Dietary glucosinolates and risk of type 2 diabetes in 3 prospective cohort studies

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ABSTRACT

Background: Glucosinolates are a group of phytochemicals that are abundant in cruciferous vegetables and precursors of the potentially chemopreventive isothiocyanates. Isothiocyanates may reduce oxidative stress and inflammation, but little is known regarding the association between glucosinolate intake and risk of type 2 diabetes (T2D).

Objective: To evaluate the association between the intake of glucosinolates and the incidence of T2D in US men and women.

Design: This prospective cohort study investigated 200,907 women and men [71,256 women from the Nurses' Health Study (NHS; 1984–2012), 88,293 women from the NHS II (1991–2013), and 41,358 men from the Health Professionals Follow-Up Study (1986–2012)] who were free of diabetes, cardiovascular disease, and cancer at baseline. Diet was assessed using validated semiquantitative food frequency questionnaires. Self-reported T2D incidence was confirmed by a supplementary questionnaire.

Results: During follow-up in the 3 cohorts, we accumulated 4,303,750 person-years and 16,567 incident cases of T2D. After adjustment for major lifestyle and dietary risk factors for T2D, participants in the highest quintile of total glucosinolate intake had a 19% higher risk (95% CI: 13%, 25%; $P_{trend} < 0.001$) of T2D than did those in the lowest quintile. The intake of 3 major glucosinolate subtypes was consistently and significantly associated with T2D risk, with pooled HRs ranging from 1.13 to 1.18 (all $P_{trend} < 0.001$). A significant association was also observed between total cruciferous vegetable consumption and T2D (HR: 1.16; 95% CI :1.07, 1.25; $P_{trend} < 0.001$). These associations persisted in subgroups defined by demographic, lifestyle, and other dietary factors.

Conclusions: Dietary glucosinolate intake was associated with a moderately higher risk of T2D in US adults. These results need to be replicated in further investigations, including biomarker-based studies. Mechanistic research is also needed to understand the relation between exposures to glucosinolates, isothiocyanates, and other metabolites with T2D risk. This trial was registered at clinicaltrials.gov as NCT03366532. *Am J Clin Nutr* 2018;107:617–625.

Keywords: Glucosinolate, isothiocyanate, cruciferous vegetable, diet, type 2 diabetes

INTRODUCTION

Increasing consumption of vegetables has been widely recommended for the primary prevention of major chronic diseases, although epidemiologic studies have found mixed results regarding total vegetable intake and risk of type 2 diabetes (T2D) (1, 2). Most recently, emerging data have indicated that individual vegetables may not be equally associated with risk of chronic diseases, which may be attributed to the different micronutrient and phytochemical profiles of the various vegetable subgroups (3, 4).

Glucosinolates are a diverse group of secondary plant metabolites that are particularly abundant in cruciferous vegetables. Glucosinolates per se are not biologically active, although upon hydrolysis by plant myrosinase and/or by human gut microbiota they give rise to several groups of metabolites, of which isothiocyanates (ITCs) are the most common (5, 6). Abundant evidence has suggested that ITCs are inhibitors of phase I enzymes and potent inducers of phase II enzymes (7, 8). Because of these properties of ITCs, extensive research has been dedicated to evaluating the role of ITCs in the chemoprevention of cancers (9). Evidence regarding ITC intakes in relation to other chronic diseases is limited, although laboratory research has suggested that

Supported by research grants CA186107, CA176726, CA167552, and DK058845 from the National Institutes of Health. QS was supported by ES021372, ES022981, and HL035464.

The funders had no role in the study design, implementation, analysis, decision to publish, or preparation of the manuscript.

Supplemental Figures 1 and 2 and Supplemental Tables 1–3 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/ajcn/.

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Abbreviations used: AHEI, alternative healthy eating index; CVD, cardiovascular disease; FFQ, food frequency questionnaire; GSH, reduced glutathione; HPFS, Health Professionals Follow-up Study; ITC, isothiocyanate; MET, metabolic equivalent; NHS, Nurses' Health Study; Nrf2, nuclear factor E2-related factor; P:S, polyunsaturated to saturated fat; ROS, reactive oxygen species; T2D, type 2 diabetes.

Received August 17, 2017. Accepted for publication December 26, 2017.

First published online April 9, 2018; doi: https://doi.org/10.1093/ajcn/nqy003.

these phytochemicals may also modulate the risk of T2D. Sulforaphane, one of the primary ITCs, exerts antioxidative and anti-inflammatory effects by activating the nuclear factor E2-related factor (Nrf2), which subsequently induces phase II enzymes (10). Accumulating evidence has shown that the activation of the Nrf2 pathway plays an important role in preventing T2D and reducing insulin resistance (11). To date, prospective studies examining dietary intake of glucosinolates and T2D risk are largely lacking.

