The Journal of Nutrition
Volume 152, Issue 11, November 2022, Pages 2419-2428

Cumulative Consumption of Sulfur Amino Acids and Risk of Diabetes: A Prospective Cohort Study

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https://doi.org/10.1093/jn/nxac172

ABSTRACT

Background

Cross-sectional studies have suggested that consumption of sulfur amino acids (SAAs), including methionine and cysteine, is associated with a higher risk of type 2 diabetes (T2D) in humans and with T2D-related biomarkers in animals. But whether higher long-term SAA intake increases the risk of T2D in humans remains unknown.

Objectives

We aimed to investigate the association between long-term dietary SAA intake and risk of T2D.

Methods

We analyzed data collected from 2 different cohorts of the Framingham Heart Study, a long-term, prospective, and ongoing study. The Offspring cohort (1991–2014) included participants from fifth through ninth examinations, and the Third-Generation cohort (2002–2011) included participants from first and second examinations. After excluding participants with a clinical history of diabetes, missing dietary data, or implausible total energy intake, 3222 participants in the Offspring cohort and 3205 participants in the Third-Generation cohort were included. Dietary intake was assessed using a validated FFQ. The relations between energy-adjusted total SAA (methionine and cysteine) intake or individual SAA intake (in quintiles) and risk of incident T2D were estimated via Cox proportional hazards models after adjusting for dietary and nondietary risk factors. Associations across the 2 cohorts were determined by direct combination and meta-analysis.

Results

During the 23 y of follow-up, 472 participants reported a new diagnosis of T2D in the 2 cohorts. In the meta-analysis, the HRs of T2D comparing the highest with the lowest intake of total SAAs, methionine, and cysteine were 1.8 (95% CI: 1.3, 2.5), 1.7 (95% CI: 1.2, 2.3), and 1.4 (95% CI: 1.0, 2.1), respectively. The association of SAA intake with T2D was attenuated after adjusting animal protein intake in sensitivity analyses.

Conclusions

Our findings show that excess intake of SAAs is associated with higher risk of T2D. Dietary patterns that are low in SAAs could help in preventing T2D.

Previous Next

Keywords:

sulfur amino acid intake; methionine; cysteine; diabetes; sulfur amino acid restriction

Abbreviations used:

CVD, cardiovascular disease; EAR, Estimated Average Requirement; FGF-21, fibroblast growth factor-21; FHS, Framingham Heart Study; PH, proportional-hazards; PP2A, protein phosphatase 2A; P/S fat ratio, ratio of polyunsaturated fat to saturated fat; SAA, sulfur amino acid; T2D, type 2 diabetes.

Introduction

Epidemiological studies have shown that dietary patterns with high intakes of protein are associated with high risk of type 2 diabetes (T2D) (1). The association between T2D and protein intake depends on the protein quantity and source. Higher intakes of proteins from legumes and seafood have been inversely associated with T2D risk, whereas that from red meat showed positive association with T2D even after further adjustment for total energy intake (2). Overall, these studies suggest that with their role in protein synthesis, the source and composition of sulfur amino acids (SAAs) are important in determining the risk of T2D.

In general, plant proteins have a lower concentration of the 2 proteinogenic SAAs, methionine and cysteine. For example, it is estimated that SAA concentrations in legumes, which are considered high in SAAs among plant protein sources, are only ~25% of the SAA content found in most animal-derived foods. This value drops to ~10% for most other plant protein sources (3, 4).

Accumulating evidence from animal and observational studies has suggested that higher consumption of SAAs is associated with a higher T2D risk (5, 6). Methionine is an essential amino acid that cannot be synthesized in vivo, whereas cysteine is considered "conditionally essential" because individuals with specific disease conditions cannot synthesize it (5, 7). However, both SAAs are abundant in the diet, particularly in meat and fish proteins (8). According to the consensus of DRIs, the RDA for total SAAs in adults aged ≥19 y is 19 mg/kg/d (9). According to the NHANES, the consumption of SAAs for American adults well exceeds this recommendation (9, 10). Our previous study using NHANES data found that higher dietary SAA intake was positively associated with diabetes-related mortality (11). Studies also suggested that dietary intake of SAAs was positively associated with T2D-related risk factors and biomarkers in humans (12, 13, 14, 15, 16). In NHANES participants, we also found that serum cholesterol, glucose, uric acid, blood urea nitrogen, insulin, and glycated hemoglobin concentrations were higher in

adult individuals with greater SAA intakes (10). Similar results were observed in a Chinese study (16). A limitation of these previous observational studies is that dietary intake was assessed only once, and no data were available on the impact of long-term SAA consumption on incident risk for T2D. Therefore, we investigated the association between long-term habitual dietary SAA intake and risk of T2D in 2 ongoing prospective cohorts, the Offspring cohort and the Third-Generation cohort of the Framingham Heart Study (FHS).

Methods

Study population

Participants were selected from the prospective community-based FHS Offspring and Third-Generation cohorts, which have been extensively characterized (17, 18, 19). The FHS Offspring cohort began in 1971 by enrolling adult offspring (and offspring's spouses) of the original FHS cohort participants. The Offspring cohort follow-up visits occurred every 3–4 y and included physical examinations, anthropometric measurements, laboratory tests, health-related questionnaires, and continuous surveillance for some diseases. Dietary data were collected using validated FFQs from examination 5 (1991–1995) (17). In 2002, adults with ≥1 parent in the Offspring cohort were enrolled in the FHS Third-Generation cohort. They underwent similar examinations, questionnaires, and surveillance as the Offspring cohort (19).

