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The Associations of Habitual Intake of Sulfur Amino Acids, Proteins and Diet Quality with Plasma Sulfur Amino Acid Concentrations: The Maastricht Study

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A B S T R A C T

Background: Plasma sulfur amino acids (SAAs), i.e., methionine, total cysteine (tCys), total homocysteine (tHcy), cystathionine, total glutathione (tGSH), and taurine, are potential risk factors for obesity and cardiometabolic disorders. However, except for plasma tHcy, little is known about how dietary intake modifies plasma SAA concentrations.

Objective: To investigate whether the intake of SAAs and proteins or diet quality is associated with plasma SAAs.

Methods: Data from a cross-sectional subset of The Maastricht Study (n = 1145, 50.5% men, 61 interquartile range: [55, 66] y, 22.5% with prediabetes and 34.3% with type 2 diabetes) were investigated. Dietary intake was assessed using a validated food frequency questionnaire. The intake of SAAs (total, methionine, and cysteine) and proteins (total, animal, and plant) was estimated from the Dutch and Danish food composition tables. Diet quality was assessed using the Dutch Healthy Diet Index, the Mediterranean Diet Score, and the Dietary Approaches to Stop Hypertension score. Fasting plasma SAAs were measured by liquid chromatography (LC) tandem mass spectrometry (MS) (LC/MS-MS). Associations were investigated with multiple linear regressions with tertiles of dietary intake measures (main exposures) and z-standardized plasma SAAs (outcomes).

Results: Intake of total SAAs and total proteins was positively associated with plasma tCys and cystathionine. Associations were stronger in women and in those with normal body weight. Higher intake of cysteine and plant proteins was associated with lower plasma tHcy and higher cystathionine. Higher methionine intake was associated with lower plasma tGSH, whereas cysteine intake was positively associated with tGSH. Higher intake of methionine and animal proteins was associated with higher plasma taurine. Better diet quality was consistently related to lower plasma tHcy concentrations, but it was not associated with the other SAAs.

Conclusion: Targeted dietary modifications might be effective in modifying plasma concentrations of tCys, tHcy, and cystathionine, which have been associated with obesity and cardiometabolic disorders.

Keywords: sulfur amino acids, habitual dietary intake, proteins, diet quality, plasma concentrations

Abbreviations used: DAG, directed acyclic graph; DASH, Dietary Approaches to Stop Hypertension; DHD-index, Dutch Healthy Diet Index; FFQ, Food frequency questionnaire; IQR, Interquartile range; LC/MS-MS, Liquid chromatography tandem mass spectrometry; MDS, Mediterranean Diet Score; MICE, Multiple imputation by chained equations; NEVO, Dutch Food Composition Database; SAA(s), Sulfur amino acid(s); SD, Standard deviation; tHcy, Total homocysteine; tCys, Total cysteine; tGSH, Total glutathione; T2DM, Type 2 diabetes mellitus.

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Introduction

Plasma concentrations of sulfur amino acids (SAAs), which include the essential amino acid methionine and its derivatives cysteine, homocysteine, cystathionine, glutathione, and taurine (Figure 1) [1, 2], have been reported to be associated with obesity and cardiometabolic disorders [2, 3]. Specifically, higher plasma total cysteine (tCys) was consistently associated with higher BMI and whole-body fat mass [2–9], whereas higher plasma methionine has been related to higher intrahepatic fat [9, 10]. Furthermore, positive associations have been reported for total homocysteine (tHcy) or cystathionine with cardiovascular diseases [11, 12], whereas higher plasma total glutathione (tGSH) has been related to lower obesity and cardiometabolic risk [2, 4, 5, 13]. Finally, plasma taurine has been inconsistently associated with obesity and cardiometabolic risk [14–16]. These findings call for effective strategies to regulate plasma SAA concentrations.

Of all SAAs, only methionine and cysteine are proteinogenic and important constituents of any protein. In food, they are found in greater concentrations in animal-derived proteins [17, 18], which are consumed in excess within a Western-style diet. Additionally, taurine is commonly present as a free amino acid in animal-derived food products [19]. Consequently, it has been shown [20–22] that the SAA intake (i.e., the combined intake of methionine and cysteine) often exceeds the recommended levels for the adult population (i.e., 19 mg/kg of body weight/day [22]).

It has been hypothesized that higher protein intake, especially from animal sources, would result in higher plasma concentrations of SAAs. Yet, to date, literature reports conflicting results. Although people who followed a vegan diet had substantially lower methionine intake than omnivores, there were minimal differences in plasma methionine concentrations [23–25]. Plasma concentrations of cystathionine and taurine might be more responsive to short-term abstinence from animal proteins [26], whereas the literature is less clear regarding the other SAAs [5, 27–32]. Studies specifically examining long-term SAA intake are limited. Weak or null associations have been reported for methionine and cysteine intake with plasma concentrations of methionine, tCys, tHcy, and tGSH [29, 32–34]. No studies have investigated the long-term intake of methionine and cysteine on taurine. Finally, some studies have examined diet quality, generally characterized by higher intake of fruit and vegetables, (whole) grains and legumes, and lower intake of meat, saturated fat and processed foods, in relation to plasma methionine [35–37], tCys [37, 38], tHcy [39–44], tGSH [32, 45], cystathionine and taurine [38]. Overall, although better diet quality was consistently associated with lower plasma tHcy [39–44], weak evidence is available for the other SAAs.

Given the mounting evidence of the relationships between plasma SAAs and obesity and cardiometabolic disorders [2–16],

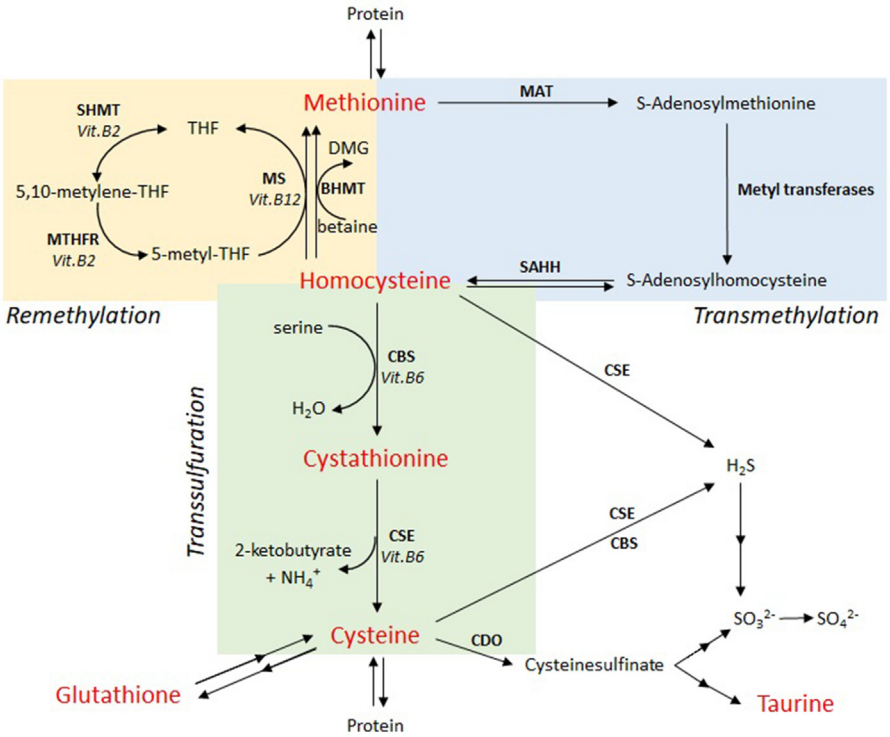

FIGURE 1. Schematic representation of the sulfur amino acids metabolic pathway. Examined compounds are written in red. Colored boxes indicate the three metabolic pathways involved: i) the transmethylation (blue), which converts methionine into homocysteine, ii) the remethylation (yellow), which regenerate methionine from homocysteine, and iii) the transsulfuration, which converts irreversibly homocysteine into cystathionine and cysteine. MAT: Methionine Adenosyltransferase; SAHH: S-adenosylhomocysteine hydrolase; BHMT: Betaine-homocysteine S-methyltransferase; DMG: dimethylglycine; MS: methionine synthase; THF: tetrahydrofolate; SHMT: serine hydroxymethyltransferase; MTHFR: methylenetetrahydrofolate reductase; CBS: cystathionine β-synthase; CDO: cysteine dioxygenase; CSE: cystathionine γ-lyase or gamma-cystathionase. Adapted from [1].

a better understanding of the effects of habitual dietary intake on plasma SAAs could yield more effective preventive or therapeutic strategies through dietary recommendations. Therefore, a comprehensive investigation of the dietary determinants of plasma SAAs is needed. Here, we examined the associations of dietary SAAs, total protein, protein source, and diet quality with plasma SAAs in a Dutch population of middle-aged adults.

