings on indispensable amino acids such as branched chained amino acidss, lysine, methionine) and Factor 3 (with high loadings on non-indispensable amino acids namely arginine, glycine, aspartate+asparagine) remained significant after further adjustment for nutrient intake and for the five protein source patterns identified previously ($HR_{Q5\text{-}Q1}$: 1.56 [0.99 2.45] and 0.55 [0.35 0.85] *P*-trends<0.01).

Conclusions: Indispensable AA have a positive and some non-indispensable AA have a negative, independent, strong association with the risk of cardiovascular mortality.

Keywords: nutritional epidemiology; factor analysis; cardiovascular disease; amino acids; dietary protein;

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Key messages

- Amino acids dietary patterns derived by factor analysis were found to be strongly associated with mortality from cardiovascular disease.

- One factor consisting of indispensable amino acids, in particular branched chained amino acidss, Lysine and Methionine, was found positively associated with cardiovascular mortality. Conversely, two factors that have major contributions from non-indispensable amino acids were found negatively associated with cardiovascular mortality

- These associations seems largely mediated by the specific effect of the amino acids per se and not by potential confounders such as patterns of protein food intakes.

Introduction

Protein from plant and animal sources has been found to differentially affect cardiovascular health [1]. Among potential mechanisms it is likely that the amino acid profile of specific foods may affect cardiovascular disease (CVD) risk by their unique physiological effects [1–3]. Plant-based proteins are lower in indispensable amino acids (methionine, lysine, and tryptophan) but higher in non-indispensable amino acids (arginine, glycine, alanine, and serine) [4]. Previous studies also documented that intake of amino acids like glutamine, arginine, glycine, cysteine and histidine may lower blood pressure by improving endothelial function and arterial stiffness [5–9] but data on other CVD risk factors or final endpoints such as cardiovascular mortality are limited [10]. Furthermore, this literature has evaluated each amino acid in isolation not taking into account the multicollinearity with the intake of other amino acids, protein source, total protein, and the overall dietary pattern. Recently it was reported that the associations between the intake of some amino acids and blood pressure vary depending on whether they originate from plant or animal protein sources [11]. Thus, the beneficial effect of plant proteins on cardiovascular health may not be assigned to a specific amino acid but rather to more complex associations of the amino acids or other substances tightly associated with those proteins. Experiments in rodents and randomized controlled trials have tested the effect of specific amino acids, or associations between two amino acids [12–17], on cardiovascular risk factors, lending credence to the possible beneficial or adverse effect of some amino acids, but the data provide no information about associations with amino acids categorized by protein sources.

This study aims to identify how different patterns of amino acids intakes may affect cardiovascular health by investigating their associations with cardiovascular mortality in participants from the Adventist Health Study-2 (AHS-2) cohort. The wide diversity of dietary status in this unique population and the study design allow deeper insights into the complex

associations between amino acids patterns and cardiovascular mortality at different levels of animal and plant protein intakes. We also adjusted for the usual confounders that are associated with the consumption of a plant vs an animal-food-based diet and studied if amino acid patterns could mediate the effect of protein patterns.

Method

Study population

All individuals were participants in the AHS-2, a large prospective cohort study investigating the relationship between lifestyle factors and several disease outcomes. Full details on the study design, recruitment methods, and responses were published previously [18]. Briefly, participants consisted of Adventists throughout the USA and Canada who completed a self-administrated lifestyle and dietary questionnaire between 2002 and 2007. The current study includes 79,838 participants (83% of all participants). Exclusion criteria were age younger than 25 years (n = 248); improbable response patterns in questionnaire data and/or estimated energy intake less than 500 kcal/d or greater than 4500 kcal/d (n = 6963); body mass index (BMI) out of range (<14 kg/m² or >60 kg/m², n = 2547), self-reported history of cardiovascular events at baseline (i.e. coronary bypass, angioplasty/stent, carotid artery surgery; or heart attack, angina pectoris, congestive heart failure, or stroke diagnosed by a physician; n = 6277). Written informed consent was obtained from all participants upon enrolment. Written informed consent was obtained from all participants upon enrolment. The institutional review board of Loma Linda University approved the study.

Dietary assessment

Usual daily dietary intake was estimated by a food-frequency questionnaire containing questions on the frequency and amount of consumption of more than 200 food items during the year preceding enrollment. Detailed descriptions of the methods of dietary measurement using the questionnaire and its validation against multiple 24-hour recalls have been published elsewhere [19,20].

CVD mortality ascertainment

Deaths through December 31, 2013 were identified by linkage with the National Death Index. The International Classification of Diseases, Tenth Revision, was used to classify underlying cause of deaths death from cardiovascular disease (I00–I99).