At baseline, 5497 and 4578 participants were enrolled in the Offspring cohort and Third-Generation cohort, respectively. Participants were excluded if they had a history of diabetes (n = 2197 in Offspring cohort and 174 in Third-Generation cohort), missing dietary data (n = 78 in Offspring cohort and 1141 in Third-Generation cohort), or invalid FFQs due to implausible total energy intake (<600 or >4200 kcal/d for males and <600 or >4000 kcal/d for females: n = 58 in Third-Generation cohort) (20). After exclusions, data from 6427 participants (3222 for the Offspring cohort and 3205 for the Third-Generation cohort) were available for analysis. **Supplemental Figure 1** shows the flow chart of study participants.

Dietary assessment

Dietary intake was repeatedly assessed by using a validated semiquantitative FFQ at the

fifth through ninth examination cycles in the Offspring cohort and the first and second examination cycles in the Third-Generation cohort (21). Before each examination cycle, the FFQs were mailed to each participant along with instructions for completing the questionnaire for foods consumed over the past year. During the examination, participants returned their completed questionnaires to trained staff, who checked them for accuracy. The FFQs contained 131 food items and queried participants on the frequency of consumption and serving sizes of these foods in the past year. Participants were also asked to provide information on the use of dietary supplements. Absolute intake of nutrients and nonnutrients was computed by multiplying each food item's frequency of consumption by the nutrient content of the specified portions.

A direct evaluation of the validity of dietary SAA intake from the FFQ used in the current study has not been performed. However, the validity of food intake measurements based on a comparison between the FFQ and two 7-d diet records collected during the year time interval covered by the FFQ has been previously documented (20, 21, 22, 23). Because absolute methionine and cysteine intake tended to be strongly correlated with each other and with total energy and protein intake, exposures (methionine, cysteine, or total SAAs; milligrams per day) were adjusted for energy intake using the residual method (24).

Identification of T2D events

The study outcome was the occurrence of T2D between the date of return of the baseline FFQ and the cutoff date (last follow-up available): April 2014 (Offspring cohort) or February 2011 (Third-Generation cohort). In these cohorts, individuals who had a nonfasting blood glucose concentration ≥200 mg/dL or fasting blood glucose concentration ≥126 mg/dL in laboratory tests, or self-reported diabetes treatment (validated by medication bag with medication or bottles/packs) (25), were identified as diabetic in the follow-up (26, 27, 28). Due to extremely low rates of type 1 diabetes in the cohorts (26, 29), a high proportion of T2D in diabetes in the United States (26), and the age of participants, it was assumed that all identified diabetes cases were T2D, as previously described (26).

Covariates

Age, sex, education, income, physical activity, and smoking status were self-reported.

Height and weight were measured using standardized methods, and BMI was calculated as kg/m^2. History of cardiovascular disease (CVD) was self-reported and included nonfatal myocardial infarction and stroke. To ensure that other dietary components would not confound the association between SAAs and T2D, we adjusted for total energy, alcohol, magnesium, sodium, and energy-adjusted polyunsaturated fat to saturated fat ratio (P/S fat ratio), calcium, vitamin A, vitamin C, vitamin B-6, and energy-adjusted animal and plant protein, separately. These covariates were selected based on clear trends in the distribution of these end points across SAA groups. The correlation coefficients between energy-adjusted total SAA intake and the main confounders are displayed in **Supplemental Table 1**.

Statistical analysis

Although the sample mean of SAA intake in each examination was stable and the correlation for energy-adjusted SAA intake between each visit was moderate (~0.5), we used the cumulative average of SAA intake from baseline to the censoring events (30) to minimize within-person variation. We generated quintile categories of energy-adjusted intakes of methionine, cysteine, and total SAAs. Follow-up time was calculated from the return of the baseline questionnaire to the date of diagnosis of T2D, death, loss of follow-up, or end of study period, whichever occurred first. Cox proportional hazards models were used to estimate the HRs and 95% CIs for associations between intakes of total SAAs or individual SAAs (in quintiles) and risk of T2D. The proportional-hazards (PH) assumption was evaluated based on the Schoenfeld residuals, and the PH assumption has been found to be supported by a nonsignificant relation between residuals and time in both cohorts. For multivariable analyses, model 1 was adjusted for basic characteristics including age, sex, and total energy intake (kilocalories per day); model 2 was further adjusted for lifestyle and dietary and nondietary factors that included BMI (BMI <18.5, 18.5 to <25, 25 to <30, and >30), income (income <US$50,000, and income ≥ US$50,000), education (bachelor's degree or above, or not), physical activity (moderate to vigorous activity ≥3 times/wk, or not), smoking status (yes/no), alcohol intake (grams per day), energy adjusted P/S fat ratio, calcium (milligrams per day), vitamin A (international units per day), vitamin C (milligrams per day), and vitamin B-6 (milligrams per day), plus magnesium (milligrams per day), and sodium (grams per day). Model 3 was additionally adjusted for baseline health conditions including history of CVD. To address the possibility of residual confounding from protein, we further tested models adjusted for

energy-adjusted animal and plant protein intake based on fully adjusted models, and additionally constructed a basic model using absolute total SAA intakes, absolute animal and plant protein intake, and energy. Tests for trends were conducted by assigning the median value to each quintile category and modeling this value as a continuous variable.

To summarize the associations across the 2 cohorts, we conducted a fixed-effect meta-analysis because no heterogeneity by Cochran Q test was apparent between cohorts (P value for heterogeneity >0.1 for all). Besides, direct combination of the 2 cohorts was done to test the robustness of the summary of the associations. Additionally in a sensitivity analysis, to test the robustness of the associations under different calculation methods of exposure (31), we studied baseline total SAAs in relation with T2D and also relating the cumulative average total SAA intake from examination 5 to examination 8 to T2D diagnosed from examination 8 to examination 9. To further test for the robustness of the findings, we conducted subgroup analysis. Individuals reporting dietary intake of SAAs below the Estimated Average Requirement (EAR; 15 mg/kg/d) (32) were excluded (n = 45 for Offspring cohort and 20 for Third-Generation cohort). All statistical tests were 2-sided with $P < 0.05$ considered significant, and were conducted using SAS 9.4 (SAS Institute Inc).