Methods

Study population

We used data from The Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology have been described previously [46]. In brief, the study focuses on the etiology, pathophysiology, complications, and comorbidities of type 2 diabetes mellitus (T2DM) and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 y and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known T2DM status, with an oversampling of individuals with T2DM, for efficiency reasons. A subcohort of 1200 participants was then selected for measuring plasma SAAs among the first 3451 participants who completed the baseline survey between November 2010 and September 2013. This subcohort comprised all participants with T2DM or prediabetes (a category that included people with impaired fasting glucose and impaired glucose tolerance) as well as a random sample of participants with normal glucose tolerance who underwent abdominal magnetic resonance imaging assessment. The latter selection criteria were defined to obtain a subsample with an extensive range of assessed characteristics and sufficient power to investigate the associations of plasma SAAs with measures of obesity. Of these, 1145 were included in the current study; individuals with missing data on dietary intake (n = 54) or plasma SAAs (n = 1) were not included (Figure 2). Examinations of each participant were performed within a time window of 3 mo. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Ministery of Health, Welfare and Sports of the Netherlands (Permit 131088-105234-PG). All participants gave written informed consent.

Dietary intake assessment

Dietary intake during the preceding 12 mo was assessed with a validated FFQ, which consisted of 253 food items with questions on frequency and quantity [47]. Frequency questions used

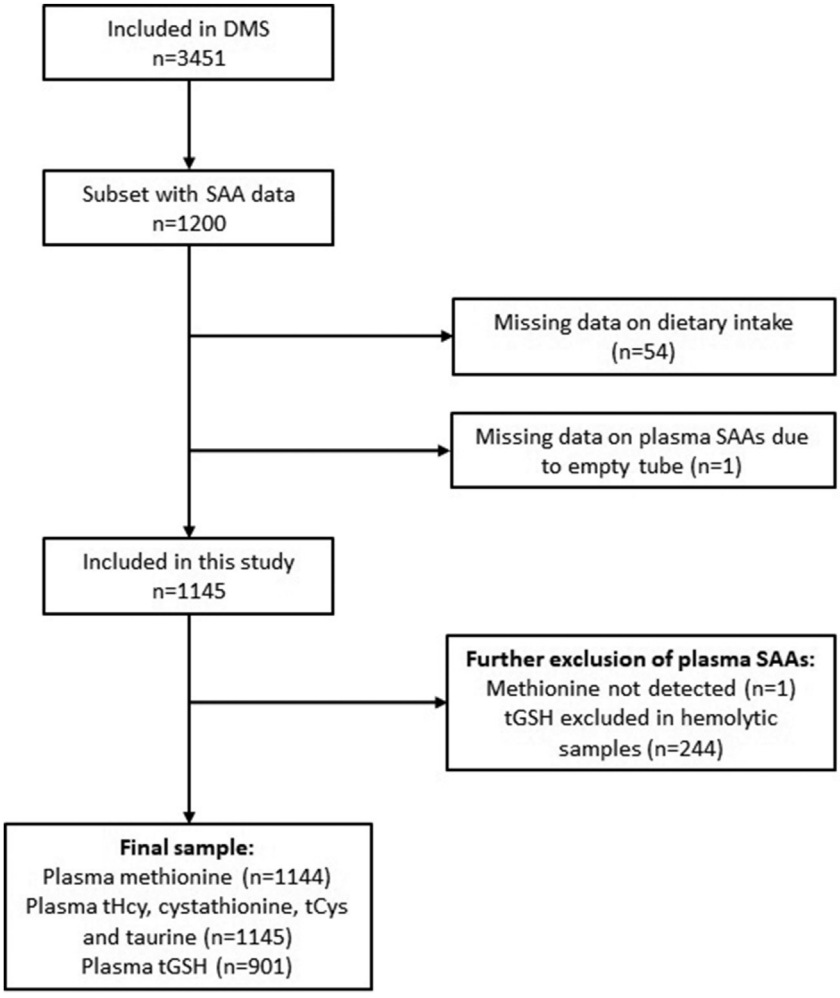

FIGURE 2. Flowchart of study participants.

an answer model with 11 options, from "not used" to "7 d/wk". Each of the frequency questions was combined with a quantity question that used an answer model with 14 standard household servings, from "<1/day" to ">12/day". Average daily consumption of food items was calculated by multiplying frequency and quantity and by transcribing food items into food codes using the Dutch Food Composition Database (NEVO) table 2011 to calculate total energy and nutrient intakes. Concentrations of SAAs in food items were not available in the NEVO table and were therefore obtained from the Danish Food Composition table [48] or, if missing, from the UK McCance and Widdowson Food Composition table [49].

Diet quality measures

Diet quality was assessed by measuring the adherence to the Dutch Healthy Diet Index (DHD-index) [50], the Mediterranean Diet Score (MDS) [51] and the Dietary Approaches to Stop Hypertension (DASH) score [52]. Higher scores on these 3 measures reflect better adherence, which has been consistently associated with positive health outcomes [53–56].

The DHD-index ranks participants based on their adherence to the 2015 Dutch dietary guidelines according to the intake of 15 food items (i.e., vegetables, fruits, wholegrain products, legumes, nuts, fish, tea, red meat, processed meat, sweetened beverages and fruit juices, alcohol, salt, fats, and oils, dairy products, and coffee). For each item, a score between 0 and 10 points was granted according to criteria previously described [50]. The DHD-index used in the current study consists of 14 items as information on type of coffee (filtered vs. unfiltered) was not included in the FFQ used in The Maastricht Study [47]. For this reason, the total index range in the current study was 0 to 140.

The MDS [51] is based on the traditional Mediterranean dietary pattern and calculates the frequencies of intake (in grams) of 9 food groups that characterize such pattern (i.e., vegetables, legumes, fruits and nuts, fish, grains, dairy products, meat, the ratio of unsaturated to saturated fatty acids, and ethanol). A score of 0 or 1 was assigned for intake below or above the cohort- and sex-specific median intake, as previously described [51]. Consequently, the overall range of the MDS was 0 to 9.

The DASH score [52] is based on the Dietary Approaches to Stop Hypertension diet plan, which has been developed for the prevention and treatment of hypertension. The score comprises 8 food groups (i.e., vegetables, fruits, nuts and legumes, wholegrain products, low-fat dairy, red and processed meat, sugar sweetened beverages, and sodium) and uses cohort- and sex-specific quintiles as cut-off values. For each food component, the score can range between 1 and 5 assigned proportionally to the intake level, as previously described [52]. Therefore, the overall DASH score ranges from 8 to 40.