Assessment of other relevant variables

Sociodemographic and lifestyle covariates were obtained from the baseline questionnaire and included age, gender, race (black or white), vigorous physical activity (none, ≤ 75 min/week, >75 min), smoking status (current smoker, quit <1 year, quit 1-4 years, quit 5-9 years, quit 10-19 years, quit 20-29 years, quit 30 years, and never smoked), alcohol consumption (never, past, current), education (≤ high school, some college, ≥ Bachelor's degree), personal income (≤ 10 000, > 10 000–30 000 and ≥ 30 000 USD per year) and marital status (single, divorced and widowed, married and common law).

Statistical analysis

All analyses were performed with SAS version 9.4 (SAS Institute Inc, Cary, NC). Values for the 18 amino acids (AA) were expressed as the percentages that they contributed to total protein intake. Factor analysis, using varimax rotation, was used to identify the AA dietary patterns as described previously [21]. Briefly, factor analysis aims to summarize large data sets containing many variables, simultaneously, into a few underlying factors by analyzing their covariance structure [22]. The number of factors retained were based on the following criteria : eigenvalues >1, the Scree test [23] and the interpretability of the factors [24]. Factor scores were calculated for each derived AA dietary pattern as a linear combination of the standardized values of the amino acid intake variables that are factored [25]. Participants were then grouped into quintiles of factor scores. Descriptive statistics of participant characteristics and diet at baseline were calculated across quintiles of each AA factor by means and standard deviations (SDs) or numbers and percentages.

Cox proportional hazard models were used to explore the relationship between AA factors and CVD mortality. Time-on-study, terminated either by cardiovascular death or censoring, was used as the time-variable, with age at baseline included as a covariate in the model [26]. HRs were calculated across quintiles of AA factors and tests for trend were performed by treating the quintiles as an ordinal variable. Bonferroni correction was applied to investigate differences across quintiles. P-trends were calculated across quintiles and correspond to the p-values associated with the type-3 Wald chi-square statistics. Adjustment for confounders was done gradually. Model 1 was adjusted for mean-centered age, sex, race and energy intake, BMI and total protein intake. Model 2 was further adjusted for physical activity, smoking status, alcohol consumption, education, personal income and marital status. In model 3, we further adjusted for nutrients known to be related to CVD outcomes, namely unsaturated fatty acids [27,28], fiber [29,30], sodium [31,32], vitamin B6, B12, folates [33,34] and antioxidants (vitamins A, C, E) [35]. All nutrients were energy adjusted using the residual method [36].

In a previous study, we examined the specific effect of protein dietary patterns (derived by factor analysis) on CVD mortality using the same approach [21]. Briefly, intakes of the 18 proteinogenic amino acids (in grams) were estimated using the Nutrition Coordinating Center (NCC) Food and Nutrient Database. To estimate protein intake from different sources, protein content of food items were classified into 18 distinct proteins sources depending on their animal or plant origin, as described in a previous work [21]. Food items having multiple sources of protein were broken down into their constitutive ingredients using representative recipe and the amount of protein of each ingredient was then assigned to the corresponding protein sources. Ingredients were finally grouped into eight major protein food groups: meat, eggs, dairy, grains, nuts and seeds, legumes, fruits and vegetables, potatoes. Five protein factors were identified by factor analysis, each having a high contribution of protein from meat; grains; processed foods; legumes fruits & vegetables; and nuts and seeds products,

respectively. Two of these factors – the “Meat” and “Nuts & Seeds” protein factors were found to be related to CVD outcomes [21]. To investigate to which extent the effects of the identified AA factors may be indeed mediators of the protein factors, a fourth model further adjusted for the five protein factors was performed. The Cox proportional hazards assumption was evaluated using tests and plots based on the Schoenfeld Residuals. Statistical significance was set at $P \leq 0.05$.

Results

Identification of the amino acids dietary patterns

Three AAs dietary patterns were identified by using factor analysis, which together explained 84.9% of the total variance in AA dietary intake. A Scree plot displaying the eigenvalues is shown in **Supplementary Figure 1**. The factor loadings and the variances explained by each pattern are presented in **Table 1**. Factor 1 showed high positive loadings for indispensable AAs – branched chained amino acids (BCAAs; leucine, isoleucine and valine), lysine, methionine, histidine and threonine – tyrosine but also alanine. Factor 2 was characterized by high positive loads for aromatic indispensable AAs (phenylalanine, tryptophan), and for the non-indispensable glutamate+glutamine, serine, proline and cysteine. Finally, Factor 3 consisted mostly in non-indispensable amino acids and was mainly characterized by high positive loadings for arginine, glycine and aspartate+asparagine and a negative loading for proline.

