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Associations between dietary amino acid intakes and blood concentration levels

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Abstract

Background and aims: Emerging evidence suggests a role of amino acids (AAs) in the development of various diseases including renal failure, liver cirrhosis, diabetes and cancer. However, mechanistic pathways and the effects of dietary AA intakes on circulating levels and disease outcomes are unclear. We aimed to compare protein and AA intakes, with their respective blood concentrations in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.

Methods: Dietary protein and AA intakes were assessed via the EPIC dietary questionnaires (DQ) and 24-h dietary recalls (24-HDR). A subsample of 3,768 EPIC participants who were free of cancer had blood AA concentrations measured. To investigate how circulating levels relate to their respecitve intakes, dietary AA intake was examined in quintiles and ANOVA tests were run. Pearson correlations were examined for continous associations between intakes and blood concentrations.

Results: Dietary AA intakes (assessed with the DQ) and blood AA concentrations were not strongly correlated ($-0.15 \leq r \leq 0.17$) and the direction of the correlations depended on AA class: weak positive correlations were found for most essential AAs (isoleucine, leucine, lysine, methionine, threonine, tryptophan, and valine) and conditionally essential AAs (arginine and tyrosine), while negative associations were found for non-essential AAs. Similar results were found when using the 24-HDR. When conducting ANOVA tests for essential AAs, higher intake quintiles were linked to higher blood AA concentrations, except for histidine and phenylalanine. For non-essential AAs and glycine, an inverse relationship was observed. Conditionally-essential AAs showed mixed results.

Conclusions: Weak positive correlations and dose responses were found between most essential and conditionally essential AA intakes, and blood concentrations, but not for the non-essential AAs. These results suggest that intake of dietary AA might be related to physiological AA status,

particularly for the essential AAs. However, these results should be further evaluated and confirmed in large-scale prospective studies.

Abbreviations: amino acids (AAs); European Prospective Investigation into Cancer and Nutrition (EPIC); dietary questionnaires (DQ); 24-h dietary recalls (24-HDR).

Keywords: amino acids; dietary questionnaire; dietary intake, blood levels; 24-hour dietary recall.

Introduction

Emerging evidence suggests a role of several dietary or circulating amino acids (AAs) in the development of various diseases including renal failure, liver cirrhosis, diabetes, and cancer [1-3]. However, mechanistic pathways and effects of dietary AA intake on circulating levels and disease outcomes are still unclear [4-6].

Each AA may have specific effects on metabolism in targeted organs/tissues via specific AA signalling pathways. Therefore, adequate AA concentrations are needed in order to maintain optimal organ and tissue functionality [7]. Circulating concentrations of AAs are subject to protein turnover (via AA oxidation and protein synthesis regulations); however, external factors like diet can also influence plasma levels of AAs. As a result of these physiological and environmental factors, concentration biomarkers do not provide absolute reference measurements of dietary intakes and therefore expected correlations between intakes and blood levels may be masked[8]. Specifically, essential AAs (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) cannot be synthesized by human metabolism and consequently they must be obtained through diet [7]. Other AAs like arginine, glycine, proline and tyrosine may be considered conditionally essential because the body cannot synthesize them in sufficient quantities during certain pathological conditions and/or physiological periods of growth, including pregnancy or adolescent growth [9]. As such, well-balanced AA intakes are presumed to be crucial for essential plasma AA concentrations needed [5]. To the best of our knowledge only a few studies, all of small sample size (n<150), have investigated the relationship between dietary and circulating AA concentrations in humans [10-12]. Therefore, the aim of this study was to examine these associations in a larger number of subjects within the European Prospective Investigation into Cancer and Nutrition (EPIC). In addition, to allow investigation of potential measurement error inherent to dietary intake assessment methods, two different instruments, the EPIC dietary questionnaires (DQ) and the 24-hour dietary recall (24-HDR) were used.