Measurement of plasma SAAs

Blood samples were collected after an overnight fast into precooled EDTA tubes and kept on ice until centrifugation (within 3 h) at 1950 g for 15 min at 4°C to collect plasma and then stored at −80°C until use. Concentrations of plasma SAAs were measured by LC–tandem mass spectrometry (LC-MS/MS) using a modified version of a previously described method [57]. Briefly, isotopically labeled internal standards were added to plasma, followed by the reduction of disulfides using dithioerythritol and then protein precipitation using 5-sulfosalicyclic acid. The extracts were diluted with an aqueous solution of formic acid [0.5%] and heptafluorobutyric acid (HFBA) [0.3%] before analysis. LC-MS/MS was carried out using a Shimadzu LC-20ADXR Prominence LC system (Kyoto, Japan) coupled to a Sciex QTRAP5500 mass spectrometer with a Turbo V ion source and TurboIonspray probe (Framingham, MA, USA). Chromatographic separation was achieved on a Phenomenex Kinetex Core Shell C18 (100 x 4.6 mm, 2.6 μm) LC column (Torrance, CA, USA) with an aqueous solution of formic acid [0.5%] and HFBA acid [0.3%] and acetonitrile gradient mobile phase. Positive mode multiple reaction monitoring was used for detection. Linear calibration curves of peak area ratios of the analyte and internal standard were used for quantification. Interassay coefficients of variation for the analytes were 3.4 to 6.7%. Internal quality control and external quality assurance were used to ensure the overall quality.

In one sample, plasma methionine concentrations were below the detection limits and could not be measured (Figure 2). Furthermore, given that hemolysis affects the measurements of plasma concentrations of tGSH [58], we excluded all plasma tGSH values for those participants for whom hemolysis was reported ($n = 244$, Figure 2), identified based on visual inspection.

Covariate assessment

Glucose concentrations were measured in fasting plasma and after a standard 75-gram oral glucose tolerance test with venous blood sampling [46]. Glucose metabolism status was defined according to the World Health Organization [59] as normal glucose tolerance, prediabetes (impaired fasting glucose and impaired glucose tolerance combined), T2DM, and other types of diabetes. Smoking behavior and education were selfreported and categorized as a never, former, or current smoker and low, medium, or high, respectively. Height (cm) was measured with a wall-mounted stadiometer, and body weight in kilograms (kg), rounded to the nearest 0.1 kg, was assessed with subjects standing on calibrated electronic scales and wearing light clothes; their ratio was used to calculate BMI (kg/m^2). The amounts of light-intensity and moderate-to-vigorous physical activity were measured using the activPAL3 physical activity monitor (PALTechnologies) as previously described [60]. Total body lean mass in grams (g), excluding bone mass, was collected with whole-body dual-energy X-ray absorptiometry following the provider's instructions [61]. Prior cardiovascular disease was defined as a selfreported history of myocardial or cerebrovascular infarction, hemorrhage, percutaneous artery angioplasty of the coronary, abdominal, peripheral, or carotid arteries, or vascular surgery on the coronary, abdominal, peripheral, or carotid arteries. The glomerular filtration rate was estimated using the short Modification of Diet in Renal Disease equation [62].

Statistical analyses

Statistical analyses were performed in R version 4.1.0 with statistical significance set at $P < 0.05$. Mean and standard deviation (SD) and median and interquartile range were used to describe normally distributed or skewed continuous variables, respectively, whereas number and percentage were used for categorical variables.

To reduce bias because of missing data, multiple imputations of missing covariate data were performed using the chained

equations method (MICE), with 50 complete datasets being generated [63, 64]. An imputation model was created that comprised all exposures, outcomes, covariates, moderators, and additional auxiliary variables. The resulting sample size, i.e., $n = 1145$, or $n = 901$ for analyses with plasma tGSH as outcome, ensured a power of 80% to detect a statistically significant effect size of f=0.10 or higher (calculated with G*Power v.3.1.9.7).

Dietary intake of SAAs and proteins was adjusted for energy intake using the energy density method and expressed as grams of SAAs per 1000 kcal or percentage of energy intake from protein (E%), respectively. Pairwise Spearman's correlations adjusted for age, sex, glucose metabolism status, and total energy intake were used to investigate the relationships among the measures of dietary intake. Next, associations of each measure of dietary intake (main exposures) with plasma SAAs (outcomes) were investigated in multiple linear regressions with z-standardized (i.e., to a mean of 0 and an SD of 1) continuous outcomes. All assumptions of linear regressions, including linearity, normality of residuals, heteroscedasticity, and multicollinearity, were assessed and met before analysis. The linearity of each association was examined with generalized additive models and visual inspection with continuous main exposures and outcomes; no significant departure from linearity was detected. For the main analyses, all primary exposure variables (i.e., intake of total SAAs, methionine, cysteine, total protein, protein from animal sources and protein from plant sources, and adherence to the DHD-index, MDS, and DASH score) were categorized to reflect 3 levels of intake or adherence. Categorization was based on tertiles of intake, except the MDS for which a priori, literature-based cut-off values have been defined (i.e., low: ≤ 3, medium: 4–5, high: ≥ 6)[65]. Primary outcomes were defined as plasma concentrations of methionine, tCys, tHcy, and cystathionine. These were selected based on the previously reported associations with obesity, intrahepatic fat, or cardiometabolic risk [2–12]. Additionally, plasma cystathionine has been shown to be highly responsive to dietary changes [26, 66–69] and could represent a suitable marker for differences in the intake of SAAs and proteins between groups. Plasma tGSH and taurine were considered secondary outcomes because they are largely intracellular compounds [70, 71].

Covariate selection was based on a literature-informed directed acyclic graph (DAG) Supplementary Figure 1 [72]. Associations were first adjusted for age (years), sex (male, female), glucose metabolism status (prediabetes yes/no, diabetes yes/no), and total energy intake (kcal/day) (Model 1). In Model 2, additional adjustment was made for smoking status (former yes/no, current yes/no), coffee consumption (g/day), light-intensity, and moderate-to-vigorous physical activity (min/day). Alcohol consumption (g/day) was additionally controlled for in models with the intake of SAAs, proteins, or DASH score as primary exposures; it was excluded in models with DHD-index or MDS because alcohol consumption is one of the underlying items of these scores. Moreover, analyses with intake of SAAs and proteins were adjusted for intake of vitamin B2 (mg/day), vitamin B12 (μg/day), and folate (μg/day) in models with plasma methionine for vitamin B6 (mg/day) in models with plasma cystathionine and tCys, and for intake of all 4 vitamins B in models with plasma tHcy as outcomes. As shown in Figure 1, these vitamins are important cofactors of enzymes involved in the metabolic pathway of SAAs. The intake of methionine and cysteine and animal and plant proteins were mutually adjusted for each other. Finally, in Model 3, we additionally adjusted for the progenitor amino acid (i.e., the sulfur amino acid immediately preceding the primary exposure in the causal path in SD). Although methionine is an essential amino acid, cysteine has a sparing effect on methionine [73], and therefore plasma tCys was included in analyses with plasma methionine as an outcome. Model 2 was considered the main model because it has the lowest combined risk of overadjustment and residual confounding.

To identify any specific driver of the associations between dietary intake and plasma SAAs, we investigated the associations with protein-rich food groups (in tertiles of intake measured in g/1000 kcal) as main exposures. Finally, to investigate whether the associations changed depending on participants' sex, obesity, or (pre)diabetes status, we tested the interaction between each primary exposure and sex, BMI, or glucose metabolism status, one at a time, on plasma SAAs. Interactions were tested with continuous main exposure variables to avoid spurious findings [74]. Associations for which a significant interaction was found were then stratified according to the effect modifier categories. All additional analyses were adjusted for Model 2.