Diet and lifestyle of participants across AA dietary factors

The AA dietary patterns were significantly associated with baseline characteristics (**Table 2**). Factor 1 was positively associated with smoking, alcohol consumption, being single and negatively associated with age and physical activity (Ps <0.0001). Conversely, Factors 2 and 3 showed opposite trends. Furthermore participants in upper quintile for Factor 1 were more likely to be women, black, and to have a lower educational background (Ps <0.0001). Participants in upper quintile for Factor 3 tended to have lower BMIs and total protein intake (Ps <0.0001).

Protein dietary-source patterns characterized by the protein factors varied widely among the AAs factors (**Figure 1**). Factor 1 was positively correlated with both the 'Meat' and 'Processed Foods' protein factors and had a strong negative correlation with the 'Grains'

protein factor. Factors 2 and 3 were inversely associated with the 'Meat' and 'Processed Foods' protein factors respectively. Factor 3 was also highly positively correlated with the 'Legumes Fruits & Vegetables' (LFV) protein factor. Partial correlation coefficients between the AAs factors and nutrients related to cardiovascular disease outcomes varied from -0.47 to 0.45 (**Supplementary Table 1**).

During a median of 9.9 years of follow-up, 2216 CVD deaths cases were identified. The plots of the Schoenfeld Residuals were consistent with the Cox proportional hazards assumption. After adjustment for potential confounders (model 2), Factor 1 was positively associated with CVD mortality; and Factors 2 and 3 were inversely associated with CVD mortality (**Table 3**). In multivariate adjustment (model 2), the HRs of CVD mortality for the highest vs. lowest quintile of AAs factors were 1.62 (98.75% CI: 1.15 2.28; *P*-trend: <0.001) for Factor 1, 0.74 (98.75% CI: 0.53 1.04; *P*-trend: 0.002) for Factor 2 and 0.65 (98.75% CI: 0.44 0.95; *P*-trend: 0.024) for Factor 3. When further adjusting for nutrients that have been related to CVD outcomes, the association numerically increased for Factor 1, and little changed for Factor 2 and 3. With further adjustment for the five protein factors, so controlling for food-related patterns of protein intake and potential confounders, such as nutrients closely related to protein sources, associations with CVD mortality remained significant for Factor 1 (HR: 1.56, 98.75% CI: 0.99 2.45; *P*-trend <0.01), became non-significant for Factor 2 (HR: 0.89, 98.75% CI: 0.59 1.32, *P*-trend >0.01); and an even stronger protective association was found for Factor 3 (HR: 0.55, 98.75% CI: 0.35 0.85; *P*-trend: <0.001) (**Table 4**). We have also run models analyzing each amino acid individually, and all together as independent variables (see **Supplementary Table 2**) but this latter model proved to be highly multicolinear, with variance inflation factors >10.

Discussion

In this study, we identified factors that describe three patterns of amino acid intake in the population and revealed their strong associations with cardiovascular mortality. Factor 1, consisting of indispensable amino acids, in particular BCAAs, Lysine, Methionine, is positively associated with cardiovascular mortality. Conversely, Factor 2 and especially Factor 3 that have major contributions from non-indispensable amino acids, namely glutamate+glutamine, serine and proline for Factor 2 and aspartate+asparagine, arginine and glycine for Factor 3, are negatively associated with cardiovascular mortality. Furthermore, Factors 1 and 3 continue to be highly predictive after adjustment for major food sources of dietary proteins, so more closely isolating amino acids, as distinct from substances closely associated with protein.

Interpretation and potential explanatory mechanisms

Among the amino acids that contribute to the apparently hazardous amino acid patterns (Factor 1), BCAAs have been widely studied since it was evidenced that plasma concentrations of BCAAs are elevated in obese subjects with insulin resistance [37]. Plasma BCAA concentrations are associated with cardiovascular risk factors [38–40] and can be predictive of diabetes and CVD [41,42]. BCAA intake has also been associated with the incidence of insulin resistance and diabetes, but BCAA intake could simply be a marker of total and animal protein intake [43,44]. Lysine and methionine have long been suggested to be atherogenic in animal model [45,46], but results are conflicting. In humans, data suggest that dietary methionine may increase cardiovascular risk by its conversion to homocysteine [47]. However, evidence is weak for such an effect in humans who consume methionine as protein or together with other individual amino acids [48,49]. Recent data suggest that the adverse

effect of methionine may be explained by its impact on metabolic regulations including methylation processes [50,51]