Methods

Study design and Participants

EPIC is a large on-going multicentre prospective cohort study consisting of 521,324 adults (366,521 women and 153,437 men) mostly aged 35-70 years from whom diet, and lifestyle data were collected at baseline. The objective of this cohort is to investigate the role of diet, lifestyle, metabolic factors and genetics in cancer development as well as other chronic diseases in a European sample. The participants were enrolled between 1992 and 2000 from 23 centres in 10 European countries: Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and the United Kingdom [13]. The rationale, study population and data collection have been described elsewhere [14]. All participants provided written informed consent and the ethical review boards from the International Agency for Research on Cancer (IARC) and from all local centers approved the study.

In order to study associations between dietary AA intakes and blood levels DQ data and AA blood concentrations from 3,768 men and women who served as controls in any of five EPIC nested case-control studies on colorectal (n = 491) [15], hepatobiliary (n = 327) [16], kidney (n = 633) [17], breast cancer (N=796) [18] and prostate cancer (n = 1,521) [19] were used (**Figure 1**). In addition, data from 334 men and women with 24-HDR data and plasma AA information were analysed. Only control participants were included in this study to avoid any potential bias due to metabolic changes induced by pre-clinical cancer development at the time of blood collection in participants that were subsequently diagnosed with cancer (i.e., cases from the nested studies). None of the control participants analysed were included in multiple case-control studies, therefore avoiding duplication. These case-control studies will hereafter be referred to as 'sub-studies'. For all sub-studies, controls were mostly selected from the full cohort of individuals who were alive and did not have a previous cancer (except non-melanoma skin cancer) at the time of diagnosis of the cases, using incidence density sampling and with controls matched to cases by key confounding factors: age (±6 months at recruitment), sex, study center, follow-up time since blood collection, time of day and fasting status at time of blood collection (<3, 3–6, >6 hours-h-). For women,

153 additional matching criteria included menopausal status, phase of menstrual cycle for
154 premenopausal women and current hormone replacement therapy use at blood collection (for
155 postmenopausal women). Although fasting status was not required in any of the studies, 40.0%
156 of the study sample had fasting blood samples (≥6 h since last meal) vs. <3h (n=40.1%); 3-6h
157 (17.6%) or non-reported/missing (2.3%) (Table 1).

158 ***Blood sampling and laboratory analysis***

159 A standardised protocol for blood collection and processing was followed. More details can be
160 found elsewhere [13,20]. Briefly, AAs in plasma (serum for the hepatobiliary cancer study, and
161 citrated plasma for all other sub-studies; after two or three freeze–thaw cycles in all cancer sub-
162 studies) were assayed at the IARC, using the AbsoluteIDQ p180 Kit (Biocrates Life Sciences AG,
163 Innsbruck, Austria) and following the procedure recommended by the supplier. Samples were
164 analyzed by ultra-high performance liquid chromatography (LC; 1290 Series HPLC; Agilent, Les
165 Ulis, France) coupled to a tandem mass spectrometer (MS/MS; QTrap 5500; AB Sciex, Les Ulis,
166 France, for the hepatobiliary and kidney cancer sub-studies; and Triple Quad 4500; MesoScale
167 Diagnostics, Rockville, MD, USA for breast cancer sub-studies and AB Sciex, Framingham, MA,
168 USA, for the colorectal and prostate cancer sub-studies). AA concentrations were determined
169 using isotope-labelled internal standards. The assay showed excellent reliability and
170 reproducibility for AAs measured in different laboratories on plasma or serum with different MS
171 instruments [21]. In total, there were 89 analytical batches with 30–39 quality control samples per
172 batch.

173 Less than 0.5% of the samples were below the limit of quantification. AA values below the limit
174 of quantification were set to half the limit of quantification. Measurements above the highest
175 calibration standards (set for the mass-spectrometer) were truncated to the highest standard.