Several sensitivity analyses were performed to assess the robustness of our results, all based on Model 2. We repeated the analyses with continuous main exposures. Since no significant deviation from linearity was found, beta estimates from multiple linear regression analyses were reported. Additionally, we repeated the analyses with tertiles of the absolute intake of SAAs or proteins (g/day) as main exposures. We additionally adjusted all associations for the remaining plasma SAAs to avoid spurious findings because of the complex regulation of the SAA metabolic pathway in which both upstream and downstream compounds might affect plasma concentrations of the target SAAs. However, these analyses might be overadjusted. We additionally adjusted for levels of education as a proxy for socioeconomic status. Furthermore, total fiber intake was additionally adjusted for in the analyses with intake of SAAs or proteins as main exposures. Fiber intake was not added to the analyses with diet quality as main exposure because fiber was already accounted for in the diet quality measures. We additionally adjusted for lean mass, a proxy of muscle mass, which is a source of plasma amino acids in the fasted state [75], and for health conditions, which comprised prevalent cardiovascular disease and kidney function. The latter 2 analyses were potentially overadjusted because it could not be excluded that lean mass or prevalent health conditions were not affected, at least partly, by overall obesity and unhealthy fat accumulation [76–79], which was associated with plasma SAAs [2–16]. We excluded participants who reported gastrointestinal problems within the preceding 2 mo ($n = 138$). The gastrointestinal tract is an important site for the metabolism of SAAs, and its disorders might affect plasma concentrations of SAAs [80]. Finally, we repeated the analyses adjusted for Model 2 in the nonimputed dataset.

Results

Characteristics of the study population

Table 1 shows the participants' characteristics by tertiles of total SAA intake. Overall, the intake of total SAAs was 34.8 (28.7, 41.2) mg/kg body weight/day. Compared with the first and

TABLE 1
Characteristics of the study participants by tertiles of total SAA intake.

General characteristics and lifestyle	Total SAAs			
	Low intake ($N = 381$)	Medium intake ($N = 382$)	High intake ($N = 382$)	Overall ($N = 1145$)
Sex, % men	207 (54.2%)	198 (51.8%)	173 (45.3%)	578 (50.5%)
Age (y)	61.0 [56.0, 66.0]	61.0 [55.0, 66.0]	61.0 [54.0, 66.0]	61.0 [55.0, 66.0]
Glucose metabolism status (%)				
Normal glucose tolerance	171 (45.0%)	179 (46.9%)	144 (37.7%)	494 (43.2%)
Prediabetes	88 (23.1%)	76 (19.9%)	94 (24.6%)	258 (22.5%)
T2DM	122 (32.0%)	127 (33.2%)	144 (37.7%)	393 (34.3%)
Smoking status (%)				
Never smoker	120 (31.5%)	133 (34.8%)	139 (36.4%)	392 (34.2%)
Former smoker	198 (52.0%)	203 (53.1%)	200 (52.4%)	601 (52.5%)
Current smoker	56 (14.7%)	43 (11.3%)	38 (9.9%)	137 (12.0%)
BMI (kg/m^2)	26.0 [23.4, 28.7]	26.4 [23.8, 29.4]	27.5 [24.7, 30.9]	26.6 [24.0, 29.9]
Lean mass (kg)	50.2 [41.2, 58.8]	50.1 [41.1, 59.0]	49.5 [43.1, 59.0]	50.0 [41.7, 58.9]
Light-intensity physical activity (min/d)	321 [271, 388]	319 [260, 391]	333 [270, 380]	324 [266, 386]
Moderate-to-vigorous physical activity (min/d)	52.0 [36.3, 73.3]	50.6 [38.6, 71.5]	50.0 [35.4, 66.2]	50.6 [36.8, 70.6]
Coffee consumption (g/d)	488 [250, 650]	488 [250, 650]	488 [250, 625]	488 [250, 650]
Alcohol consumption (g/d)	11.6 [1.84, 25.9]	9.10 [1.62, 17.9]	5.26 [0.822, 13.5]	8.56 [1.33, 19.0]
Dietary intake				
Total calorie intake (kcal/d)	2310 [1940, 2800]	2110 [1770, 2460]	1860 [1540, 2190]	2090 [1730, 2490]
Total SAA intake (g/1000 kcal)	1.08 (0.09)	1.28 (0.06)	1.56 (0.16)	1.31 (0.23)
Total SAA intake (mg/kg/d)	33.0 [26.7, 39.7]	34.9 [29.9, 41.2]	36.5 [29.6, 43.3]	34.8 [28.7, 41.2]
Methionine intake (g/1000 kcal)	0.70 (0.07)	0.84 (0.06)	1.05 (0.14)	0.86 (0.17)
Methionine intake (mg/kg/d)	21.5 [17.4, 25.4]	22.5 [19.2, 26.7]	24.2 [19.8, 29.0]	22.6 [18.6, 26.9]
Cysteine intake (g/1000 kcal)	0.38 (0.04)	0.45 (0.04)	0.52 (0.06)	0.45 (0.07)
Cysteine intake (mg/kg/d)	11.6 [9.43, 14.3]	12.1 [10.3, 14.4]	11.8 [9.70, 14.7]	11.8 [9.75, 14.4]
Total protein intake (E%)	15.3 (1.34)	17.7 (1.04)	21.1 (2.18)	18.1 (2.88)
Animal protein intake (E%)	8.09 [6.94, 9.01]	10.1 [9.01, 11.1]	12.7 [11.6, 14.5]	10.1 [8.39, 12.0]
Plant protein intake (E%)	7.16 [6.44, 8.15]	7.61 [6.91, 8.48]	7.80 [7.04, 8.76]	7.51 [6.76, 8.50]
Total fat intake (E%)	17.5 (3.05)	17.2 (2.90)	16.5 (2.97)	17.0 (3.00)
Total carbohydrate intake (E%)	48.5 [44.7, 54.3]	48.7 [43.3, 53.3]	47.5 [43.3, 52.3]	48.2 [43.6, 53.3]
Total fiber intake (g/1000 kcal)	11.6 [10.2, 13.6]	12.3 [11.0, 14.2]	13.4 [11.4, 15.4]	12.5 [10.8, 14.5]
Dutch Healthy Diet Index	80.8 [71.3, 92.1]	84.6 [74.9, 95.5]	86.2 [75.5, 96.7]	83.7 [73.8, 94.8]
Mediterranean diet score	5.00 [3.00, 6.00]	5.00 [4.00, 6.00]	4.00 [3.00, 5.00]	5.00 [3.00, 6.00]
DASH diet score	24.0 [21.0, 27.0]	24.0 [21.0, 27.0]	24.0 [21.0, 28.0]	24.0 [21.0, 27.0]
Plasma SAAs				
Plasma methionine (μM)	22.0 (3.78)	22.1 (3.38)	21.8 (3.67)	22.0 (3.61)
Plasma tHcy (μM)	10.2 [8.76, 12.4]	10.0 [8.41, 11.8]	9.78 [8.41, 11.7]	9.99 [8.51, 12.0]
Plasma cystathionine (μM)	0.20 [0.15, 0.28]	0.21 [0.15, 0.30]	0.24 [0.16, 0.33]	0.22 [0.15, 0.31]
Plasma total cysteine (μM)	323 [299, 351]	322 [300, 352]	329 [307, 354]	325 [302, 352]
Plasma total glutathione (μM)	3.05 [2.58, 3.61]	3.17 [2.74, 3.65]	3.07 [2.62, 3.57]	3.10 [2.63, 3.62]
Plasma taurine (μM)	58.1 [50.9, 69.9]	59.9 [51.6, 71.7]	59.5 [49.7, 72.1]	59.4 [50.6, 71.6]

Note: Total SAA intake ranged from 0.49 g/1000 kcal to 2.52 g/1000 kcal in the study population. Low total SAA intake was defined as intakes ≤1.19 g/1000 kcal, medium intake as >1.19 and ≤1.39 g/1000 kcal and high intake corresponded to >1.39 g/1000 kcal. Data are expressed as mean (SD), median [IQR] or number (%).

second tertiles, participants with a higher intake of total SAAs were more likely to be women, never-smokers, and to have prediabetes or T2DM. Furthermore, these participants reported lower total calorie intake, although the intake of total and animal proteins was the highest in this group. This group was also characterized by the highest BMI and a somewhat higher total body lean mass. Adherence to the diet-quality indices was comparable among tertiles of total SAA intake. Plasma SAA concentrations did not differ substantially among tertiles, although slightly higher cystathionine and tCys concentrations were observed in the group with higher total SAA intake.