Ethical

The original data collection protocols were approved by the Institutional Review Board at Boston University Medical Center, and written informed consent was obtained from all participants. The present study protocol was reviewed and approved by the Pennsylvania State University Institutional Review Board.

Results

At baseline, the average age of participants was 54.3 ± 9.80 y (women: 54.3 ± 9.74 y; men: 54.3 ± 9.86 y) with an average BMI of 27.1 ± 4.71 (women: 26.4 ± 5.19; men: 28.0 ± 3.93) in the Offspring cohort; participants were younger (all: 40.3 ± 8.64 y; women: 40.0 ± 8.69 y; men: 40.5 ± 8.58 y) and had similar BMI distribution (all: 26.6 ± 5.27; women: 25.6 ± 5.74; men: 27.7 ± 4.42) in the Third-Generation cohort. During the 23 y of follow-up, 472 participants reported a new diagnosis of T2D (401 from the Offspring cohort and 71 from the Third-Generation cohort). The average annual incidence of T2D in the Offspring and Third-Generation cohorts was 5.4 per 1000 persons and 2.5 per 1000

persons, respectively.

Baseline characteristics of participants in both cohorts, categorized by quintiles of intake of total SAAs, are presented in **Table 1** as combined and separate for each cohort. In the Offspring cohort, the means of the highest quintile of total SAAs, methionine, and cysteine intake were 1.6-fold, 1.8-fold, and 1.6-fold greater than those of the lowest quintile. Similarly, in the Third-Generation cohort, the means of the highest quintile of total SAAs, methionine, and cysteine intake were 1.7-fold, 1.7-fold, and 1.9-fold greater than those of the lowest quintile. The means of all quintiles of total SAAs, methionine, and cysteine intake in the Third-Generation cohort were higher than corresponding means of quintiles in the Offspring cohort. In the combined cohort, total SAA intake was positively associated with BMI, physical activity, and intake of total protein, animal and plant protein, vitamin A, vitamin B-6, vitamin C, calcium, magnesium, and sodium. Participants with higher SAA intake were less likely to be smokers, alcohol drinkers, or have a history of CVD compared with those with the lowest intake. This was consistently observed in both cohorts.

TABLE 1. Baseline energy-adjusted characteristics of participants in the Offspring, Third-Generation, and combined cohorts according to quintiles of intake of total SAAs[1]

Characteristics	**Offspring cohort (*n* = 3222)**			**Third-Generation cohort (*n* = 3205)**			**Combined (*n* = 6427)**		
	Q1	**Q3**	**Q5**	**Q1**	**Q3**	**Q5**	**Q1**	**Q3**	**Q5**
n	644	644	644	641	641	641	1285	1285	1285
Cases, *n*	60	95	98	7	12	26	85	98	99
SAA median,[2] mg/kg/d	29.3 (22.3, 36.3)	36.8 (29.3, 43.9)	48.5 (39.4, 57.4)	34.8 (27.0, 44.4)	40.6 (32.2, 50.1)	55.1 (43.7, 68.5)	32.2 (25.0, 40.6)	37.6 (30.0, 46.4)	51.5 (42.0, 63.3)
SAA median,[2] g/d	2.23 (2.02, 2.36)	2.85 (2.79, 2.91)	3.48 (3.36, 3.71)	2.59 (2.35, 2.74)	3.27 (3.20, 3.33)	4.05 (3.87, 4.34)	2.41 (2.17, 2.53)	3.06 (3.00, 3.12)	3.76 (3.61, 4.04)
Age, y	56.5 ± 10.3	53.8 ± 9.60	52.3 ± 9.33	40.3 ± 9.08	39.9 ± 8.27	40.5 ± 8.18	49.5 ± 12.6	47.3 ± 11.0	44.4 ± 10.1

Sex, male, %	62.3	45.8	34.0	61.0	42.3	38.9	62.7	44.5	36.7
BMI, kg/m^2	26.7 ± 4.15	27.1 ± 4.87	27.6 ± 4.87	25.8 ± 4.68	26.6 ± 5.36	27.8 ± 6.00	26.4 ± 4.42	26.9 ± 5.21	27.7 ± 5.51
Smoking, yes, %	27.6	16.2	15.4	18.3	14.6	11.5	23.0	15.5	12.7
History of CVD, yes, %	30.0	28.1	22.1	4.37	2.96	3.12	19.1	14.3	9.26
Physical activity, moderate to vigorous activity ≥3 times/wk, %	29.3	23.5	27.3	77.9	77.5	79.9	49.6	48.9	60.5
Dietary intake									
Energy, kcal/d	1960 ± 595	1790 ± 523	1980 ± 546	2230 ± 658	1899 ± 581	2160 ± 615	2120 ± 634	1830 ± 536	2070 ± 590
Polyunsaturated fat/saturated fat	0.576 ± 0.200	0.567 ± 0.151	0.586 ± 0.154	0.613 ± 0.243	0.589 ± 0.159	0.586 ± 0.188	0.588 ± 0.216	0.586 ± 0.167	0.588 ± 0.180
Protein, g/d	61.0 ± 7.27	78.6 ± 2.44	96.6 ± 9.58	71.3 ± 8.29	88.9 ± 3.48	110 ± 14.1	60.0 ± 7.98	.83.8 ± 2.91	103 ± 12.4
Animal protein, g/d	38.4 ± 7.79	54.1 ± 4.31	71.9 ± 11.0	43.0 ± 10.6	60.5 ± 5.40	82.1 ± 15.5	40.7 ± 9.33	57.3 ± 5.26	76.9 ± 13.8
Plant protein, g/d	22.6 ± 5.43	24.5 ± 4.40	24.7 ± 5.01	28.3 ± 10.2	28.4 ± 5.47	28.2 ± 8.29	25.2 ± 8.19	26.5 ± 5.35	26.7 ± 7.01
Alcohol, g/d	18.8 ± 21.9	8.85 ± 11.6	7.1 ± 9.28	17.8 ± 21.0	9.30 ± 9.92	8.14 ± 9.75	18.4 ± 21.4	9.20 ± 10.8	7.66 ± 9.24
Vitamin A, IU/d	9880 ± 6420	12,800 ± 6210	16,100 ± 7870	10,400 ± 7440	12,300 ± 5890	14,800 ± 8440	10,300 ± 7130	12,500 ± 6310	15,100 ± 7997
Vitamin B-6, mg/d	5.99 ± 15.0	7.44 ± 16.0	11.1 ± 22.1	7.41 ± 16.6	7.31 ± 18.0	9.59 ± 20.1	6.58 ± 15.5	7.76 ± 17.7	9.89 ± 20.0
Vitamin C, mg/d	262 ± 262	285 ± 244	332 ± 299	209 ± 239.7	205 ± 200	261 ± 374	243 ± 253	251 ± 237	284 ± 338