176 ***Assessment of diet***

177 For a total of 514,487 participants, the diet was assessed at study baseline using validated
178 country/centre-specific methods, including food frequency questionnaires (FFQs) spanning the

179 previous 12 months [13]. In most centres, FFQs were self-administered, with the exception of
180 Ragusa (Italy), Naples (Italy) and Spain, where face-to-face interviews were performed.
181 Extensive quantitative FFQs were used in northern Italy, the Netherlands, Germany and Greece
182 that were structured by meals in Spain, France and Ragusa. Semi-quantitative FFQs were used in
183 Denmark, Norway, Naples (Italy), Umeå (Sweden) and the United Kingdom, while a non-
184 quantitative FFQ was combined with a 7-day record on hot meals in Malmö (Sweden) [13]

185 In addition, a computer-assisted single 24-HDR interview program (EPIC-soft) was used to
186 conduct interactive, face-to-face dietary interviews following a highly standardised procedure.
187 The 24-HDR was collected from a representative sample (calibration cohort n = 36,994) from the
188 entire EPIC cohort [22] and from which 36,972 had valid information.

189 Individual dietary intakes of AAs were estimated by matching the dietary assessment data of the
190 EPIC cohort with the U.S. nutrient database (National Nutrient Database for Standard Reference
191 of the U.S. Department of Agriculture – USDA) [23] following standardized procedures [24]. In brief,
192 the U.S. nutrient database food composition data were first matched with the food list derived
193 from the 24-HDR, which was afterwards used as a basis for the matching with the foods reported
194 in the DQ. Specific foods and recipes that were not included in the U.S. nutrient database were
195 decomposed in ingredients which were available in the U.S. nutrient database table. Different
196 quality controls (such as double data entry/matching by two independent dietitians; checking of
197 outliers within food groups, comparison with independently estimated AA intakes in the Oxford
198 arm of EPIC [25] etc.) were carried out to achieve high quality of the food matching and to avoid
199 errors, leading to reliable nutrient intake data.

200

201 ***Statistical analysis***

202 Mean and standard deviation (SD) for continuous variables and the percentages for categorical
203 variables were calculated to study the demographic characteristics of the participants. Moreover,
204 the mean, the SD, and the percentiles 25th, 50th and 75th of dietary AAs were calculated.

All AA concentrations and intakes were log-transformed to approximate the normal distribution before statistical testing. Pearson's correlation coefficients were used to compare blood AA concentrations with (i) their respective dietary intakes of AA (g/d, assessed with the DQ and the 24-HDR), following previous and published analyses from the Oxford cohort [25], and (ii) dietary density intakes of AAs (proportion of energy intake, assessed with the DQ and the 24-HDR), and (iii) dietary intakes of AAs per kg of body weight (assessed with the DQ and the 24-HDR). AA density was calculated as the log transformed proprotion of energy coming from the respective AAs [log (AA in gram*4/energy intake in kcal)]. As a sensitivty analysis, partial Pearson correlations (g/d, assessed with the DQ and the 24-HDR) were performed, adjusting for age at blood collection, sub-study, laboratory batch, body weight and fasting status, and center were used to examine the correlations between blood AA concentrations and dietary intakes of AAs. Furthermore, correlation coefficients (g/d, assessed with the DQ and the 24-HDR) between blood AA concentrations and their respective dietary intakes were calcuated stratified by fasting status (≤ 6h fasting, > 6h fasting).

In order to investigate how blood AA concentrations (μmol/l) relate to increasing intakes, dietary intakes of AAs (in grams per day assessed with the DQ and the 24-HDR) were converted into quintiles of intakes and then one way analysis of variance (ANOVA) tests were run.

Conventional two-sided P-values are shown, but all results have been interpreted after allowance for multiple testing using the Bonferroni method; the per-test significance level was 0.05 divided by the number of tests (N=17 and thus $p<0.0029$). All analyses were conducted using SAS version 9.4 and SPSS v.20.

Results

Table 1 shows the main demographic characteristics of the study participants with both biomarker information and dietary information (assessed with the DQ; N=3,768 and the 24-HDR; N=334), respectively. Among the 3,768 participants, most were females (59.2% vs 40.8% males), most

had a low or medium level of education (43.4% and 34.7%, respectively), and the average body mass index (BMI) was 26.5 kg/m^2.

Mean, SD, 25th, 50th, 75th percentile of blood AAs and dietary AA intakes (assessed with the DQ; N=3,768 and the 24-HDR; N=334), are shown in **Table 2**. Overall, mean dietary AA from DQ intakes ranged from 0.87 g/d (tryptophan) to 14.42 g/d (glutamic acid). Very similar results were found when using the 24-HDR instead of the DQ to assess dietary AA intakes. Blood AA concentrations ranged from 8.81 µmol/l (aspartic acid) to 342.44 µmol/l (alanine).