Relationships among measures of dietary intake

Correlations among measures of dietary intake adjusted for age, sex, glucose metabolism status, and total energy intake are shown in Supplementary Figure 2. As expected, the intake of SAAs and of total and animal proteins were strongly correlated with each other (ρ ranged from 0.69 to 0.96), apart from the intake of cysteine and animal proteins, whose correlation was of only medium strength (ρ = 0.38). Weaker correlations were found between the intake of plant proteins and of total SAAs (ρ = 0.12), methionine (ρ = -0.07), total proteins (ρ = 0.13), and animal proteins (ρ = -0.33), whereas the intake of cysteine and plant proteins was strongly correlated (ρ = 0.53). The 3 measures of diet quality were not or only weakly correlated with intake of SAAs or proteins, the only exceptions being the positive correlations with plant proteins (ρ ranged from 0.38 to 0.47). Positive trends were observed between the 3 measures of diet quality and cysteine intake (ρ ranged from 0.11 to 0.17), whereas inverse trends were found with animal protein intake (ρ ranged from

-0.09 to -0.21). Finally, although positive correlations with medium-to-large effect sizes were observed among measures of diet quality, correlations with the MDS were weaker (ρ = 0.43 with the DHD-index and 0.49 with the DASH score) than between the DHD-index and the DASH score (ρ = 0.61).

Analyses between measures of dietary intake and plasma SAAs

Results of the adjusted associations between tertiles of SAA intake, protein intake or diet quality measures, and plasma concentrations of SAAs are presented in Figures 3-5 as well as Supplementary Tables 1A-1C. Analyses with protein-rich food groups are reported in Supplementary Table 2, whereas results of the analyses stratified by sex and overweight/obesity status are reported in Supplementary Tables 3 and 4. The results of the sensitivity analyses are shown in Supplementary Tables 5-13. Unless otherwise specified, estimates from Model 2 are reported below. Results are expressed as estimates and 95% CIs and represent the adjusted difference in plasma SAAs (in SD) between the medium or high dietary intake level and the low intake level, which was set as a reference.

Associations between intake of SAAs and plasma SAAs

The intake of SAAs, either total SAAs or methionine and cysteine separately, was not associated with plasma methionine concentrations (Figure 3 and Supplementary Table 1A). Positive, nonsignificant trends for associations were found for the intake of total SAAs medium vs. low: 0.06 (-0.07, 0.20); high vs. low: 0.14 (-0.01, 0.28); *P*-trend= 0.07] and methionine medium vs. low: 0.05 (-0.09, 0.20); high vs. low: 0.15 (-0.03, 0.32); *P*-trend=

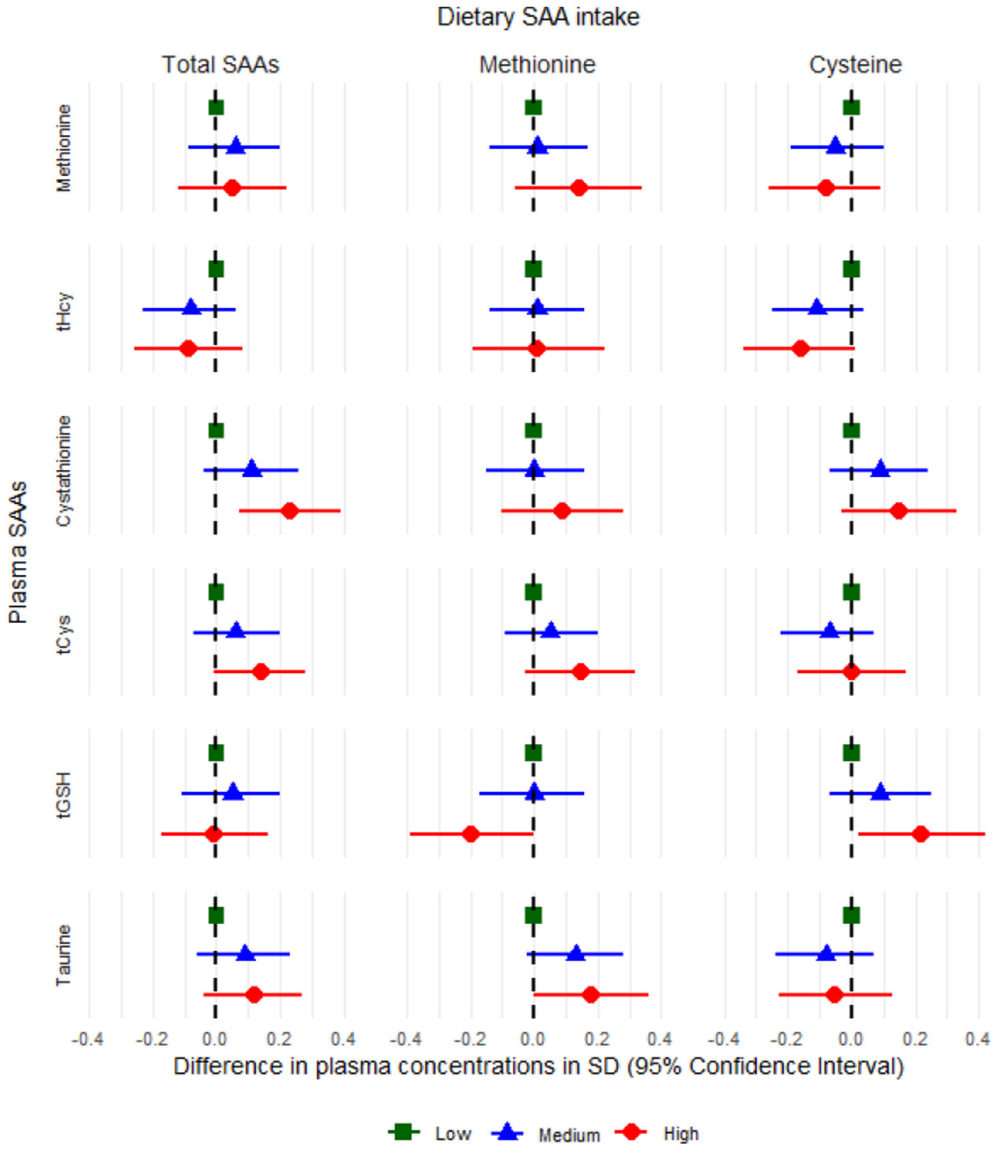

FIGURE 3. Associations between SAA intake and plasma SAAs. Note: Difference in plasma concentrations for medium and high tertiles of SAA intake compared to low intake (reference) are shown. Adjusted for age, sex, glucose metabolism status, total energy intake, smoking status, coffee consumption, light-intensity and moderate-to-vigorous physical activity, and alcohol consumption. Additional adjustment was made for vitamin B2, folate and vitamin B12 intake (in models with plasma methionine and tHcy as outcomes) and vitamin B6 (in models with plasma tHcy, cystathionine and tCys as outcomes). Intake of methionine and cysteine were mutually adjusted for each other.