Among the amino acids that contribute to the protective amino acid patterns (Factor 2 and 3), arginine (predominant in Factor 3) has been the most studied. Arginine provides the substrate for the synthesis of nitric oxide, the key mediator of vascular homeostasis [52], whose impairment has been associated with cardiometabolic risk, including coronary artery disease, stroke and diabetes [53]. The potential of arginine-rich proteins to prevent alterations of postprandial endothelial function has been documented [54]. The benefits of high arginine intake have also been studied for many years in connection with lysine levels, stemming from seminal works on plant and animal proteins and atherosclerosis [45]. More recently, in a closely controlled trial, Vega-Lopez *et al.* found that a low (vs high) lysine:arginine ratio in the diet during 35 d lowered fasting and postprandial C-reactive protein and lowered postprandial plasma levels of triglycerides in moderately hypercholesterolemic subjects [12]. Some other amino acids found in the protective factors identified here, have been also studied for their relation to cardiovascular risk. Cysteine (predominant in Factor 2) intake has been inversely associated with the incidence of stroke, [55] and possible cardioprotective effects of cysteine intake, via modulation of glutathione and redox status, have been documented [14]. Dietary and plasma glutamine has been positively and glutamate negatively associated with cardiometabolic risk [10,56] but our data could not distinguish between dietary glutamine and glutamate (Factor 2). The literature regarding other amino acids that contribute to the protective amino acid pattern that we identified, is much less abundant. However, oral glycine (Factor 3) has been shown as particularly efficient in potentiating the action of insulin [57], and plasma glycine has been inversely associated with incident type-2 diabetes [41].

There is very little published work on patterns of amino acid intakes with which to compare our results. Jennings *et al*. have reported significant associations between the intakes of 7 amino acids considered as potentially cardioprotective and arterial stiffness or central blood pressure in a cross-sectional study [11]. The results are in line with ours concerning arginine and glycine, but at variance regarding leucine, tyrosine and histidine. However, the authors found an interaction between the intake of the amino acid and the protein source, and therefore the association with each of these amino acids may be confused in part by the nature and influence of the proteins that carry them. Recently, Teymoori *et al.* used an approach similar to ours to analyze the relation between patterns of amino intake and incident hypertension in a small cohort [58]. They identified a strong positive association between the risk of hypertension and an amino acid pattern contributed to by BCAAs, tyrosine, threonine, to name those that are in line with the present findings. However, this amino acid pattern was strongly positively correlated with dietary intakes of animal protein and dairy, and negatively correlated with plant protein, fruit, and vegetable. Therefore, despite the adjustments for the intake of nutrients, the results could be ascribed to the overall intakes of plant vs animal protein. The intakes of animal/plant protein and the intake of some specific sources of animal or plant protein sources has been associated with cardiovascular and diabetes risk [21,59,60].

An original and instructive finding in our study is that the associations between amino acid patterns and cardiometabolic mortality remain significant in a model further adjusting for patterns of food-specific protein intake found to be related to cardiovascular mortality [21]. Since the protein patterns and the amino acid patterns are naturally associated, the fact that the associations persist in a model with mutual adjustment shows that the association seems to be specific to the amino acids per se, at least for two of the three AA patterns. One could also argue that, besides AA, the effect of protein could also be meditated by unidentified factors other than amino acids closely associated with protein. Given that we adjusted for many

nutrients as potential confounders, these hypothetical components are unlikely to be nutrients, and may be substances tightly associated with the dietary proteins. For example, for plant protein, and in particular proteins from nuts and seeds, a variety of phytochemicals are suspected to play a role [61]. For the meat protein factor, various putative factors other than amino acids include advanced glycation end products, nitrites, heme iron, carnitine (via trimethylamine N-oxide synthesis), and endocrine disruptor chemicals [62].

Because most dietary amino acid intakes are weakly associated with plasma concentrations across a large spectrum of dietary patterns [63] the effect of dietary amino acids are usually considered to be mediated by their impact on metabolism during the postprandial period [64]. Other mechanisms might involve the microbiome, such as changes in the gut microbiota metabolite synthesis [65].

Strengths and limitations

A notable strength of this study is the large number of participants who are followed over a long period allowing the identification of many cardiovascular deaths. Additionally, a large proportion of participants adhered to vegetarian and non-vegetarian lifestyles, thus providing a wide range of protein food sources.

Our study also has potential limitations. The self-administered food frequency questionnaire (FFQ) was used at baseline to measure dietary intake. FFQs have the advantage of assessing usual diet of participants over a long-term period but in return may have a greater risk of recall and reporting error in the data. However, nutrient intake estimates derived by the FFQ were validated against multiple 24-hour dietary recalls [19,20]. Diet patterns and lifestyle behaviors were assessed at baseline and may change over time. Furthermore, the intake of amino acids is strongly related to protein sources, and the overall dietary pattern. Despite careful efforts to adjust for these with apparently precise estimates in our modeling we cannot

be sure that the associations are not, in part, resulting from residual confounding with other strongly influential dietary and lifestyle factors.

