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

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The manuscript has been carefully edited by someone with an excellent mastery of the English language.

1 **Abstract**

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

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

10 **Results:** Three patterns of amino acids were identified. Factor 1 was positively associated
11 with cardiovascular disease (CVD) mortality [HR_{Q5-Q1}: 1.62, 98.75% CI: 1.15 2.28; P-
12 trend < 0.001]; and Factors 2 and 3 were inversely associated with CVD mortality [HR_{Q5-Q1}
13 Factor 2: 0.74, 98.75% CI: 0.53 1.04, P-trend<0.01; HR_{Q5-Q1} Factor 3: 0.65, 98.75% CI: 0.44
14 0.95, P-trend< 0.05]. The associations with Factor 1 (with high loadings on indispensable
15 amino acids such as branched chained amino acidss, lysine, methionine) and Factor 3 (with
16 high loadings on non-indispensable amino acids namely arginine, glycine,
17 aspartate+asparagine) remained significant after further adjustment for nutrient intake and for
18 the five protein source patterns identified previously (HR_{Q5-Q1}: 1.56 [0.99 2.45] and 0.55
19 [0.35 0.85] P-trends<0.01).

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

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

25 **Key messages**

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

28 - One factor consisting of indispensable amino acids, in particular branched chained amino
29 acidss, Lysine and Methionine, was found positively associated with cardiovascular mortality.

30 Conversely, two factors that have major contributions from non-indispensable amino acids
31 were found negatively associated with cardiovascular mortality

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

34

35 **Introduction**

36 Protein from plant and animal sources has been found to differentially affect cardiovascular
37 health [1]. Among potential mechanisms it is likely that the amino acid profile of specific
38 foods may affect cardiovascular disease (CVD) risk by their unique physiological effects [1–
39 3]. Plant-based proteins are lower in indispensable amino acids (methionine, lysine, and
40 tryptophan) but higher in non-indispensable amino acids (arginine, glycine, alanine, and
41 serine) [4]. Previous studies also documented that intake of amino acids like glutamine,
42 arginine, glycine, cysteine and histidine may lower blood pressure by improving endothelial
43 function and arterial stiffness [5–9] but data on other CVD risk factors or final endpoints such
44 as cardiovascular mortality are limited [10]. Furthermore, this literature has evaluated each
45 amino acid in isolation not taking into account the multicollinearity with the intake of other
46 amino acids, protein source, total protein, and the overall dietary pattern. Recently it was
47 reported that the associations between the intake of some amino acids and blood pressure vary
48 depending on whether they originate from plant or animal protein sources [11]. Thus, the
49 beneficial effect of plant proteins on cardiovascular health may not be assigned to a specific
50 amino acid but rather to more complex associations of the amino acids or other substances
51 tightly associated with those proteins. Experiments in rodents and randomized controlled
52 trials have tested the effect of specific amino acids, or associations between two amino acids
53 [12–17], on cardiovascular risk factors, lending credence to the possible beneficial or adverse
54 effect of some amino acids, but the data provide no information about associations with amino
55 acids categorized by protein sources.

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

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

64 **Method**

65 *Study population*

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

80 *Dietary assessment*

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

86 *CVD mortality ascertainment*

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

90 *Assessment of other relevant variables*

91 Sociodemographic and lifestyle covariates were obtained from the baseline questionnaire and
92 included age, gender, race (black or white), vigorous physical activity (none, ≤ 75 min/week,
93 >75 min), smoking status (current smoker, quit <1 year, quit 1-4 years, quit 5-9 years, quit 10-
94 19 years, quit 20-29 years, quit 30 years, and never smoked), alcohol consumption (never,
95 past, current), education (\leq high school, some college, \geq Bachelor's degree), personal income
96 ($\leq 10\,000$, $> 10\,000$ – $30\,000$ and $\geq 30\,000$ USD per year) and marital status (single, divorced
97 and widowed, married and common law).

98 *Statistical analysis*

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

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

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

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

142 **Results**

143 *Identification of the amino acids dietary patterns*

144 Three AAs dietary patterns were identified by using factor analysis, which together explained
145 84.9% of the total variance in AA dietary intake. A Scree plot displaying the eigenvalues is
146 shown in **Supplementary Figure 1**. The factor loadings and the variances explained by each
147 pattern are presented in **Table 1**. Factor 1 showed high positive loadings for indispensable
148 AAs – branched chained amino acids (BCAAs; leucine, isoleucine and valine), lysine,
149 methionine, histidine and threonine – tyrosine but also alanine. Factor 2 was characterized by
150 high positive loads for aromatic indispensable AAs (phenylalanine, tryptophan), and for the
151 non-indispensable glutamate+glutamine, serine, proline and cysteine. Finally, Factor 3
152 consisted mostly in non-indispensable amino acids and was mainly characterized by high
153 positive loadings for arginine, glycine and aspartate+asparagine and a negative loading for
154 proline.

155 *Diet and lifestyle of participants across AA dietary factors*

156 The AA dietary patterns were significantly associated with baseline characteristics (**Table 2**).
157 Factor 1 was positively associated with smoking, alcohol consumption, being single and
158 negatively associated with age and physical activity (Ps <0.0001). Conversely, Factors 2 and
159 3 showed opposite trends. Furthermore participants in upper quintile for Factor 1 were more
160 likely to be women, black, and to have a lower educational background (Ps <0.0001).
161 Participants in upper quintile for Factor 3 tended to have lower BMIs and total protein intake
162 (Ps <0.0001).

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

166 protein factor. Factors 2 and 3 were inversely associated with the ‘Meat’ and ‘Processed
167 Foods’ protein factors respectively. Factor 3 was also highly positively correlated with the
168 ‘Legumes Fruits & Vegetables’ (LFV) protein factor. Partial correlation coefficients between
169 the AAs factors and nutrients related to cardiovascular disease outcomes varied from -0.47 to
170 0.45 (**Supplementary Table 1**).