Calcium, mg/d	748 ± 328	956 ± 356	1140 ± 433	920.2 ± 357	1080 ± 366	1340 ± 517	829 ± 346	1030 ± 370.2	1250 ± 483
Magnesium, mg/d	296 ± 97.6	312 ± 93.1	375 ± 106	355 ± 124	339 ± 111	422 ± 139	328 ± 115	325 ± 105	401 ± 128
Sodium, g/d	2.08 ± 0.760	2.02 ± 0.633	2.26 ± 0.694	2.25 ± 0.727	2.08 ± 0.672	2.42 ± 0.760	2.19 ± 0.750	2.03 ± 0.629	2.32 ± 0.725

[1]

Values are presented as percentage of the selected category or means ± SDs, unless otherwise indicated. Energy-adjusted total SAAs is equal to the sum of energy-adjusted methionine and cysteine. CVD, cardiovascular disease; Q, quintile; SAA, sulfur amino acid.

[2]

Values are medians (IQRs).

In both Offspring and Third-Generation cohorts, individuals with the highest total SAA intake had 1.6-fold (95% CI: 1.1, 2.3; $P = 0.016$) and 3.9-fold (95% CI: 1.6, 9.6; $P = 0.003$) higher risks of T2D, respectively, than those with the lowest intake after further adjustments for dietary and nondietary risk factors and comorbidity (**Table 2**). In the meta-analysis, total SAA intake was associated with a 1.8-fold (95% CI: 1.3, 2.5; $P = 0.001$) higher risk of T2D in the highest compared with the lowest SAA intake quintile in the fully adjusted model (**Figure 1**A).

TABLE 2. HRs for risk of type 2 diabetes according to quintiles of energy-adjusted intakes of total SAAs in the Offspring and Third-Generation cohorts[1]

Total SAAs	**Total SAA intake**					***P*-trend**
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	
Offspring						
n	644	645	644	645	644	
SAA median, g/d	2.23 (2.02, 2.36)	2.60 (2.53, 2.66)	2.85 (2.79, 2.91)	3.09 (3.03, 3.17)	3.48 (3.36, 3.71)	

SAA mean,[7] g/d	2.15 ± 0.272	2.60 ± 0.0824	2.85 ± 0.0669	3.10 ± 0.0839	3.59 ± 0.379	
Cases, *n*	60	86	95	62	98	
Cases, *n* × 10^5/person-y	697	917	985	600	969	
Model 1[3]	1.0[2]	1.4 (1.0, 2.0)*	1.6 (1.2, 2.3)*	1.1 (0.7, 1.5)	1.9 (1.3, 2.6)***	<0.001
Model 2[4]	1.0[2]	1.4 (1.0, 1.9)	1.5 (1.1, 2.2)*	1.0 (0.7, 1.4)	1.6 (1.1, 2.4)*	0.074
Model 3[5]	1.0[2]	1.4 (1.0, 1.9)	1.5 (1.1, 2.1)*	0.9 (0.6, 1.4)	1.6 (1.1, 2.3)*	0.111
Third-Generation						
n	641	641	641	641	641	
SAA median, g/d	2.59 (2.35, 2.74)	3.01 (2.92, 3.08)	3.27 (3.20, 3.33)	3.54 (3.46, 3.63)	4.05 (3.87, 4.34)	
SAA mean,[7] g/d	2.50 ± 0.324	3.00 ± 0.0883	3.27 ± 0.0717	3.55 ± 0.0997	4.22 ± 0.535	
Cases, *n*	7	10	12	16	26	
Cases, *n* × 10^5/person-y	176	254	303	408	665	
Model 1[3]	1.0[2]	1.5 (0.6, 4.0)	2.0 (0.8, 5.2)	3.00 (1.2, 7.4)*	5.1 (2.2, 11.8)***	<0.001
Model 2[4]	1.0[2]	1.3 (0.5, 3.4)	1.6 (0.6, 4.2)	2.2 (0.9, 5.6)	4.1 (1.6, 10.1)**	<0.001
Model 3[5]	1.0[2]	1.3 (0.5, 3.4)	1.6 (0.6, 4.2)	2.2 (0.9, 5.6)	3.9 (1.6, 9.6)**	<0.001

[6] Values are medians (IQRs).

[1] HRs are presented with the format of HR (95% CI). *,**,***Significantly different from the reference group: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. The numbers of participants in the Offspring

cohort and Third-Generation cohort were 3222 and 3205, respectively. P/S fat ratio, ratio of polyunsaturated fat to saturated fat; Q, quintile; SAA, sulfur amino acid.

2

The reference group.

3

Model 1: adjusted for age, sex, and total energy (kcal/d). Race is not included because of limited information.