Table 3 shows Pearson's correlations coefficients between blood AA concentrations and dietary intakes assessed with the DQ (in (i) g/d, by (ii) dietary density intakes of AAs and by (iii) kg of body weight). When using the DQ (g/d), unadjusted models showed some significant but weak correlations ($-0.15 \leq r \leq 0.17$).

Statistically significant positive correlations were found for most essential AAs (isoleucine, leucine, lysine, methionine, threonine, tryptophan, and valine) and conditionally essential AAs (arginine, glycine and tyrosine), after controlling for multiple testing (Bonferroni correction). For non-essential AAs (alanine, aspartic acid, glutamic acid), statistically significant small negative correlations were found.

We did not observe any significant correlation between circulating levels and dietary intake levels for the essential AAs histidine and phenylalanine, the conditionally essential AA proline, and non-essential AA serine.

Similar results were found when investigating the correlation between blood AA concentrations and (ii) dietary density intakes of AA or (iii) dietary intakes of AA per kg of body weight. For essential AAs attenuated results were found but still remained significant for lysine, methionine, threonine and the conditionally essential arginine and proline. On the other hand, for the non-essential AAs stronger correlations were shown.

Supplementary Table 1 presents correlations between dietary AA intakes (assessed with the 24-HDR, N=334) and blood concentrations of AAs. Although results with the 24-HDR presented

similar characteristics to the DQ results, only correlation coefficients for the essential AAs isoleucine, leucine, lysine and valine and the conditional essential AA arginine were significant , while almost all correlations tests (including also most essential AAs) were significant for the DQ data (N=3,768).

Supplementary Table 2 shows partial Pearson's correlation coefficients between blood AA concentrations (µmol/l) and and dietary intakes of AA (g/d) (assessed with the DQ and 24-HDR) adjusted for age at blood collection, sub-study, laboratory batch, body weight, fasting status, and center. Results remained similar in terms of directions and magnitudes of the relationships between blood concentrations and the respective dietary intakes. Adjusted correlations (Supplementary Table 2) showed similar but slightly attenuated correlations compared to unadjusted models (Table 3). Nonetheless, for both unadjusted and adjusted models, statistically significant positive correlations were found for most essential AAs (isoleucine, leucine, lysine, methionine, tryptophan, and valine) and the conditionally essential AA tyrosine, after controlling for multiple testing (Bonferroni correction). For non-essential AAs, after adjustments, none of the correlations remained significant.

In **Supplementary Table 3**, Pearson's correlations between blood AA concentrations (µmol/l) and dietary intakes of AA (g/d) (assessed with the DQ and 24-HDR) stratified for fasting status using 2 categories: ≤6h and >6h are presented. For those correlations that were positive (mainly essential AAs and the conditionally essential arginine and tyrosine) higher positive correlations were detected for those who fasted > 6h compared to those with ≤6 h of fasting. For those that had a negative correlation (mainly the non-essential AAs alanine and glutamic acid and the conditionally essential glycine and proline) lower negative correlations were found for those who fasted >6h.

Supplementary Figures 1 and 2 show the relationship between dietary AA intakes (quintiles) (assessed with the DQ and 24-HDR respectively) and mean blood AA concentrations (log transformed) using the ANOVA statistics. Statistically significant associations between blood AA concentrations and AA intakes in quintile categories were found for most AA, except for essential

AAs phenylalanine and threonine, conditionally essential AAs glycine and proline and non-essential AA aspartic acid. These results are showing weak positive associations for essential AAs (higher quintiles of AA dietary intakes were linked to higher blood AA concentrations), except for histidine and phenylalanine for which an initial increase of dietary AA intake (from quintile 1 to quintile 2) was related to an increase in blood AA levels but not successive increases. The conditionally-essential AAs were showing mixed results (arginine and tyrosine showing a positive trend while glycine a negative trend). For non-essential AAs weak inverse relationships were observed. Although results with the quintiles from 24-HDR presented similar characteristics to the DQ results, only lysine and valine were statistically significant for the ANOVA tests performed on the 24-HDR.