To fill this knowledge gap, in the current study we examined the associations of dietary intake of total and subgroups of glucosinolates, as well as cruciferous vegetables, with T2D risk in 3 large prospective cohort studies. We hypothesized that a higher consumption of glucosinolates and cruciferous vegetables is associated with a lower risk of T2D.

SUBJECTS AND METHODS

Study population

The Nurses' Health Study (NHS) consisted of 121,700 female registered nurses aged 30–55 y from 11 US states who were enrolled in 1976 (12). The NHS II was initiated in 1989 with the recruitment of 116,671 younger female registered nurses, 24–44 y of age, from 14 states (13). The Health Professionals Follow-up Study (HPFS) was established in 1986 and comprised 51,529 US male health professionals ranging in age from 40 to 75 y at enrollment from 50 states (12). For the current analysis, we used 1984 for the NHS ($n = 81{,}712$), 1991 for the NHS II ($n = 97{,}604$), and 1986 for the HPFS ($n = 51{,}529$) as our baselines, when the detailed dietary information was first collected. We excluded participants who had died or reported a diagnosis of diabetes (including type 1 diabetes, T2D, and gestational diabetes for women), cardiovascular disease (CVD), or cancer at baseline for the dietary analyses ($n = 9392$ in NHS, $n = 6155$ in NHS II, and $n = 6926$ in HPFS). Participants were also excluded if they left >70 of the 131 food items blank on the baseline food frequency questionnaire (FFQ), reported unusual total energy intake levels (<3347 or >17,573 kJ/d for men and <2092 or >14,644 kJ/d for women), or had missing baseline information on cruciferous vegetable intake ($n = 346$ in NHS, $n = 2481$ in NHS II, and $n = 1692$ in HPFS). In addition, we excluded subjects who completed only the baseline questionnaire ($n = 718$ in NHS, $n = 675$ in NHS II, and $n = 1553$ in HPFS). A total of 200,907 participants (71,256 women in the NHS, 88,293 women in the NHS II, and 41,358 men in the HPFS) were included in the analysis (**Supplemental Figure 1**). The study protocol was approved by the Institutional Review Board of the Brigham and Women's Hospital and the Human Subjects Committee Review Board of Harvard T.H. Chan School of Public Health, Boston, MA.

Dietary assessment

Diet was assessed using validated FFQs every 2–4 y. The FFQs inquired about the average consumption frequency of selected food items during the previous year with a standardized portion size using 9 categories of intake frequency, ranging from never or <1 time/mo to ≥6 times/d. Glucosinolate composition in relevant food items was obtained primarily from the Harvard University Food Composition Database and published data (14). Intakes of individual glucosinolates were estimated by multiplying the reported intake frequency of each food by the content of specific glucosinolates for each food item with a prespecified serving size and then summing the contributions from across all food items. Average daily intakes of individual glucosinolates were summed to compute intake of subgroups and total glucosinolates. Glucosinolate intakes were energy-adjusted by using the residual method. We calculated total cruciferous vegetable consumption in the current analysis by summing the consumptions of the individual cruciferous vegetables (broccoli, cabbage, cauliflower, Brussels sprouts, kale, and mustard or chard greens). To better represent long-term dietary and lifestyle patterns and to minimize within-person variation, we used the cumulative averages from the baseline to the end of follow-up. We stopped updating dietary variables upon a report of cancer or CVD because diagnosis of these conditions might lead to changes in diet. The reproducibility and validity of the FFQs in measuring primary food sources of dietary glucosinolates were assessed by comparing with data from multiple weeks of diet records (15, 16). Correlation coefficients between FFQ and diet record assessments were 0.69 for broccoli, 0.55 for cabbage, and 0.51 for Brussels sprouts in a validation study among NHS participants (15, 16). Reasonable correlation coefficients were also found in a validation study in the HPFS (17). Overall, these validation studies suggested that the FFQ assessments were able to differentiate participants by their glucosinolate intake levels. In a pilot study among a subgroup of the NHS participants, higher intake of glucosinolates was significantly associated with higher urinary concentration of total ITCs (tertile 1: 1.83 μM/L; tertile 3: 3.92 μM/L; $P_{\text{trend}} = 0.04$; **Supplemental Figure 2**).