4

Model 2: adjusted for covariates in model 1 and lifestyle, dietary, and nondietary factors including BMI (BMI <18.5, 18.5 to <25, 25 to ≤30, and >30), income (<US$50,000, and ≥US$50,000), education level (lower than bachelor's degree, and bachelor's degree or above), physical activity (moderate to vigorous activity ≥3 times/wk, or not), smoking status (yes/no), and intakes of alcohol (g/d), magnesium (mg/d), sodium (g/d), energy-adjusted P/S fat ratio, calcium (mg/d), vitamin A (IU/d), vitamin C (mg/d), and vitamin B-6 (mg/d). Race is not included because of limited information.

5

Model 3: adjusted for covariates in model 2 and baseline comorbidities including history of cardiovascular disease. Race is not included because of limited information.

7

Values are presented as means ± SDs.

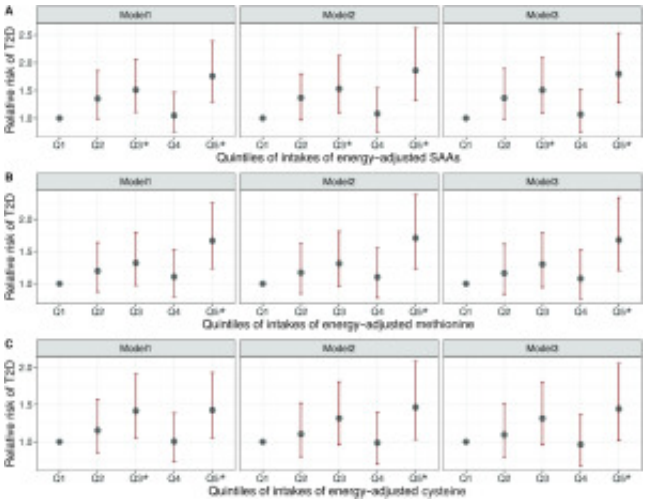

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FIGURE 1. HRs of type 2 diabetes according to quintiles of intakes of energy-adjusted SAAs, methionine, and cysteine via meta-analysis for the Offspring and the Third-Generation cohorts. A, B, and C show RRs of type 2 diabetes according to quintiles of intakes of energy-adjusted SAAs, methionine, and cysteine in meta-analysis, respectively. *Significantly different from the reference group. The vertical lines represented the 95% CIs for each quintile compared with the lowest quintile (Q1) as the reference group. Model 1: adjusted for age, sex, and total energy (kcal/d). Model 2: adjusted for covariates in model 1 and lifestyle, dietary, and nondietary factors including BMI (BMI <18.5, 18.5 to <25, 25 to ≤30, and >30), income (income <US$50,000, and income ≥US$50,000), education level (lower than bachelor's degree, and bachelor's degree or above), physical activity (moderate to vigorous activity ≥3 times/wk, or not), smoking status (yes/no), and intakes of alcohol (g/d), magnesium (mg/d), sodium (g/d), energy-adjusted P/S fat ratio, calcium (mg/d), vitamin A (IU/d), vitamin C (mg/d), and vitamin B-6 (mg/d). Model 3: adjusted for covariates in model 2 and baseline comorbidities including history of cardiovascular disease. P/S fat ratio, ratio of polyunsaturated fat to saturated fat; Q, quintile; SAA, sulfur amino acid; T2D, type 2 diabetes.

Associations between the intake of methionine and cysteine individually with incident T2D in the Offspring and Third-Generation cohorts are shown in **Table 3**. Comparing the highest quintiles of intake with the lowest quintiles in model 3, the HRs for T2D for the Offspring cohort were 1.5 (95% CI: 1.0, 2.1; $P = 0.030$) for methionine and 1.2 (95% CI: 0.8, 1.8; $P = 0.327$) for cysteine, and for the Third-generation cohort were 4.1 (95% CI: 1.6, 10.4; $P = 0.003$) for methionine and 4.5 (95% CI: 1.7, 11.7; $P = 0.002$) for cysteine. In the meta-analysis of the fully adjusted models for both cohorts, the HRs of T2D comparing the highest with the lowest intakes of methionine and cysteine were 1.7 (95% CI: 1.2, 2.3) and 1.4 (95% CI: 1.0, 2.1), respectively (**Figure 1**B,C). Tests for linear trend across increasing quintiles were significant in the meta-analysis and Third-Generation separately (all P-trend < 0.05).

TABLE 3. HRs for risk of type 2 diabetes according to quintiles of energy-adjusted intakes of individual methionine and cysteine in the Offspring and Third-Generation cohorts[1]

Cohort	Q1	Q2	Q3	Q4	Q5	*P*-trend
Individual methionine intake						
Offspring						
n	644	645	644	645	644	
Methionine, median, g/d	1.39 (1.26, 1.48)	1.64 (1.59, 1.68)	1.81 (1.77, 1.84)	1.98 (1.93, 2.02)	2.24 (2.15, 2.39)	
Methionine, mean,[7] g/d	1.34 ± 0.181	1.63 ± 0.0520	1.81 ± 0.0448	1.98 ± 0.0572	2.31 ± 0.269	
Cases, *n*	66	77	92	67	99	
Cases, *n* × 10^5/person-y	753	817	974	645	986	
Model 1[3]	1.0[2]	1.12 (0.9, 1.7)	1.5 (1.1, 2.0)*	1.1 (0.8, 1.5)	1.8 (1.3, 2.4)***	<0.001
Model 2[4]	1.0[2]	1.2 (0.8, 1.6)	1.3 (0.9, 1.8)	1.0 (0.7, 1.4)	1.5 (1.0, 2.1)*	0.096
Model 3[5]	1.0[2]	1.1 (0.8, 1.6)	1.3 (0.9, 1.8)	1.0 (0.7, 1.4)	1.5 (1.0, 2.1)*	0.116
Third-Generation						
n	641	641	641	641	641	
Methionine, median, g/d	1.56 (1.42, 1.66)	1.83 (1.78, 1.87)	2.00 (1.96, 2.04)	2.17 (2.12, 2.23)	2.48 (2.37, 2.69)	
Methionine, mean,[7] g/d	1.51 ± 0.205	1.83 ± 0.0526	2.00 ± 0.0470	2.18 ± 0.0600	2.60 ± 0.374	
Cases, *n*	6	10	10	17	28	
Cases, *n* × 10^5/person-y	151	255	254	430	719	