Discussion

This study aimed to investigate associations between dietary intakes and blood concentrations of AA in a large sample of EPIC participants. Weak although statistically significant positive correlations were observed between intakes and blood concentrations of most essential AAs (isoleucine, leucine, lysine, methionine, threonine, tryptophan, and valine) and conditionally essential AA (arginine and tyrosine). On the contrary, correlations were negative among most non-essential AA (alanine, aspartic acid, glutamic acid) intakes and blood concentrations. Additional analyses were conducted to investigate the variations in blood AA levels across quintiles of dietary AA intake. Higher intakes of non-essential AAs and glycine were related to lower blood AA levels, while for increasing quintiles of essential AA, higher blood AA concentrations were observed. Only histidine and phenylalanine had initial increase of dietary AA intake (from quintile 1 to quintile 2) but no successive increases; which is in agreement with a previous study [26]. Kriengsinyos et al. demonstrated that a lack of histidine in the diet for a prolonged period of time resulted in an accommodation of protein turnover and phenylalanine oxidation, leaving in doubt the essential role of histidine in healthy adults [26].

These results suggest that dietary AA intakes might have some impact on physiological AA status, particularly the essential AAs. Although the circulating concentrations of AAs are subject to homeostatic control, they are also dependent on diet, as well as absorption and gut microbial synthesis and utility, BMI, lifestyle (i.e. physical activity) and genetic factors [7]. Therefore, it is accepted that AA intakes are not directly reflected in AA blood concentrations [27].

Few studies have investigated the relationship between dietary AA intakes and concentrations in serum or plasma and have yield inconsistent results [2,9-11,28-32]. Moreover, these studies were mainly conducted in animals, usually had a small sample size and special conditions (such as dietary supplementation of specific AAs during a specific period of time). Similar to our findings, a previous publication using data from the EPIC-Oxford cohort (N=392, with little overlap with the data in the current analysis), found that dietary AA and plasma AA concentrations were not strongly correlated and only significant positive correlations were found for leucine, lysine, methionine, tryptophan and tyrosine [25]. A recent study examined serum metabolites associated with dietary protein intake and found that many metabolites associated with protein intake were related to the metabolism of essential AAs and branched-chain AAs (a group of three essential amino acids: leucine, isoleucine and valine) in particular [29]. However, in another study that included 1997 female twins, no associations were found between branched chain AA intakes and plasma concentrations [2]. Iwasaki et al. investigated the relationship between dietary and plasma AA levels [25,31]; contrary to our study, no significant positive correlations were observed between AA intakes (from weighed dietary records or DQ) and plasma levels for either men (N=68) or women (N=71). Most of the correlations observed were inverse, though rarely statistically significant [25,31]; although in agreement with our study, significant inverse correlations were found for the conditionally essential (cysteine and glycine) and non-essential (alanine) AAs [31]. Although measurement errors related to the 4-day weighed dietary recalls and the DQ cannot be ruled out, the small sample size in the study of Iwasaki et al., could potentially explain their non-significant findings. Most of the studies therefore, have concluded that correlations between dietary intakes

of AA and plasma levels are low or have no correlation, even after excluding subjects with non-fasting samples [25,31]

In spite of being a conditionally essential AA, dietary intake of glycine was found to be negatively related with glycine levels in plasma. Contrary to our results, some studies including animals supplemented with glycine reported that plasma glycine and serine concentrations were closely associated with the presence or absence of these AAs in the diet through supplementation [33,34].

The weak positive correlations observed in this study between essential AA intake and concentrations in plasma can be explained by the fact that essential AAs (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) cannot be made by the body and consequently they must be found in food. On the other hand, the negative correlations found mostly between non-essential or conditionally essential AA intake and concentration levels in plasma could be expected because they don´t have to be consumed as they can be synthesized by the body.