Assessment of covariates

In the biennial follow-up questionnaires, we collected and updated information on risk factors for T2D, such as body weight, smoking status, physical activity, medication or multivitamin use, and a family history of diabetes, as well as a history of chronic diseases, including hypertension and hypercholesterolemia. Total physical activity was expressed as metabolic equivalent (MET) hours per week by summing the product of the duration of moderate or vigorous forms of exercise with the MET value specific to each activity. Alcohol intake was calculated based on the frequency of consumption of beer, wine, and liquor during the previous year. The validity and reproducibility of alcohol consumption assessments have been published elsewhere (18). Among the NHS and NHS II participants, menopausal status, postmenopausal hormone use, and oral contraceptive use (NHS II only) were also ascertained. To evaluate overall diet quality, an alternative healthy eating index (AHEI) score was calculated as an indicator of adherence to healthy eating behavior by summarizing consumption of 11 foods and nutrients that are most predictive of chronic disease risk in general: vegetables, fruits, whole grains, sugar-sweetened beverages and fruit juice, nuts and legumes, red and processed meat, *trans* fat, long-chain n–3 fats, polyunsaturated fats, sodium, and alcohol. In the current study, we excluded cruciferous vegetables from the calculation of this index because cruciferous vegetables are the predominant source of glucosinolates in the diet.

Ascertainment of T2D

The primary endpoint for this study was incident T2D. Participants reporting a physician diagnosis of T2D on the biennial main questionnaire were sent a validated supplementary questionnaire regarding symptoms, diagnostic tests, and diabetes treatment. The diagnosis was confirmed if participants reported ≥1 of the following National Diabetes Data Group criteria before 1998 (19): *1*) ≥1 classic symptom plus elevated plasma glucose concentrations (fasting concentrations ≥7.8 mmol/L, random concentrations ≥11.1 mmol/L, and/or 2-h plasma glucose concentrations ≥11.1 mmol/L during oral glucose tolerance testing), *2*) ≥2 elevated blood glucose concentrations as defined above on different occasions in the absence of symptoms, or *3*) treatment with insulin or oral hypoglycemic medication. For cases diagnosed in 1998 or later, a fasting glucose concentration of 7.0 mmol/L was considered the threshold on the basis of the American Diabetes Association criteria (20). Only cases confirmed by the supplemental questionnaires were included in the current analysis. In NHS and HPFS, questionnaire-confirmed diagnosis of T2D was reconfirmed by medical record review in <97% of the cases (21, 22). In addition, another study assessing the prevalence of undiagnosed diabetes suggested a very low rate of false-negative diabetes status (0.5%) (23).

Statistical analysis

For each participant, person-years were calculated from the date of return of the baseline questionnaire to the date of diagnosis of T2D, death, or the end of follow-up (30 June 2012 in NHS, 30 June 2013 in NHS II, and 31 January 2012 in HPFS), whichever came first. Incidence rates were calculated by dividing the number of cases by person-years of follow-up. The HRs and 95% CIs of incident T2D were estimated for dietary glucosinolate intake by using time-dependent Cox proportional hazards regression within each cohort. The regression models included age in years as the time scale, stratified by calendar time in 2-y intervals, and allowed for a possible interaction between calendar time and age in the baseline hazards to be accounted for nonparametrically. In multivariate analyses, we further adjusted for ethnicity, family history of diabetes, smoking status, alcohol intake, physical activity, menopausal status and postmenopausal hormone use, oral contraceptive use, multivitamin use, hypertension, hypercholesterolemia, BMI, and total energy intake. To assess whether overall diet quality is a potential mediator or confounder of the association between glucosinolate intake and T2D, we included the modified AHEI score in our final model. Tests for linear trend were conducted by assigning the median value to each quintile or category as a continuous variable in the regression model. For the primary analyses, to obtain overall estimates for both sexes and to increase statistical power, the HRs from the multivariable-adjusted models across the 3 cohorts were combined using an inverse variance-weighted fixed-effects meta-analysis. *P* values for heterogeneity of study results were calculated using the Cochran *Q* test. We also examined cruciferous vegetables in relation to T2D using the same analytic approaches. We conducted analyses stratified by race/ethnicity (Caucasians compared with others), age (<65 compared with ≥65 y), BMI [(kg/m^2)<30 or ≥30], modified AHEI score (below median level compared with at or above median level), physical activity (below median level compared with at or above median level), smoking (never compared with ever), and alcohol consumption (abstainer compared with drinker) in our fully adjusted model to assess whether any potential interactions exist between diabetes risk factors and glucosinolate intake. The likelihood ratio test was used to assess the significance of cross-product terms. We also examined the possible dose-response relation between glucosinolate intake and T2D by using restricted cubic spline regression with 4 knots. In this analysis, we excluded participants within the highest and lowest 5% of glucosinolate concentrations to minimize the potential impact of outliers. To test the robustness of our findings, we performed several sensitivity analyses: *1*) using baseline glucosinolate intake instead of cumulative averages of intake level, *2*) evaluating the influence of adjustment for major dietary components, including polyunsaturated to saturated fat (P:S) ratio, and intakes of *trans* fat, red meat, whole grains, and fruits (all in quintiles) instead of the modified AHEI score, *3*) continuing updating diet after diagnosis of CVD or cancer when calculating the cumulative averages, and *4*) placing a 4- or 8-y lag period between the assessment of glucosinolate intake and T2D ascertainment. In addition, we also performed separate secondary analyses to evaluate the associations of 3 major glucosinolate subgroups and 5 major individual glucosinolates with risk of T2D. Data were analyzed using SAS 9.3 (SAS Institute., Cary, NC). All *P* values were 2-sided, with statistical significance defined as $P < 0.05$.