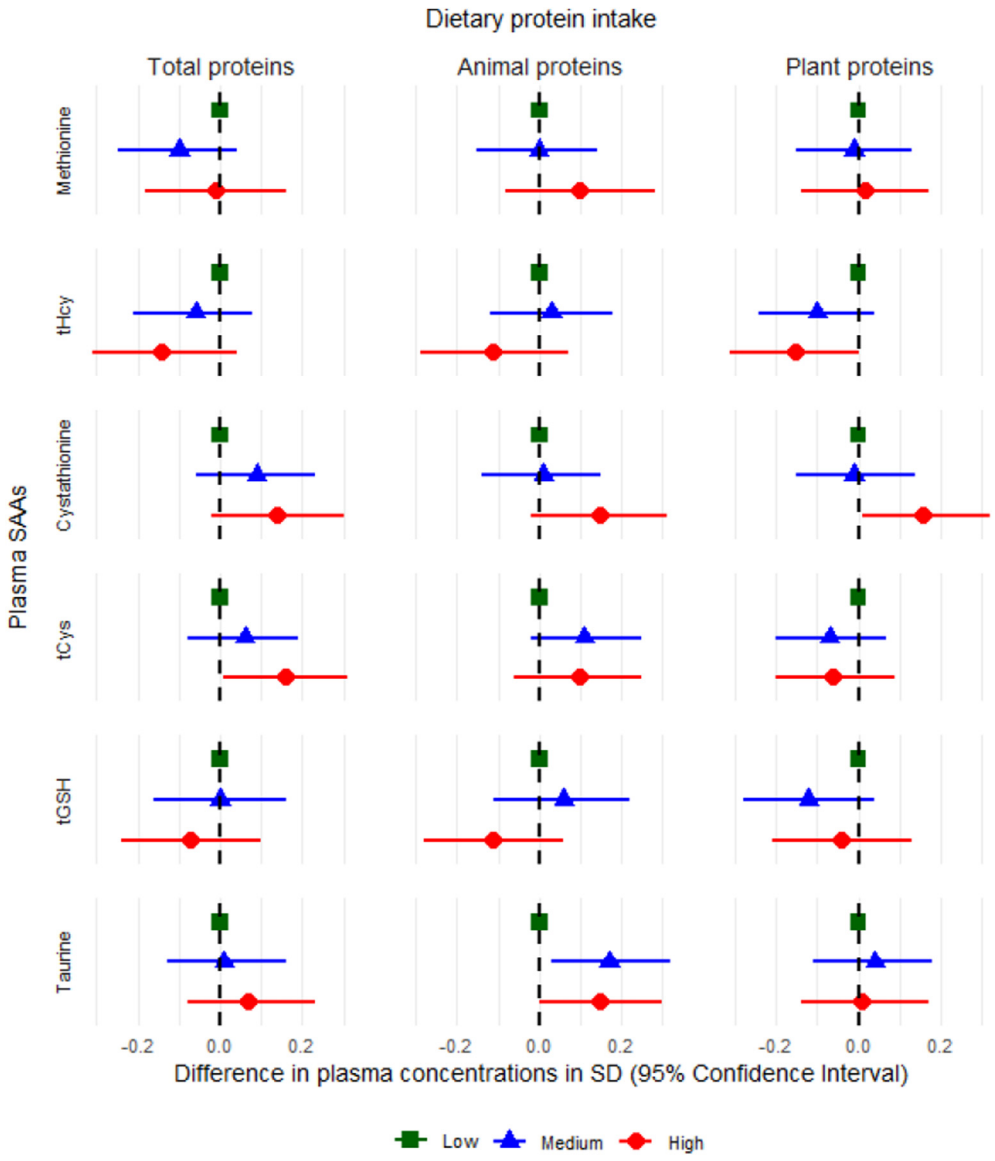

FIGURE 4. Associations between protein intake and plasma SAAs. Note: Difference in plasma concentrations for medium and high tertiles of protein intake compared to low intake (reference) are shown. Adjusted for age, sex, glucose metabolism status, total energy, intake smoking status, coffee consumption, light-intensity and moderate-to-vigorous physical activity, and alcohol consumption. Additional adjustment was made for vitamin B2, folate and vitamin B12 intake (in models with plasma methionine and tHcy as outcomes) and vitamin B6 (in models with plasma tHcy, cystathionine and tCys as outcomes). Intake of animal and plant proteins were mutually adjusted for each other.

0.10] with plasma tCys. No association was observed between cysteine intake and plasma tCys concentrations medium vs. low: -0.07 (-0.22, 0.07); high vs. low: 0.00 (-0.17, 0.17); *P*-trend= 0.99].

The intake of total SAAs was associated with lower tHcy concentrations in Model 1 but not after adjustment for lifestyle factors and vitamin B intake (Supplementary Table 1A). However, an inverse trend was found for the association of cysteine intake and plasma tHcy medium vs. low: -0.11 (-0.25, 0.04); high vs. low: -0.16 (-0.34, 0.01); *P*-trend= 0.07]. Positive associations were noted between total SAA intake and plasma cystathionine: participants in the middle and highest tertiles of total SAA intake had 0.11 SD and 0.23 SD higher plasma cystathionine levels, respectively than those in the lowest tertile medium vs. low: 0.11 (-0.04, 0.26); high vs. low: 0.23 (0.07, 0.39); *P*-trend= 0.004]. This association was mainly driven by cysteine intake and was strengthened by adjustment for tHcy (Supplementary Table 1A, Model 3).

The intake of total SAAs was not associated with plasma tGSH or taurine levels, but some associations were noted for the intake of individual SAAs. Cysteine intake was positively associated with tGSH [medium: 0.09 (-0.07, 0.25); high: 0.22 (0.02, 0.42); *P*-trend= 0.03] whereas methionine intake was inversely associated with plasma tGSH [medium: 0.00 (-0.17, 0.16); high: -0.20 (-0.39, 0.00); *P*-trend= 0.05] and positively associated with taurine [medium: 0.13 (-0.02, 0.28); high: 0.18 (0.00, 0.36); *P*-trend= 0.05].

In summary, the intake of total SAAs was not associated with plasma methionine, tHcy, tGSH, or taurine but was associated with higher plasma cystathionine and a trend for higher plasma