Apart from the limitations of estimating nutrient intakes with DQ and/or 24-HDR [35], the homeostatic regulation can partly explain the small or the negative correlations found between dietary AA intakes and plasma concentrations. Therefore, although the consumption of foods with the highest amount of AAs could be correlated with higher levels of these AAs in plasma, levels in plasma seemed not necessarily representative of AA intake [36]. For example, the gastrointestinal tract is responsible for digestion of proteins and absorption of small peptides and AAs, and plays an important role in the synthesis, conversion and catabolism of AAs. Moreover, there are other internal organs such as liver, kidney and muscles that play an important role affecting the plasma AA profile through inter-organ AA metabolism. The liver, for example, can synthesize most of the non-essential AAs and is the most important organ to catabolise AAs. Thus, if the liver is damaged, the total plasma AA concentrations will increase [36]. Similarly, the kidneys are not only able to synthesize some AAs (e.g. glycine from hydroxyproline) but is a key factor in the homeostatic control of AA concentrations through filtration, reabsorption, synthesis, degradation and urinary excretion of free AAs and peptides [37]. Finally, muscles contribute largely to the

363 homeostatic control of AA plasma concentrations since depending on its metabolism status,
364 muscles are using free AAs from the plasma to synthesize their own proteins and liberating AAs
365 when catabolizing proteins.

366 To the best of our knowledge, this is the largest study to date comparing dietary intakes of AAs
367 with circulating AA levels using two different methods to calculate AA intake (DQ and 24-HDR)
368 and the first study to examine a dose-response dimension in humans. Moreover, great care was
369 taken in matching the U.S. nutrients database with the EPIC food list including many different
370 quality controls, such as independent matching by the EPIC Oxford team.

371 This study also has several limitations. Although the gastrointestinal tract and other organs such
372 as the liver, kidney or skeletal muscle are implicated in AA metabolism, we were not able to
373 account for these factors. Moreover, the DQs were not designed to measure single AAs, which
374 could result in measurement errors when assessing dietary AA. However, given that we observed
375 small but positive correlation coefficients for AA intake from the single 24-HDR and DQs and
376 that the correlations between the circulating levels and both dietary intake assessments (24-HDR
377 and DQs) were similar, these possible measurement errors might not be the major reason for the
378 absence of moderate/strong positive correlations between dietary and plasma concentrations;
379 instead it may be due to the interpersonal differences in the absorption and metabolism of the AA.
380 Matching European foods with the U.S. nutrient database may also have some limitations,
381 although the U.S. nutrient database includes many foods from different regions in the world.
382 Nevertheless, our study results demonstrated very good comparison between protein and energy
383 intakes calculated via the U.S. nutrient database and the protein and energy intake values obtained
384 in the “reference” EPIC Nutrient DataBase (ENDB) that was compiled by combining different
385 country-specific food composition databases following standardised procedures (data not shown)
386 [38]. An important disadvantage of our analysis is that only one blood measurement, as well as only
387 one measurement for the 24-HDR, were taken at baseline which may not be representative for
388 long-term status.

Another limitation is that around 60% of our study sample did not have fasting blood samples (with fasting ≥6 h since last meal). Using liquid chromatography-tandem mass spectrometry (LC-MS), Townsend et al. compared multivariable-adjusted geometric mean metabolite LC-MS peak areas across fasting time, season of blood collection, and time of day of blood collection categories. Their results showed that fasting status, was not an important source of variability in measurements of most AAs in their study[39]. Another recent investigation showed that percent differences in AAs were negligible across fasting status at the time of blood collection [40]. Nevertheless, in order to minimize the possible confounding by nutritional influence, correlations were adjusted for fasting status[41]. Additionally, we stratified our results by fasting status to investigate potential differences in associations between the AA intakes and blood AA concentrations.

In conclusion, weak positive correlations were found between most essential and conditionally essential AA intakes and plasma AA concentrations, while weak inverse associations were observed for the non-essential AAs. While metabolic mechanisms regulating plasma AA homeostasis may play a major part in the association between dietary AA intake and plasma concentration levels, these results might suggest a potential association between dietary AA intakes on AA status, particularly for the essential AAs.

IARC disclaimer

Where authors are identified as personnel of the International Agency for Research on Cancer / World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer / World Health Organization.