171 During a median of 9.9 years of follow-up, 2216 CVD deaths cases were identified. The plots
172 of the Schoenfeld Residuals were consistent with the Cox proportional hazards assumption.

173 After adjustment for potential confounders (model 2), Factor 1 was positively associated with
174 CVD mortality; and Factors 2 and 3 were inversely associated with CVD mortality (**Table 3**).

175 In multivariate adjustment (model 2), the HRs of CVD mortality for the highest vs. lowest
176 quintile of AAs factors were 1.62 (98.75% CI: 1.15 2.28; *P*-trend: <0.001) for Factor 1, 0.74
177 (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:
178 0.024) for Factor 3. When further adjusting for nutrients that have been related to CVD

179 outcomes, the association numerically increased for Factor 1, and little changed for Factor 2

180 and 3. With further adjustment for the five protein factors, so controlling for food-related

181 patterns of protein intake and potential confounders, such as nutrients closely related to

182 protein sources, associations with CVD mortality remained significant for Factor 1 (HR: 1.56,

183 98.75% CI: 0.99 2.45; *P*-trend <0.01), became non-significant for Factor 2 (HR: 0.89, 98.75%

184 CI: 0.59 1.32, *P*-trend >0.01); and an even stronger protective association was found for

185 Factor 3 (HR: 0.55, 98.75% CI: 0.35 0.85; *P*-trend: <0.001) (**Table 4**). We have also run

186 models analyzing each amino acid individually, and all together as independent variables (see

187 **Supplementary Table 2**) but this latter model proved to be highly multicollinear, with

188 variance inflation factors >10.

189

190 **Discussion**

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

201 *Interpretation and potential explanatory mechanisms*

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

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

215 Among the amino acids that contribute to the protective amino acid patterns (Factor 2 and 3),
216 arginine (predominant in Factor 3) has been the most studied. Arginine provides the substrate
217 for the synthesis of nitric oxide, the key mediator of vascular homeostasis [52], whose
218 impairment has been associated with cardiometabolic risk, including coronary artery disease,
219 stroke and diabetes [53]. The potential of arginine-rich proteins to prevent alterations of
220 postprandial endothelial function has been documented [54]. The benefits of high arginine
221 intake have also been studied for many years in connection with lysine levels, stemming from
222 seminal works on plant and animal proteins and atherosclerosis [45]. More recently, in a
223 closely controlled trial, Vega-Lopez *et al.* found that a low (vs high) lysine:arginine ratio in
224 the diet during 35 d lowered fasting and postprandial C-reactive protein and lowered
225 postprandial plasma levels of triglycerides in moderately hypercholesterolemic subjects [12].

226 Some other amino acids found in the protective factors identified here, have been also studied
227 for their relation to cardiovascular risk. Cysteine (predominant in Factor 2) intake has been
228 inversely associated with the incidence of stroke, [55] and possible cardioprotective effects of
229 cysteine intake, via modulation of glutathione and redox status, have been documented [14].

230 Dietary and plasma glutamine has been positively and glutamate negatively associated with
231 cardiometabolic risk [10,56] but our data could not distinguish between dietary glutamine and
232 glutamate (Factor 2). The literature regarding other amino acids that contribute to the
233 protective amino acid pattern that we identified, is much less abundant. However, oral glycine
234 (Factor 3) has been shown as particularly efficient in potentiating the action of insulin [57],
235 and plasma glycine has been inversely associated with incident type-2 diabetes [41].

236

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

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

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

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

273 *Strengths and limitations*

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

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

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

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440

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
	<i>Branched-chain AA</i>		
Isoleucine	0.91	0.33	0.15
Valine	0.86	0.38	0.14
Leucine	0.84	0.48	0.04
	<i>Other indispensable AA</i>		
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
	<i>Semi-indispensable AA</i>		
Cysteine	0.15	0.67	-0.17
Tyrosine	0.83	0.45	-0.08
	<i>Non indispensable AA</i>		
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

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

	<i>Factor 1</i>		<i>P-value^a</i>	<i>Factor 2</i>		<i>P-value^a</i>	<i>Factor 3</i>		<i>P-value^a</i>
	<i>Q1</i>	<i>Q5</i>		<i>Q1</i>	<i>Q5</i>		<i>Q1</i>	<i>Q5</i>	
	<i>Participant characteristics</i>								
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
	<i>Protein dietary patterns, N° participants (column %)</i>								
'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.

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	<i>P-trend</i>
<i>Factor 1</i>				
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
<i>Factor 2</i>				
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
<i>Factor 3</i>				
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, ≤ 75 min/wk, > 75 min/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 ($\leq 10\ 000$, $> 10\ 000$ – $30\ 000$ and $\geq 30\ 000$ USD per year), education (\leq high school, some college, \geq 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
<u>AA factors model</u>				
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
<u>Protein factors model</u>				
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
<u>AA factors + Protein factors model (mutually adjusted)</u>				
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) ^o	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

	Factor 1	Factor 2	Factor 3
'Meat' protein factor	0,37	-0,51	0,14
'Grains' protein factor	-0,61	0,10	-0,06
'LFVs' protein factor	-0,2	-0,14	0,55
'Processed Foods' protein factor	0,36	0,15	-0,39
'Nuts & Seeds' protein factor	-0,32	0,03	0,16

LFVs: Legumes, Fruits & Vegetables.

^aThe 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)
<i>Branched-chain AA</i>				
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)
<i>Other indispensable AA</i>				
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)
<i>Semi-indispensable AA</i>				
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)
<i>Non indispensable AA</i>				
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