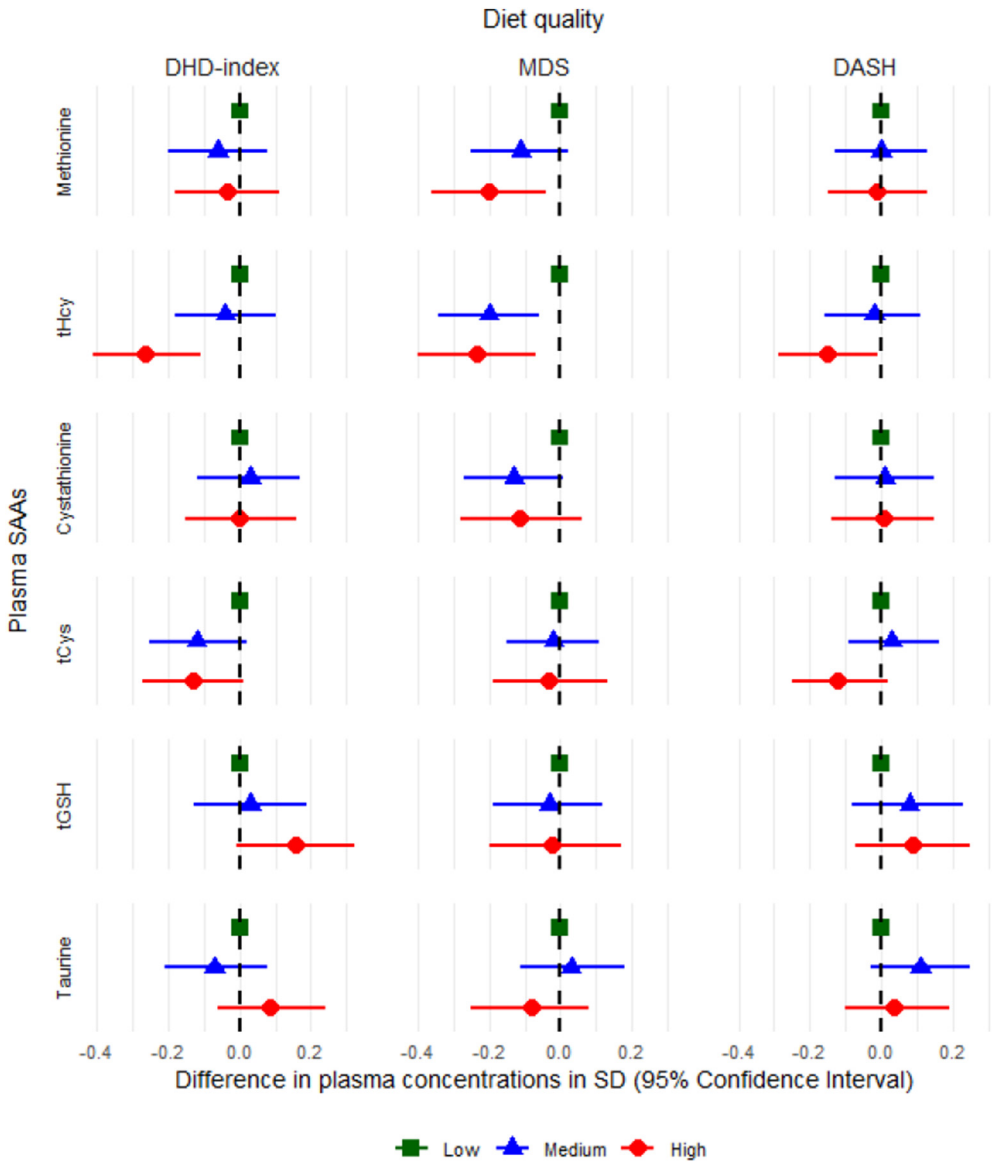

FIGURE 5. Associations between diet quality and plasma SAAs. Note: Difference in plasma concentrations for medium and high tertiles of diet-quality measures compared to low intake (reference) are shown. Adjusted for age, sex, glucose metabolism status, total energy intake, smoking status, coffee consumption, and light-intensity and moderate-to-vigorous physical activity. Additional adjustment was made for alcohol consumption in models with the DASH score as main exposure.

tCys concentrations. Methionine intake was associated with lower tGSH and higher taurine concentrations in plasma, whereas cysteine intake was associated with higher tGSH and a tendency for lower tHcy and higher cystathionine.

Associations between protein intake and plasma SAAs

No associations were found between the intake of total, animal, or plant proteins and plasma methionine concentrations (Supplementary Table 1B and Figure 4). Total protein intake was positively associated with plasma tCys [medium: 0.06 (-0.08, 0.19); high: 0.16 (0.01, 0.31); *P*-trend= 0.04]. This association appeared to be driven more by animal than plant protein intake, but neither association was statistically significant.

Intake of plant proteins was inversely associated with plasma tHcy [medium vs. low: -0.10 (-0.24, 0.04); high vs. low: -0.15 (-0.31, 0.00); *P*-trend = 0.05], but positively associated with plasma cystathionine [medium vs. low: 0.11 (-0.03, 0.26); high vs. low: 0.20 (0.04, 0.36); *P*-trend = 0.03], with nonsignificant trends for total and animal protein intake with tHcy (inverse) and cystathionine (positive). The association of protein intake with plasma cystathionine was strengthened by adjusting for plasma tHcy (Supplementary Table 1B; Model 3).

No associations were found between protein intake and plasma tGSH or taurine, except for a positive trend between animal protein intake and taurine [medium vs. low: 0.17 (0.03, 0.32); high vs. low: 0.15 (0.00, 0.30); *P*-trend= 0.06].

In summary, total protein intake was positively associated with plasma tCys and cystathionine but not with plasma methionine, tHcy, tGSH, or taurine. Higher intake of animal protein was positively associated with plasma taurine, whereas higher intake of plant protein was associated with lower plasma tHcy and higher cystathionine concentrations.

Associations between measures of diet quality and plasma SAAs

MDS [medium vs. low: -0.11 (-0.25, 0.02); high vs. low: -0.20 (-0.36, -0.04); *P*-trend= 0.01], but not the DHD-index or the DASH score, was inversely associated with plasma methionine (Supplementary Table 1C and Figure 5).

The DHD-index [medium vs. low: -0.04 (-0.18, 0.10); high vs. low: -0.26 (-0.41, -0.11); *P*-trend <0.001], MDS [medium vs. low: -0.20 (-0.34, -0.06); high vs. low: -0.23 (-0.40, -0.07); *P*-trend <0.01], and DASH score [medium vs. low: -0.02 (-0.16, 0.11); high vs. low: -0.15 (-0.29, -0.01); *P*-trend= 0.04] were inversely associated with plasma tHcy. No significant associations were found between diet quality measures and plasma concentrations of cystathionine, tCys, tGSH, or taurine.

Associations between protein-rich food groups and plasma SAAs

Consumption of protein-rich food groups was not associated with plasma methionine concentrations, whereas consumption of meat [medium vs. low 0.12 (-0.02, 0.26); high vs. low 0.17 (0.02, 0.32)] and legume [medium vs. low(0.03 (-0.11, 0.16); high vs. low(0.19 (0.06, 0.33)] was positively associated with plasma tCys concentrations. Egg [medium vs. low: -0.04 (-0.18, 0.10); high vs. low: -0.18 (-0.32, -0.03)] and fish [medium vs. low: -0.16 (-0.30, -0.02); high vs. low: -0.13 (-0.29, 0.04)] consumption was inversely associated with plasma tHcy. Egg consumption was inversely associated [medium vs. low: -0.15 (-0.30, -0.01); high vs. low: -0.06 (-0.21, 0.09)] with plasma cystathionine, whereas a positive association was found between wholegrain consumption [medium vs. low 0.10 (-0.05, 0.24); high vs. low 0.19 (0.03, 0.34)] and plasma cystathionine. Finally, consumption of refined grains was inversely associated with plasma tGSH [medium vs. low: -0.03 (-0.20, 0.13); high vs. low: -0.20 (-0.38, -0.03)] (Supplementary Table 2).

Interactions by glucose metabolism, sex, and overweight/obesity

No consistent effect modification by glucose metabolism status was observed (results not shown).

The associations between intake of total SAAs and plasma cystathionine and between methionine intake and plasma tGSH were stronger in women than in men. Similarly, the associations of total and animal proteins with plasma cystathionine and tGSH were stronger in women. On the contrary, the associations of MDS with plasma methionine and cystathionine and of the DHD-index with plasma tCys were stronger in men (Supplementary Table 3).

Overweight/obesity status significantly interacted with total SAA intake on plasma tCys and cystathionine concentrations and with methionine intake on plasma tCys and tGSH. A significant interaction was also found between overweight/obesity status and the intake of total and animal proteins on plasma cystathionine. All these associations were stronger in participants with normal weight (BMI < 25) (Supplementary Table 4).

Sensitivity analyses

Additional adjustment for all plasma SAAs resulted in the strengthening of the inverse trends of the intake of cysteine, total proteins, and plant proteins with plasma tHcy identified in the main analyses. In contrast, the associations of plant protein intake with cystathionine and of methionine intake with plasma taurine became nonsignificant (Supplementary Table 7).

Excluding participants who reported gastrointestinal problems during the preceding 2 mo strengthened the associations of intake of total and methionine SAAs and proteins (total and from animal sources) with plasma tCys. Similarly, stronger associations were observed between MDS and plasma methionine, between the DASH score and plasma tCys, between all 3 diet-quality measures and plasma tHcy, and between the DASH score and plasma taurine (Supplementary Tables 12). Other sensitivity analyses had little effect on the results (Supplementary Tables 5–13).

Discussion

This study examined the relationships between several aspects of the habitual diet and fasting plasma SAAs in a Dutch population of middle-aged adults by design enriched for prediabetes and T2DM. The main findings are that (1) higher total protein intake was associated with higher plasma tCys concentrations. This association was driven by a higher intake of animal proteins, specifically meat consumption, rather than plant protein. (2) Higher intake of total SAAs and cysteine as well as total animal and plant proteins were associated with higher plasma cystathionine concentrations, and (3) no significant associations were observed between intake of SAAs or proteins and plasma methionine concentrations, despite it being an essential amino acid. Finally, (4) higher plant protein intake and better diet quality were inversely associated with plasma tHcy.