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Authorship

The authors' contributions were as follows: I.I and I.H designed the research; I.I, I.H conducted the research; I.I and I.H analysed the data and I.I and I.H, A.C and J.A.S wrote the paper. The manuscript was drafted and prepared, reviewed and revised by all authors. All authors made substantial contributions to the paper and approved the final version of the manuscript.

Availability of data and materials: For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at http://epic.iarc.fr/access/index.php

Declaration of interests

The authors declare no conflicts of interests.

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Table 1. Demographic characteristics of the control subjects in five nested case-control cohorts within the EPIC study with available measures of blood concentrations of amino acids and dietary intake data measured by dietary questionnaires (DQ) (N=3768) or 24-h dietary recalls (24-HDR) (N=334).

	N= 3,768 (control subjects with DQ data)		**N= 334** (control subjects with 24-HDR data)	
Demographic characterictics	**Mean**	**SD**	**Mean**	**SD**
Age (years)	57.0	8.3	58.0	6.9
BMI (kg/m^2)	26.5	3.9	26.4	3.8
	n	**%**	**n**	**%**
Sex				
Female	2229	59.2	228	68.3
Male	1539	40.8	106	31.7
Education				
Low	1634	43.3	159	47.6
Medium	1306	34.6	105	31.4
High	688	18.2	65	19.4
Not reported or missing	140	3.7	5	1.4
Fasting status				
< 3h fasting	1510	40.1	112	33.5
3 – 6h fasting	662	17.6	57	17.1
> 6h fasting	1508	40.0	156	46.7
Missing	88	2.3	9	2.7

DQ: Dietary Questionnaire; 24-HDR: 24h-Dietary Recall.

Table 2. Table 2: Mean, SD, 25th, 50th, and 75th percentile of dietary amino acid (AA) intakes (g/day) reported for the 24-hour dietary recall data (24-HDR) and the dietary questionnaire data (DQ) and blood AA concentrations (µmol/l) for the control subjects in five nested case-control cohorts within the EPIC study.

	Dietary amino acid intakes (DQ – g/d); N= 3,768					**Dietary AA (24-HDR – g/d); N=334**					**Blood AA (µmol/l); N= 3,768**				
AA	**Mean**	**SD**	**P25**	**P50**	**P75**	**Mean**	**SD**	**P25**	**P50**	**P75**	**Mean**	**SD**	**P25**	**P50**	**P75**
Essential															
Histidine	2.1	0.7	1.6	2.0	2.5	2.0	0.9	1.6	2.0	2.5	72.9	13.5	64.3	70.9	79.3
Isoleucine	3.3	1.1	2.5	3.1	3.9	3.2	1.5	2.5	3.1	3.97	68.7	22.3	53.8	64.4	78.2
Leucine	5.8	1.9	4.4	5.6	6.9	5.7	2.6	4.4	5.6	6.9	127.4	36.8	103.0	120.0	144.0
Lysine	5.2	1.8	3.9	5.0	6.3	5.1	2.5	3.9	5.0	6.3	189.6	44.8	158.0	184.0	215.0
Methionine	1.6	0.5	1.2	1.6	2.0	1.6	0.7	1.2	1.6	2.0	20.5	6.0	16.5	19.6	23.2
Phenylalanine	3.3	1.1	2.5	3.1	3.9	3.2	1.5	2.5	3.1	3.9	59.0	13.0	50.1	56.4	65.5
Threonine	2.9	0.9	2.2	2.7	3.4	2.8	1.3	2.2	2.7	3.4	110.0	27.5	90.5	106.0	125.0
Tryptophan	0.8	0.2	0.6	0.8	1.0	0.8	0.4	0.6	0.8	1.0	54.1	11.0	46.6	52.8	60.4
Valine	3.9	1.3	3.0	3.7	4.6	3.8	1.7	3.0	3.7	4.6	205.4	45.8	173.0	200.0	232.0
Conditionally essential															
Arginine	3.8	1.3	2.9	3.6	4.5	3.8	2.0	2.5	3.5	5.0	60.1	27.7	43.2	57.3	72.7
Glycine	2.8	0.9	2.3	2.6	3.3	2.8	1.4	2.1	2.6	3.3	202.5	68.5	149.0	155.0	185.0
Proline	5.1	1.8	3.8	4.8	6.1	5.0	2.2	3.8	4.8	6.1	184.8	60.6	141.0	175.0	218.0
Tyrosine	2.6	0.9	2.0	2.5	3.1	2.5	1.2	2.0	2.5	3.1	64.5	16.8	53.0	62.1	73.1
Non-essential															
Alanine	3.4	1.1	2.5	3.2	4.0	3.4	1.6	2.2	3.1	4.2	342.4	91.8	278.0	330.0	395.0