RESULTS

We accumulated 1,684,221 person-years of follow-up in the NHS, 1,781,825 person-years in the NHS II, and 837,704 person-years in the HPFS. We documented a total of 16,567 incident cases of T2D (7586 cases in the NHS, 5438 in the NHS II, and 3543 in the HPFS). The age-adjusted baseline characteristics of the study population by quintiles of glucosinolate intake were presented in **Table 1**. In all 3 cohorts, participants with higher glucosinolate intake were older and tended to be more physically active. They were also more likely to have a better diet quality, as reflected by a higher AHEI score. Higher glucosinolate intake was associated with lower intake of *trans* fats and higher P:S ratio. Participants with higher glucosinolate intake tended to consume more fruits and vegetables, but less red meat.

The pooled results showed that a higher intake of glucosinolates was significantly associated with T2D risk in the age-adjusted model (**Table 2**). Further adjustment for demographic and lifestyle factors only slightly attenuated this association, and the positive association remained statistically significant. The pooled multivariable-adjusted HR comparing extreme categories was 1.08 (95% CI: 1.02, 1.13; $P_{trend} = 0.007$). After adjustment for the modified AHEI score, this association was further strengthened: individuals in the highest quintile of glucosinolate intake had 19% increased risk of T2D (HR: 1.19; 95% CI: 1.13, 1.25; $P_{trend} < 0.001$) compared with those in the lowest quintile.

In stratified analyses, the association between glucosinolate intake and the risk of T2D persisted in all subgroups, and no significant effect modification was observed between glucosinolate intake and race, BMI, modified AHEI score, physical activity, smoking status, or alcohol consumption (all $P_{interaction} > 0.10$; **Table 3**).

TABLE 1
Age-adjusted baseline characteristics of participants according to quintiles of total glucosinolate intake in the NHS, NHS II, and HPFS[1]

	NHS			NHS II			HPFS		
	Q1	Q3	Q5	Q1	Q3	Q5	Q1	Q3	Q5
Participants, *n*	14,266	14,239	14,256	17,628	17,687	17,663	8271	8270	8274
Glucosinolate intake, mg/d	3.31[2]	10.5	28.9	2.07	7.66	25.8	2.19	9.92	29.4
Age,[3] y	49.2	50.2	51.1	35.2	36.2	37.0	52.6	52.9	54.2
Caucasians, %	98	98	97	96	96	95	96	95	94
Current smoker, %	26	24	23	13	12	13	11	10	8
Alcohol intake, g/d	6.80	7.32	6.57	3.01	3.10	3.11	11.0	12.7	10.5
Physical activity, MET/wk	11.8	14.1	17.3	18.0	20.6	25.4	19.3	20.8	23.8
BMI, kg/m^2	24.6	24.9	25.2	24.6	24.5	24.7	24.9	24.9	25.0
Family history of diabetes, %	25	25	26	16	16	17	19	19	19
Multivitamin use, %	64	63	59	44	42	44	59	61	64
Hypertension, %	19	20	22	6	6	6	19	18	21
Hypercholesterolemia, %	7	8	8	14	14	15	9	10	11
Ever menopausal hormone use, %	21	22	22	3	3	3	—	—	—
Current use of oral contraceptive, %	—	—	—	11	11	10	—	—	—
Total energy intake, kcal/d	1778	1781	1686	1882	1700	1691	2032	2085	1904
Modified AHEI score	42.6	46.4	53.0	40.7	45.9	51.5	45.1	48.4	54.6
trans Fat intake, % energy	2.04	1.95	1.71	1.81	1.66	1.44	1.40	1.31	1.08
Polyunsaturated fat–to–saturated fat ratio	0.52	0.55	0.59	0.49	0.52	0.57	0.53	0.56	0.63
Total fruit intake, servings/d	1.82	2.11	2.46	0.96	1.12	1.41	2.02	2.32	2.70
Total vegetable intake, servings/d	2.16	2.88	4.37	2.15	2.88	4.59	2.18	2.90	4.31
Cruciferous vegetable intake, servings/d	0.12	0.36	1.00	0.09	0.32	0.94	0.14	0.40	1.02
Red meat intake, servings/d	1.24	1.18	0.98	0.92	0.76	0.63	1.29	1.25	0.93