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Tables

Table 1. Identification of amino acids dietary patterns from factor loadings for 18 amino acids from the Adventist Health Study (n = 79 838) [a]

	Factor loadings		
	Factor 1	Factor 2	Factor 3
		Branched-chain AA	
Isoleucine	0.91	0.33	0.15
Valine	0.86	0.38	0.14
Leucine	0.84	0.48	0.04
		Other indispensable AA	
Lysine	0.95	-0.11	0.13
Methionine	0.93	0.01	-0.13
Histidine	0.79	0.18	0.43
Threonine	0.78	0.17	0.51
Phenylalanine[b]	0.28	0.82	0.40
Tryptophan[b]	0.23	0.74	0.32
		Semi-indispensable AA	
Cysteine	0.15	0.67	-0.17
Tyrosine	0.83	0.45	-0.08
		Non indispensable AA	
Glutamate + glutamine	-0.12	0.88	-0.11
Serine	0.34	0.81	0.31
Proline	0.25	0.75	-0.52
Alanine	0.68	-0.06	0.62
Aspartate + asparagine	0.18	0.06	0.83
Glycine	0.18	-0.05	0.85
Arginine	-0.06	0.11	0.92
Variability explained	0.50	0.20	0.15

[a] Data are factor loadings (i.e. correlation coefficients between the variables and factors) derived from factor analysis.
[b] aromatic indispensable AAs

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Table 2. Baseline characteristics among 79 838 Adventist Health Study 2 participants by extreme quintile of the AAs factors

	Factor 1		*P-value*[a]	*Factor 2*		*P-value*[a]	*Factor3*		*P-value*[a]
	Q1	*Q5*		*Q1*	*Q5*		*Q1*	*Q5*	
				Participant characteristics					
Mean (SD) age, years	59,7 (14,1)	53,7 (13,4)	*<.0001*[b]	55,4 (13,3)	56,7 (14,5)	*0.0342*[b]	57,9 (14,5)	57,7 (13,6)	*<.0001*[b]
Sex, N° women (%)	9964 (62)	11137 (70)	*<.0001*[c]	10655 (66)	10281 (64)	*0.0025*[c]	10610 (66)	10815 (68)	*0.920*[c]
Race, N° black (% black)	2740 (17)	5055 (32)	*<.0001*[c]	6741 (42)	2769 (17)	*<.0001*[c]	3016 (18)	4468 (27)	*<.0001*[c]
Mean (SD) BMI, kg/m^2	25,28 (5.0)	28,9 (6,5)	*<.0001*[b]	28,1 (6,2)	27.0 (5,6)	*<.0001*[b]	27,6 (6,0)	25,6 (5,1)	*<.0001*[b]
N° never smokers (%)	13643 (85)	11990 (75)	*<.0001*[c]	12022 (75)	13512 (84)	*<.0001*[c]	13073 (82)	13226 (83)	*0.0082*[c]
N° never drinkers (%)	11912 (75)	8185 (51)	*<.0001*[c]	8885 (55,6)	11270 (70,6)	*<.0001*[c]	10698 (67)	10771 (67)	*0.463*[c]
N° physically inactive (%)	2846 (18)	3884 (24)	*<.0001*[c]	3604 (22)	3390 (21)	*<.0001*[c]	3612 (22)	3367 (21)	*<.0001*[c]
N° currently married (%)	12263 (77)	10789 (67)	*<.0001*[c]	10774 (67)	11891 (74)	*<.0001*[c]	11280 (70)	11496 (72)	*<.0001*[c]
Personal income, USD/year:			*<.0001*[d]			*<.0001*[d]			*<.0001*[d]
< 10,000	3769 (23)	3051 (19)		3158 (19)	3301 (20)		3517 (22)	3249 (20)	
10,000–30,000	6212 (38)	5839 (36)		6118 (38)	5858 (36)		6283 (39)	5848 (36)	
> 30,000	5981 (37)	7073 (44)		6687 (41)	6804 (42)		6162 (38)	6865 (43)	
Education, N° (%):			*<.0001*[d]			*<.0001*[d]			*<.0001*[d]
High school or less	3082 (19)	3672 (23)		3838 (24)	2985 (18)		3652 (23,16)	2877 (18)	
Some college	5797 (36)	6797 (43)		6592 (41)	5891 (37)		6226 (39,48)	6224 (39)	
Bachelor's degree or higher	6901 (43)	5305 (33)		5300 (33)	6912 (43)		5893 (37,37)	6668 (42)	
Mean (SD) energy, kcal/d	1865 (681)	1799 (748)	*<.0001*[b]	1777 (730)	1891 (737)	*<.0001*[b]	1887 (724)	1843 (729)	*<.0001*[b]
Mean (SD) protein, g/d	71,9 (30,9)	72,6 (31,3)	*<.0001*[b]	71,5 (32,4)	74,1 (33,3)	*<.0001*[b]	77,6 (33,1)	67,5 (29,3)	*<.0001*[b]
				Protein dietary patterns, N° participants (column %)					
'Meat' protein factor			*<.0001*[e]			*<.0001*[e]			*<.0001*[e]
Q1	1975 (12)	1916 (12)		393 (2)	6711 (42)		4656 (29)	2251 (14)	
Q5	1074 (6)	8780 (55)		8793 (55)	632 (4)		1815 (1)	3453 (21)	