Model 1[3]	1.0[2]	1.7 (0.6, 4.8)	1.8 (0.7, 5.0)	3.6 (1.4, 9.1)**	6.4 (2.6, 15.5)***	<0.001
Model 2[4]	1.0[2]	1.4 (0.5, 3.8)	1.3 (0.5, 3.6)	2.4 (0.9, 6.4)	4.3 (1.7, 10.9)**	<0.001
Model 3[5]	1.0[2]	1.3 (0.5, 3.8)	1.3 (0.5, 3.7)	2.5 (0.9, 6.5)	4.1 (1.6, 10.4)**	<0.001
Individual cysteine intake						
Offspring						
n	644	645	644	645	644	
Cysteine, median, g/d	0.825 (0.751, 0.870)	0.957 (0.932, 0.978)	1.04 (1.02, 1.06)	1.13 (1.10, 1.15)	1.26 (1.21, 1.34)	
Cysteine, mean,[7] g/d	0.795 ± 0.0964	0.955 ± 0.0284	1.04 ± 0.0223	1.13 ± 0.0283	1.30 ± 0.124	
Cases, *n*	65	81	95	68	92	
Cases, *n* × 10^5/person-y	764	879	99	655	885	
Model 1[3]	1.0[2]	1.2 (0.9, 1.7)	1.5 (1.1, 2.1)*	1.0 (0.7, 1.4)	1.4 (1.0, 2.0)*	<0.001
Model 2[4]	1.0[2]	1.1 (0.8, 1.5)	1.3 (0.9, 1.8)	0.9 (0.6, 1.3)	1.2 (0.9, 1.8)	0.471
Model 3[5]	1.0[2]	1.1 (0.7, 1.5)	1.3 (0.9, 1.8)	0.8 (0.6, 1.2)	1.2 (0.8, 1.8)	0.586
Third-Generation						
n	641	641	641	641	641	
Cysteine, median, g/d	0.984(0.896, 1.04)	1.15 (1.11, 1.17)	1.25 (1.22, 1.28)	1.38 (1.34, 1.42)	1.63 (1.54, 1.77)	
Cysteine, mean,[7] g/d	0.946 ± 0.133	1.14 ± 0.0334	1.25 ± 0.0337	1.38 ± 0.0457	1.69 ± 0.221	

Cases, *n*	8	11	15	15	22	
Cases, *n* × 10^5/person-y	203	278	382	379	565	
Model 1[3]	1.0[2]	1.3 (0.5, 3.3)	2.2 (0.9, 5.2)	2.1 (0.9, 5.1)	3.8 (1.7, 8.6)**	<0.001
Model 2[4]	1.0[2]	1.3 (0.5, 3.3)	1.7 (0.7, 4.3)	2.1 (0.8, 5.3)	4.1 (1.6, 10.5)**	0.001
Model 3[5]	1.0[2]	1.5 (0.6, 3.8)	1.7 (0.7, 4.4)	2.3 (0.9, 5.8)	4.5 (1.7, 11.7)**	0.001

[6] Values are medians (IQRs).

1

HRs are presented with the format of HR (95% CI). *,**,***Significantly different from the reference group: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. The numbers of participants in the Offspring cohort and Third-Generation cohort were 3222 and 3205, respectively. P/S fat ratio, ratio of polyunsaturated fat to saturated fat; Q, quintile; SAA, sulfur amino acid.

2

The reference group.

3

Model 1: adjusted for age, sex, and total energy (kcal/d). Race is not included because of limited information.

4

Model 2: adjusted for covariates in model 1 and lifestyle, dietary, and nondietary factors including BMI (BMI <18.5, 18.5 to <25, 25 to ≤30, and >30), income (<US$50,000, and ≥US$50,000), education level (lower than bachelor's degree, and bachelor's degree or above), physical activity (moderate to vigorous activity ≥3 times/wk, or not), smoking status (yes/no), and intakes of alcohol (g/d), magnesium (mg/d), sodium (g/d), energy-adjusted P/S fat ratio, calcium (mg/d), vitamin A (IU/d), vitamin C (mg/d), and vitamin B-6 (mg/d). Race is not included because of limited information.

5

Model 3: adjusted for covariates in model 2 and baseline comorbidities including history of cardiovascular disease. Race is not included because of limited information.

7

Values are presented as means ± SDs.

Positive associations between the intake of total SAAs and individual SAAs with T2D risk were also significant in the combined cohort (**Supplemental Table 2**). All *P*-trend values across increasing quintiles were <0.05 in the combined cohort (Supplemental Table 2). When we additionally adjusted for both animal and plant protein intakes, the positive association of total SAA intake with T2D became attenuated, but remained significant (**Supplemental Table 3**). In additional sensitivity analysis, the association between dietary total SAAs and T2D risk remained in the same positive trend, but became nonsignificant when absolute SAA was used as the exposure (**Supplemental Table 4**). Furthermore, baseline total SAAs was positively associated with T2D risk (**Supplemental Figure 2**), and similar associations were observed when relating the cumulative average total SAA intake from examination 5 to examination 8 with T2D diagnosed from examination 8 to examination 9 (**Supplemental Figure 3**). Similar results are presented in **Supplemental Figures 4** and **5** in the meta-analysis for the sensitivity analysis. When individuals reporting intake of SAAs below the EAR were excluded, similar significant positive associations between total SAAs and individual SAA intake and risk of T2D were observed in the subgroup analysis for all cohorts (**Supplemental Table 5**).