Dietary amino acid intakes (DQ – g/d); N= 3,768						Dietary AA (24-HDR – g/d); N=334					Blood AA (µmol/l); N= 3,768				
AA	**Mean**	**SD**	**P25**	**P50**	**P75**	**Mean**	**SD**	**P25**	**P50**	**P75**	**Mean**	**SD**	**P25**	**P50**	**P75**
Aspartic acid	6.6	2.1	5.1	6.4	7.9	6.6	3.2	5.1	6.4	7.9	8.8	9.0	2.5	6.7	9.6
Glutamic acid	14.4	4.8	10.6	13.6	16.9	14.3	6.4	10.9	13.6	16.9	58.8	43.3	29.9	41.4	53.4
Serine	3.4	1.1	2.6	3.3	4.1	3.4	1.5	2.6	3.3	4.1	93.1	27.3	74.9	88.5	105.0

Table 3. Pearson's correlations* between blood AA concentrations (μmol/l) and (i) dietary intakes of AA, (ii) dietary density[+] intakes of AA, and (iii) dietary intakes of AA per kg of body weight, assessed with the dietary questionnaires data (DQ) (N=3,768) for the control subjects in five nested case-control cohorts within the EPIC study.

AA	(i) Pearson correlations (g/d)	p [a]	(ii) Pearson correlations (dietary density)	p [a]	(iii) Pearson correlations (per kg body weight)	p [a]
Essential						
Histidine	-0.016	0.315	-0.016	0.321	0.013	0.422
Isoleucine	0.107	**<0.001**	0.026	0.108	-0.041	0.011
Leucine	0.114	**<0.001**	0.026	0.112	-0.031	0.056
Lysine	0.124	**<0.001**	0.126	**<0.001**	0.070	**<0.001**
Methionine	0.110	**<0.001**	0.067	**<0.001**	0.090	**<0.001**
Phenylalanine	0.018	0.281	-0.003	0.870	-0.048	0.003
Threonine	0.053	**0.001**	0.073	**<0.001**	0.077	**<0.001**
Tryptophan	0.096	**<0.001**	0.043	0.009	0.043	0.008
Valine	0.156	**<0.001**	0.069	**<0.001**	-0.013	0.426
Conditionally essential						
Arginine	0.114	**<0.001**	0.128	**<0.001**	0.113	**<0.001**
Glycine	-0.145	**<0.001**	-0.065	**<0.001**	-0.026	0.117
Proline	0.009	0.589	-0.071	**<0.001**	-0.066	**<0.001**
Tyrosine	0.105	**<0.001**	0.059	**<0.001**	-0.010	0.537

AA	(i) Pearson correlations (g/d)	p [a]	(ii) Pearson correlations (dietary density)	p [a]	(iii) Pearson correlations (per kg body weight)	p [a]
Non-essential						
Alanine	-0.061	**<0.001**	-0.081	**<0.001**	-0.132	**<0.001**
Aspartic acid	-0.059	**<0.001**	-0.025	0.132	-0.079	**<0.001**
Glutamic acid	-0.106	**<0.001**	-0.190	**<0.001**	-0.222	**<0.001**
Serine	-0.026	0.114	0.064	**<0.001**	0.069	**<0.001**

***** AA blood concentrations and dietary intakes were log-transformed before doing the correlation analysis.

[+] AA density was calculated as the log transformed proprotion of energy coming from the respective AAs [log (AA in gram*4/energy intake in kcal)].

[a] Conventional P-values are shown and those marked in bold were significant after Bonferroni correction (N test = 17) (P<0.0029).