'Grains' protein factor			*<.0001*[e]			*<.0001*[e]			*<.0001*[e]
Q1	447 (2)	8479 (53)		5066 (31)	2302 (14)		4068 (25)	3275 (20)	
Q5	9004 (56)	718 (4)		3355 (21)	3656 (22)		4707 (29)	1490 (9)	
'Legumes, fruits & vegetables' protein factor			*<.0001*[e]			*<.0001*[e]			*<.0001*[e]
Q1	2950 (18)	5295 (33)		3224 (20)	5162 (32)		8411 (52)	758 (4)	
Q5	3774 (23)	1236 (8)		3666 (23)	1070 (7)		304 (2)	7725 (48)	
'Processed Foods' protein factor			*<.0001*[e]			*<.0001*[e]			*<.0001*[e]
Q1	4832 (30)	1844 (11)		4309 (27)	2223 (14)		1039 (6)	7290 (45)	
Q5	662 (4)	5505 (34)		1905 (12)	4658 (29,18)		5568 (34)	773 (4)	
'Nuts & Seeds' protein factor			*<.0001*[e]			*<.0001*[e]			*<.0001*[e]
Q1	901 (5)	5800 (36)		5874 (37)	2566 (16)		1871 (12)	2643 (16)	
Q5	5696 (35)	1595 (10)		2462 (15)	2738 (17)		2602 (16)	6082 (38)	

[a] Adjusted on age, sex and race.

[b] P-trend based on analysis of variance with quintiles of dietary pattern scores (ordinal variable) treated as an independent variable.

[c] P-trend based on binomial logistic regression with quintiles of dietary pattern scores (ordinal variable) treated as an independent variable.

[d] Overall model P-value of a multinomial logistic regression with participants' characteristic treated as an independent variable.

[e] P-value based on Chi-Square test.

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Table 3. Multivariate-adjusted Hazard Ratio of CVD mortality according to quintiles of identified AAs dietary pattern scores in 79 838 participants of the Adventist Health Study 2

	Q1	Q3	Q5	*P-trend*
Factor 1				
Person-years	152779	150553	149897	
Deaths, n	551	447	376	
Model 1 HR (98.75% CI)[a]	1.00 (referent)	1.07 (0.73 1.56)	1.65 (1.18 2.31)	*<.0001*
Model 2 HR (98.75% CI)[b]	1.00 (referent)	1.10 (0.75 1.62)	1.62 (1.15 2.28)	*<.001*
Model 3 HR (98.75% CI)[c]	1.00 (referent)	1.16 (0.79 1.70)	1.78 (1.24 2.57)	*<.0001*
Factor 2				
Person-years	150257	150456	151824	
Deaths, n	403	458	475	
Model 1 HR (98.75% CI)[a]	1.00 (referent)	0.69 (0.49 0.96)	0.75 (0.54 1.05)	*0.002*
Model 2 HR (98.75% CI)[b]	1.00 (referent)	0.73 (0.52 1.03)	0.74 (0.53 1.04)	*0.002*
Model 3 HR (98.75% CI)[c]	1.00 (referent)	0.73 (0.52 1.03)	0.75 (0.53 1.05)	*0.004*
Factor 3				
Person-years	151278	150987	149757	
Deaths, n	519	422	418	
Model 1 HR (98.75% CI)[a]	1.00 (referent)	0.99 (0.71 1.37)	0.67 (0.46 0.97)	*0.029*
Model 2 HR (98.75% CI)[b]	1.00 (referent)	1.02 (0.73 1.40)	0.65 (0.44 0.95)	*0.024*
Model 3 HR (98.75% CI)[c]	1.00 (referent)	1.00 (0.72 1.39)	0.62 (0.41 0.92)	*0.004*

All results are shown as HR and 98.75% CIs to account for multiple comparisons (Bonferroni correction: significance criterion 0.05/4 = 0.0125 for each quintile)

[a] adjusted on mean centered age (y), sex (men, women), race (black white), energy intake (kcal/d), BMI (kg/m2) and energy adjusted total protein intake (g/d)

[b] Model 1 further adjusted for physical activity (none, ≤ 75min/wk, > 75min/wk), smoking status (current smoker, quit <1 year, quit 1-4 years, quit 5-9 years, quit 10-19 years, quit 20-29 years, quit 30 years, and never smoked), alcohol consumption (never, past, current), income (≤ 10 000, > 10 000–30 000 and ≥ 30 000 USD per year), education (≤ high school, some college, ≥ Bachelor's degree), marital status (single, divorced and widowed, married and common law).