Discussion

In 2 large, prospective cohorts of US adult men and women, we observed consistent positive associations of long-term consumption of SAAs, including methionine and cysteine, individually and together, with the risk of T2D. These associations were independent of traditional diabetes risk factors, including CVD history. Together with previous epidemiological studies (10, 15, 16, 33 these new findings provide insights into the importance of dietary SAAs as a modifier of T2D risk and the potential for reduced SAA intake as a novel dietary intervention to prevent T2D.

The crude incidence of T2D in the Offspring cohort (1991–2014: 5.4 per 1000 persons per year) with a mean age of 54.3 y, and the Third-Generation cohort (2002–2011: 2.5 per

1000 persons per year) with a mean age of 40.2 y, were similar to those in other studies (34, 35) and somewhat lower than in the National Health Interview Survey of US adults (for 1980 and 2012: 2.0 and 3.7 per 1000 persons per year for 20–44-y-old adults and 4.6 and 12.1 per 1000 persons per year for 45–64-y-old adults) (36). This difference could be due, in part, to the selective inclusion/exclusion criteria resulting in a saturation effect (35), could reflect a regional difference because the FHS is not a nationally representative sample (37), or could result from the use of the self- or proxy report of diabetes diagnosis used in the National Health Interview Survey and our study (36).

The significant associations between SAA consumption and T2D appeared to be robust, with consistent findings observed in both the Offspring and Third-Generation cohorts, although stronger associations were found for the latter cohort. Significant associations were also observed in the combined cohort and confirmed when analyses were conducted using fixed-effects meta-analysis. Besides, whenever exposure was defined as cumulative average intake, the cumulative average exposure from examination 5 to examination 8, or baseline intake, the significantly positive associations between SAA consumption and T2D remained. Finally, significant associations were also observed for analyses of the individual SAAs, methionine and cysteine, suggesting that the effects were not specific for either one. Because the primary exposure, dietary SAA intake, is correlated with protein, especially animal protein, we included adjustments for this factor to better distinguish the specific role of SAAs. The positive relation remained when further adjusted for energy-adjusted plant protein intake in fully adjusted models, whereas adjustment for energy-adjusted animal protein intake attenuated the associations. Of note, adjustment for animal protein could be considered an overadjustment (22), because low SAA intake was associated with decreased consumption of animal-based diets. Moreover, this finding was also consistent with the fact that SAA content in plant protein is far less than in animal protein (3).

For the first time, to assess SAA consumption, the cumulative average dietary intake was calculated from FFQ results as a means of reducing within-person variation over time and to better reflect long-term dietary habits (38). Although crude, FFQs used to assess long-term dietary intake can collect complex information and achieve relatively accurate data, especially when adjusting for energy (39, 40, 41). The 24-h food recall method we have used in the previous studies, on the other hand, could accurately record the actual intake on specific days to estimate the short-term average diet of a population (39). However, when used for measuring long-term dietary intake, this method might cause

significant random measurement errors due to day-to-day variation of the diet and increase time and cost, as well as recall error (42, 43).

Mean SAA intake values were 2- to 4-fold higher than the adults' EAR for SAA of 15 mg/kg/d (32) in both Offspring and Third-Generation cohorts. It was also noteworthy that in the Third-Generation cohort, dietary SAA intake was 10–20% higher, and T2D HRs were ~2-fold higher than the Offspring cohort. These differences might reflect a greater intake of animal protein in younger adults compared with older ones (44), because the content of SAAs is generally higher in animal than vegetable proteins. In a comparison of 3 European cohorts, the younger cohort (Young Fins, mean age of 38 y) exhibited a significantly higher intake of animal protein and a higher T2D risk than the older 2 cohorts (44). In this report, the Young Finns cohort (mean age: 38.0 y) consumed 71.2 g/d animal protein, which was nearly 2-fold greater than that in Lifelines (mean age: 45.7 y) and NQplus (mean age: 53.5 y) cohorts. In a dose–response meta-analysis of prospective studies, a 5% energy increment from dietary total and animal protein intake was related to a 12% higher risk of T2D (45). We conducted additional analysis to compare the association between animal protein and T2D risk with and without adjusting for SAA intake. We found a significant association between animal protein and T2D, which became nonsignificant after adjusting for SAAs. This suggests that SAAs might contribute to the positive association seen between animal protein and T2D risk. Interestingly, the association between plant protein intake and T2D was a significant U-shaped curve, with the most risk reduction at intake of ~6% of energy intake from plant protein. Additionally, the animal protein to plant protein ratio was slightly lower in the Third-Generation cohort at quintile 1 and higher at quintiles 3 and 5 compared with the Offspring cohort. Although no significant difference between the animal protein to plant protein ratio was observed between the cohorts, it should still be noted that some studies demonstrated that the animal protein to plant protein ratio was positively associated with fasting blood glucose (46) and insulin resistance (47). Overall, the associations observed were more pronounced in the upper quintile of SAA intake, which could be indicative of a negative health impact of excessive SAA consumption. The finding is consistent with the study in US adults from the NHANES III (11), in which the associations between each of total SAAs, methionine, and cysteine intake and diabetes-specific mortality were particularly observed in the fourth and fifth quintiles.

To our knowledge, this is the first study that examined the association between long-term dietary SAA intake and risk of diabetes in humans. In a previous study, we

examined the association between dietary SAA intake and risk factors for cardiometabolic diseases in the NHANES III study (10). In that representative study of the US adult population, we found dietary SAA intake was positively associated with higher concentration of metabolic disease–related serum biomarkers, including cholesterol, insulin, and blood glucose (10). Participants who reported the lowest levels of SAA intake, which were close to the dietary requirements, had the lowest cardiometabolic disease risk, suggesting that optimal levels of SAA intake may be close to the EAR (10). The relation remained significant regardless of how much protein was consumed, indicating that the health risks were related to not only the absolute SAA intake, but also the proportion of SAAs consumed (10).