Our results show that a higher habitual SAA intake might increase the plasma concentrations of trans-sulfuration metabolites, including tCys, cystathionine, and the downstream metabolite taurine. This pattern is consistent with the hypothesis that prolonged excessive SAA intake stimulates the irreversible conversion of homocysteine into cystathionine by the enzyme cystathionine β-synthase, leading to higher concentrations of metabolites in the trans-sulfuration pathway (Figure 1) [81].

Positive associations between the intake of total or animal proteins and plasma tCys concentrations have been reported previously [5, 28]. Our data suggest that these associations might be especially driven by methionine intake, whereas no association was found with cysteine intake. Interestingly, we found that a higher methionine intake might result in higher plasma tCys only in individuals with normal weight but not in people with overweight or obesity. This suggests that not only plasma tCys might promote adiposity [2–9] but also that being overweight or obese might influence the association of dietary intake with plasma tCys concentrations. Hence, lowering plasma tCys concentrations by reducing the SAA intake might be easier for people with normal weight than in individuals with established overweight.

Plasma cystathionine concentrations, both fasted and postprandial, are highly responsive to high-protein meals [66, 67] or short-term SAA-rich diets [68, 69]. Previous studies have reported significant reductions in plasma cystathionine concentrations after a substantial decrease in the intake of animal proteins [26] or SAAs [68, 69]. This study extends these findings by showing that more subtle differences in long-term habitual SAA intake are reflected in plasma cystathionine concentrations.

Furthermore, our data suggest that the association between intake of SAAs, especially methionine, and plasma cystathionine might be differentially regulated in men than in women, as well as in individuals with or without overweight/obesity. Specifically, the overweight status seems to affect plasma cystathionine concentrations and its response to dietary intake in a similar manner, although less strongly, as for plasma tCys.

On the contrary, our findings revealed no clear associations between diet and plasma methionine. This is in line with previous studies, which showed that fasting methionine was nonresponsive to acute changes in dietary protein intake [6, 26, 82] and habitual intake of dietary proteins [5, 27] or methionine [23–25, 34]. The current findings also indicate that plasma methionine concentrations might not be affected by cysteine intake, despite the cysteine-sparing effect on methionine [73]. Strong dietary modifications, such as 80 to 90% restriction of SAA intake [68, 69, 83], might be required for noticeable changes in fasting plasma methionine concentrations, which are tightly regulated to guarantee sufficient amounts of this essential nutrient [84]. Nonetheless, the inverse association observed between MDS and plasma methionine warrants further research, as no previous study has investigated the Mediterranean dietary pattern in relation to plasma methionine concentrations.

Finally, we found that higher cysteine and plant protein intake and better diet quality were associated with lower plasma tHcy. Although the associations of intake of cysteine or plant proteins with plasma tHcy have been investigated only by 2 previous studies [28, 31], with contrasting results, the inverse association with better diet quality has been consistently reported [39–44]. Better diet quality relates to plasma folate, vitamin B12, vitamin B2, and vitamin B6 concentrations [43], which are cofactors in homocysteine catabolism (Figure 1) and targets for lowering plasma tHcy concentrations [85]. Furthermore, higher fruit and vegetable consumption, promoted by all diet quality measures, has been shown to be associated with lower tHcy concentrations independently of vitamins of group B [86, 87]. Our findings suggest that the beneficial effect of better diet quality in reducing plasma tHcy concentrations might be additionally driven by a higher intake of plant proteins and correspondingly of cysteine, which both showed a strong positive correlation with all measures of diet quality.

Clinical perspectives

Previous evidence has shown that a higher intake of SAAs is associated with overweight or obesity [88], increased cardiometabolic risk [20, 21], and mortality from T2DM [89]. Our results provide a plausible biological link to connect dietary intake to obesity and metabolic diseases through plasma SAA concentrations. Of note, habitual dietary intake was largely not associated with plasma methionine, suggesting that the damaging health effects of excessive SAA intake might not be mediated by plasma concentrations of this amino acid. This interpretation is in line with the large body of evidence showing no association between plasma methionine and obesity [3–6, 8], although we and others have previously reported a positive association with intrahepatic fat [9, 10]. In contrast, the higher plasma concentrations of tCys and cystathionine reported in relation to a higher intake of total SAAs and methionine support a potential mediatory role of these trans-sulfuration metabolites in the associations of SAA intake with obesity and metabolic diseases. Furthermore, the lower plasma tHcy concentrations observed in relation to higher plant protein and cysteine intake support and provide an explanation for the beneficial effect of plant proteins compared with animal proteins on cardiovascular diseases [90, 91]. Overall, the evidence suggests that decreasing the SAA intake to values closer to recommendations [22, 92] might result in reduced adiposity and metabolic diseases. Importantly, our data suggest that the associations between dietary intake and plasma SAA concentrations might not be substantially different in participants with prediabetes or T2DM compared with those with normal glucose metabolism.

Strengths and limitations

The main strength of this study relates to the comprehensive investigation of dietary intakes, for which we examined 3 levels of complexity, i.e., (1) the main dietary SAAs (methionine and cysteine and their cumulative intake), (2) total, animal and plant proteins, and (3) overall diet quality using three different dietary indices. Intakes of SAAs and proteins were assessed with the Dutch Food Composition Database, incorporating data on dietary SAAs from the Danish and the UK Food Composition Databases, which ensured good estimation precision for dietary intakes in the Dutch population. In addition, plasma concentrations of multiple SAAs were investigated. Another strength is the detailed phenotyping of the participants in our study cohort, which allowed for thorough adjustment for potential confounders, including multiple sensitivity analyses. We have identified some limitations with our study. Participants were middle-aged Caucasians living in the South of the Netherlands, and the study population was enriched for prediabetes and T2DM. As a consequence, 22.5% of the individuals included in this study had prediabetes, and 34.3% had T2DM. People with T2DM generally have altered concentrations of several SAAs compared with healthy individuals [93–97], which might limit the generalizability of our findings. However, interaction analyses did not suggest a clear difference in the pattern of the associations between dietary intake and plasma SAAs in individuals with prediabetes or T2DM compared with those with normal glucose tolerance, and our analyses were all controlled for confounding by glucose metabolism status. We, therefore, do not expect that the enrichment substantially influenced our results, but the confirmation of our findings in a general population is still warranted. Dietary intake was selfreported with an FFQ, which, although validated, is subject to recall bias. Despite our efforts to control for all potential confounders, residual confounding is still possible because of the observational study design. Finally, the cross-sectional study design limits any inference of the cause-effect relationship between variables.

Conclusions

Given the accumulating evidence for the associations between plasma SAAs and human obesity [2–7, 9, 15, 16], cardiometabolic risk [8–11, 13–15], and mortality [12], this study was set out to examine the associations between the intake of SAAs and proteins as well as diet quality with plasma SAAs.

These predictors were selected as potential determinants of plasma concentrations of SAAs, which could, in future, be used as potential targets for the prevention of obesity and obesity-related metabolic diseases. We found that a high intake of total SAAs or proteins was associated with higher plasma tCys and cystathionine concentrations, especially in individuals with normal weight, that a higher intake of cysteine and plant proteins was associated with lower plasma tHcy and higher plasma cystathionine, and that a better diet quality was associated with lower plasma tHcy. Future studies are warranted to confirm these findings and investigate the causality of the identified associations.

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Author disclosures

The authors report no conflicts of interest.

Data Availability

Data described in the manuscript and code book will be made available upon request to The Maastricht Study Management Team (research.dms@mumc.nl) pending application and approval. The analytic code will be available from the corresponding author upon reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2023.05.008.

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