[c] Model 1 further adjusted on fiber, PUFA, SFA, sodium , and vitamins A, C, E, B6, B9 and B12 *[intake of nutrients were energy adjusted with the residual method]*.

Table 4. Multivariate-adjusted Hazard Ratio of CVD mortality according to quintiles of identified AAs dietary pattern scores and protein dietary pattern in 79 838 participants of the Adventist Health Study 2

	Q1	Q3	Q5	*P-trend*
AA factors model				
AA factor 1 HR (98.75% CI)	1.00 (ref.)	1.16 (0.79 1.70)	1.78 (1.24 2.57)	*<.0001*
AA factor 2 HR (98.75% CI)	1.00 (ref.)	0.73 (0.52 1.03)	0.75 (0.53 1.05)	*0.004*
AA factor 3 HR (98.75% CI)	1.00 (ref.)	1.00 (0.72 1.39)	0.62 (0.41 0.92)	*0.004*
Protein factors model				
Meat protein factor HR (98.75% CI)	1.00 (ref.)	1.26 (0.85 1.84)	1.70 (1.18 2.46)	*0.001*
Grains protein factor HR (98.75% CI)	1.00 (ref.)	0.95 (0.79 1.14)	0.87 (0.72 1.06)	*0.121*
Processed foods protein factor HR (98.75% CI)	1.00 (ref.)	1.01 (0.83 1.23)	1.04 (0.81 1.34)	*0.482*
LFV protein factor HR (98.75% CI)	1.00 (ref.)	1.02 (0.83 1.24)	1.02 (0.80 1.28)	*0.492*
Nuts & Seeds protein factor HR (98.75% CI)	1.00 (ref.)	0.70 (0.49 1.01)	0.63 (0.44 0.91)	*0.001*
AA factors + Protein factors model (mutually adjusted)				
AA factor 1 HR (98.75% CI)	1.00 (ref.)	1.08 (0.71 1.62)	1.56 (0.99 2.45)	*0.010*
AA factor 2 HR (98.75% CI)	1.00 (ref.)	0.82 (0.56 1.18)	0.89 (0.59 1.32)	*0.306*
AA factor 3 HR (98.75% CI)	1.00 (ref.)	0.89 (0.63 1.27)	0.55 (0.35 0.85)	*0.0007*
Meat factor HR (98.75% CI)	1.00 (ref.)	1.28 (0.86 1.91)	1.50 (0.95 2.35)	*0.0387*
Grains factor HR (98.75% CI)	1.00 (ref.)	1.02 (0.83 1.25)	0.97 (0.76 1.25)	*0.9709*
Processed foods factor HR (98.75% CI)	1.00 (ref.)	1.03 (0.84 1.27)	1.13 (0.85 1.50)	*0.1362*
LFV factor HR (98.75% CI°	1.00 (ref.)	0.98 (0.79 1.20)	0.93 (0.71 1.21)	*0.7086*
Nuts & Seeds factor HR (98.75% CI)	1.00 (ref.)	0.74 (0.51 1.06)	0.77 (0.52 1.14)	*0.0403*

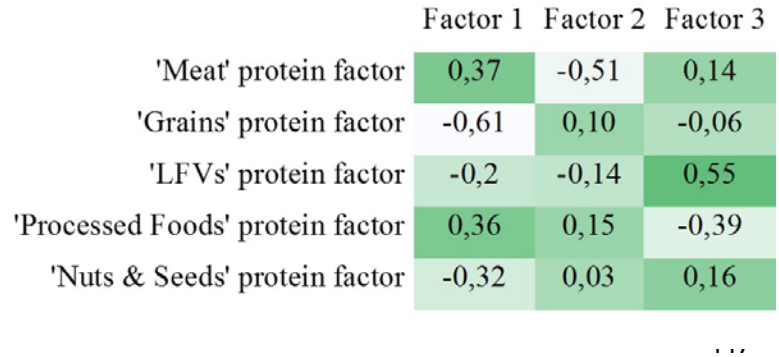
LFV: Legumes, Fruits & Vegetables

All results are shown as HR and 98.75% CIs to account for multiple comparisons (Bonferroni correction: significance criterion 0.05/4 = 0.0125 for each quintile)

Models are adjusted on mean centered age (y), sex (men, women), race (black white) and energy intake (kcal/d), BMI (kg/m2), energy adjusted total protein intake (g/d), physical activity (none, ≤ 75min/wk, > 75min/wk), smoking status (current smoker, quit <1 year, quit 1-4 years, quit 5-9 years, quit 10-19 years, quit 20-29 years, quit 30 years, and never smoked), alcohol consumption (never, past, current), income (≤ 10 000, > 10 000–30 000 and ≥ 30 000 USD per year), education (≤ high school, some college, ≥ Bachelor's degree), marital status (single, divorced and widowed, married and common law), fiber, PUFA, SFA, sodium, and vitamins A, C, E, B6, B9 and B12 *[intake of nutrients were energy adjusted with the residual method]*.