Our findings are consistent with previous laboratory animal studies, which have provided direct evidence that excessive SAA intake is related to insulin resistance, hyperglycemia, or T2D in rats, mice, and pigs (6, 48, 49, 50). These findings are also consistent with several recent human studies in which dietary SAA intake or related genetic markers were associated with diabetes risk, fasting glucose, or insulin resistance (13, 15, 16, 33). Both dietary methionine and cysteine intakes were positively related to overweight or obesity, an important risk factor for T2D (16). In an internet-based cross-sectional study of 936 participants aged 18 to 40 y in China, dietary SAA intake was positively associated with BMI, waist circumference, and the prevalence of overweight or obesity (16). In other studies, higher blood concentrations of free SAAs, which could reflect higher SAA intake (51, 52), were associated with higher risk of T2D (16, 33, 53). Evidence from a Chinese multiprovincial cohort study conducted in communities of Shandong province also revealed that higher plasma cysteine concentrations were significantly associated with a greater diabetes prevalence (33). Similarly, in a large-scale prospective cohort in older Chinese men and women in Hong Kong, each SD increment in plasma total cysteine concentration was associated with a 68% significantly higher risk of diabetes (53). Plasma methionine was also found to be associated with T2D in a prospective family-based study located in southwestern Netherlands (54). A systematic review revealed that elevated plasma homocysteine, which forms during the breakdown of methionine, was causally related to increased risk of T2D (55). The association between SAAs and T2D-related risk factors was not only found in adults, but also observed in children. In a cross-sectional study in 601 children aged 12–18 y in the United States, dietary SAA intake was demonstrated to be positively associated with general body adiposity, central obesity, and fat mass (13). In 26 Japanese children aged 9

or 10 y with moderate to severe obesity and hyperlipidemia, Suzuki et al. (15) also found that plasma methionine was positively associated with plasma glucose and insulin resistance via retrospective analysis.

The positive association of SAA consumption with T2D risk suggests that dietary SAA restriction could be a promising regime for the prevention of T2D (56). Indeed, there are biologically plausible mechanisms for the beneficial effects of dietary SAA restriction on T2D risk. Dietary SAA restriction might improve insulin secretion and its signaling pathway to benefit glucose homeostasis and insulin sensitivity (56). Dietary SAA restriction also can induce the release of fibroblast growth factor-21 (FGF-21) in animal models (6, 48, 51). Reductions in FGF-21 can impair peripheral insulin sensitivity manifesting as insulin resistance, pancreatic islet hyperplasia, and pancreas dysfunction in the mouse (57). Meanwhile, upregulation or release of FGF-21 can promote metabolism and improve glucose uptake with other biomarkers and signal pathways in or out of the pancreas (58, 59). Olsen et al. (51) revealed that restricting dietary SAAs induced elevated serum FGF-21 in a double-blind, randomized controlled pilot study in 20 overweight or obese Norwegian women. Alternatively, changes in autophagic activity have been implicated in the progression of T2D through β-cell function disorder and the development of insulin resistance (60). Methionine and its downstream metabolite *S*-adenosylmethionine have been implicated in the inhibition of autophagy via protein phosphatase 2A (PP2A) signaling in which the methylation of PP2A is a sensing mechanism of cellular methionine and its metabolite (56). In methionine-restricted mice, autophagy maintaining β-cell homeostasis is markedly activated, which improves peripheral insulin sensitivity (56, 60).

Our study had some strengths and limitations. An important strength of our study was the prospective nature of the study design, which minimized the likelihood of recall and selection biases, and the high follow-up rates, which largely reduced the concern that the results were affected by differential follow-up rates. In addition, the cumulative averages of repeated assessments of intake were used to minimize random measurement error caused by within-person variation and to account for real changes in diet over time. Moreover, considering the heterogeneity of the 2 different cohorts, we used meta-analysis to pool the overall results. We also directly combined the cohorts to test the robustness of the combined results, and overall outcomes were similar to those obtained for the meta-analysis. Residual confounding is a common and unavoidable issue in observational studies. We sought to minimize the influence of the potential confounders

by controlling for potentially confounding variables including major lifestyle, dietary risk factors, and health status. Furthermore, most of the participants in our study were Caucasians living in Massachusetts, who might not represent the overall US adult population. However, this generated within-study homogeneity. Another limitation of the study is that the T2D diagnosis date was defined as the examination date when the subject first met the T2D diagnosis criteria.

In summary, we observed consistent associations between higher dietary intake of SAAs and higher risk of T2D in 2 prospective cohorts. Our findings emphasize the need to identify the diets with more appropriate dietary SAA intake, which could provide useful approaches to public health nutrition for the prevention and treatment of T2D. More prospective studies, preferably in populations with various eating habits, and randomized feeding trials are warranted to investigate the potential roles of low-SAA dietary patterns in the prevention of T2D.

The authors' responsibilities were as follows—ZD, JPR, XG: designed the research; ZD: conducted the research and drafted the paper; ZD, BS: analyzed the data; ZD, JPR, XG, LA-S, SNN, DO: contributed to analysis and manuscript development; and all authors: read and approved the final manuscript.

Data Availability

Detailed FHS survey descriptions, methodology, and laboratory test procedures are publicly available (https://framinghamheartstudy.org/ ↗). Data tables can be applied at the same website.

SUPPORTING INFORMATION

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Supported by the Orentreich Foundation for the Advancement of Science, Inc, Project #: EPI001.

Author disclosures: The authors report no conflicts of interest.

Supplemental Figures 1–5 and Supplemental Tables 1–5 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/ ↗.