Figure

Figure 1. Partial Pearson product-moment correlation between the AAs factors and the protein factors [a]



LFVs: Legumes, Fruits & Vegetables.

[a] The AAs factors and the protein factors amino acids were derived by factor analysis. AAs and protein intake were as percentage they contributed to total protein intake. Correlations were adjusted for age, and gender and race. The colors indicate the strength and direction of the correlations.

Supplementary Table 1. Partial correlations between the AAs factors and various nutrients

	Factor 1	Factor 2	Factor 3
Total protein	0,05	-0,09	-0,22
Total carbohydrate	-0,31	-0,03	0,07
Sugar	0,00	-0,12	-0,01
Total fat	0,18	0,03	0,05
SFA	**0,45**	**0,01**	**-0,28**
TFA	**0,20**	**0,02**	**-0,27**
PUFA	**-0,14**	**0,14**	**0,27**
Omega 3	**0,09**	**0,01**	**0,21**
MUFA	**0,06**	**-0,03**	**0,14**
Fiber	**-0,47**	**-0,13**	**0,40**
Vit B6	**-0,02**	**-0,04**	**0,03**
Folate	**-0,06**	**0,01**	**0,09**
Vit B12	**0,01**	**0,01**	**0,01**
Vit C	**-0,01**	**-0,01**	**0,03**
Vit A	**-0,06**	**-0,06**	**0,19**
Vit E	**0,02**	**-0,01**	**0,06**
Sodium	**0,03**	**0,02**	**-0,14**

[a] The AAs factors and the protein factors amino acids were derived by factor analysis. AAs and protein intake were expressed as the percentage that they contributed to total protein intake. Models adjusted for age, and gender and race.

Supplementary table 2. Multivariate-adjusted Hazard Ratio of CVD mortality according to individual AA intake in 79 838 participants of the Adventist Health Study 2 [a]

	Bivariate models		Multivariate model[b]	
	HR	(95% CI)	HR	(95% CI)
Branched-chain AA				
Isoleucine	1.1	(0.95 1.27)	1,9	(0,80 4,52)
Valine	1.06	(0.93 1.20)	0,71	(0,39 1,32)
Leucine	1.03	(0.94 1.12)	1,23	(0,77 1,97)
Other indispensable AA				
Lysine	1.10	(1.03 1.19)	1,09	(0,79 1,48)
Methionine	1.32	(0.99 1.78)	1,26	(0,62 2,55)
Histidine	1.05	(0.82 1.35)	0,62	(0,24 1,62)
Threonine	1.14	(0.95 1.36)	3,25	(1,55 6,82)
Phenylalanine[c]	0.95	(0.82 1.09)	0,36	(0,14 0,87)
Tryptophan[C]	0.68	(0.42 1.10)	0,27	(0,08 0,90)
Semi-indispensable AA				
Cysteine	0.76	(0.57 1.01)	0,71	(0,43 1,16)
Tyrosine	1.06	(0.88 1.28)	0,54	(0,25 1,12)
Non indispensable AA				
Glutamate + glutamine	0.98	(0.95 1.00)	1,06	(1,00 1,12)
Serine	0.97	(0.86 1.10)	1,49	(0,82 2,69)
Proline	0.96	(0.90 1.02)	1,08	(0,89 1,31)
Alanine	1.02	(0.90 1.16)	0,41	(0,25 0,69)
Aspartate + asparagine	1.07	(1.01 1.13)	1,18	(1,03 1,35)
Glycine	0.97	(0.85 1.11)	1,22	(0,79 1,89)
Arginine	1.00	(0.92 1.08)	1,02	(0,84 1,25)

[a] Models are adjusted on mean centered age (y), sex (men, women), race (black white), energy intake (kcal/d), BMI (kg/m2), energy adjusted total protein intake (g/d), physical activity (none. ≤ 75min/wk. > 75min/wk), smoking status (current smoker, quit <1 year, quit 1-4 years, quit 5-9 years, quit 10-19 years, quit 20-29 years, quit 30 years, and never smoked), alcohol consumption (never, past, current), income (≤ 10 000, > 10 000–30 000 and ≥ 30 000 USD per year), education (≤ high school, some college, ≥ Bachelor's degree), marital status (single, divorced and widowed, married and common law), fiber, PUFA, SFA, sodium, and vitamins A, C, E, B6, B9 and B12 *[intake of nutrients were energy adjusted with the residual method]*.

[b] Variance Inflation Factor was >10 for all AAs

[c] Aromatic indispensable AAs

Supplementary Figure 1. Scree plot of the factor analysis