[1] Values were standardized to the age distribution of the study population. AHEI, alternative healthy eating index; HPFS, the Health Professionals Follow-Up Study; MET, metabolic equivalents of task; NHS, Nurses' Health Study; Q, quintile.
[2] The presented data refer to the mean values unless otherwise indicated.
[3] Values were not age adjusted.

Results from the multivariable adjusted restricted cubic spline regression suggested monotonic dose-response relations between glucosinolate intake and the incidence of T2D ($P_{linearity} < 0.001$ and $P_{curvature} = 0.73$). Every 1-SD increment of total glucosinolate intake was significantly associated with a 5% (95% CI, 3%, 6%) higher T2D risk ($P < 0.001$).

The sensitivity analysis using baseline dietary data only showed associations similar to those observed in the main analyses, and the pooled multivariable-adjusted HR for the comparison of the extreme categories was 1.16 (95% CI: 1.11, 1.22; $P_{trend} < 0.001$). Adjustment for individual dietary factors instead of modified AHEI score did not materially alter the associations, and the corresponding HR (95% CI) for glucosinolate intake was 1.12 (95% CI: 1.06, 1.18; $P_{trend} < 0.001$). When we continued updating the dietary variables even after a diagnosis of cancer or CVD, the risk estimate was similar (HR: 1.18; 95% CI: 1.11, 1.24; $P_{trend} < 0.001$) to those obtained when we stopped updating the diet upon these diagnoses. Finally, incorporating a 4-y (HR: 1.17; 95% CI: 1.11, 1.23; $P_{trend} < 0.001$) or 8-y lag (HR: 1.18; 95% CI: 1.11, 1.24; $P_{trend} < 0.001$) did not materially change the association.

We subsequently estimated the HRs of T2D associated with intake of glucosinolate subgroups (**Supplemental Table 1**). All 3 subgroups were associated with a higher risk of developing T2D after multivariable adjustment. In comparison to those in the lowest quintile of aliphatic glucosinolate intake, participants in the highest quintile had a pooled HR (95%CI) of 1.18 (1.12, 1.24; $P_{trend} < 0.001$). For indolylglucosinolate and aromatic glucosinolate intake, the corresponding multivariable-adjusted HRs (95% CIs) comparing extreme quartiles were 1.17 (1.11, 1.23; $P_{trend} < 0.001$) and 1.13 (1.07, 1.19; $P_{trend} < 0.001$), respectively. Each 1 SD of aliphatic glucosinolate, indolylglucosinolate, and aromatic glucosinolate intake was associated with a 5%, 4%, and 3% increased risk of T2D (all $P < 0.001$), respectively. Additionally, significant positive associations were also observed for all 5 main individual glucosinolates when comparing extreme quintiles, with HRs ranging from 1.06 to 1.21 (all $P_{trend} < 0.05$; **Supplemental Table 2**).

We also investigated the association of cruciferous vegetables with T2D risk and found a pooled HR of 1.21 (95% CI: 1.12, 1.29; $P_{trend} < 0.001$) for a comparison of ≥1 serving/d with <1 serving/wk. Additional adjustment for covariates slightly attenuated the result, but this association remained statistically significant. The pooled HR comparing extreme cruciferous vegetable intake levels was 1.16 (95% CI: 1.07, 1.25; $P_{trend} < 0.001$; **Table 4**). Every 2 servings/wk of cruciferous vegetable consumption was associated with a 3% increase risk of T2D (HR: 1.03; 95% CI: 1.01, 1.04). Further adjustments for dietary β-carotene, flavonoids, vitamin C, vitamin E, and fiber, nutrients that cruciferous vegetables are rich in, did not appreciably alter the results (HR: 1.12; 95% CI: 1.03,1.22; $P_{trend} < 0.001$). To further examine whether the observed association between cruciferous vegetables and T2D could be explained by glucosinolate intake, we simultaneously adjusted for glucosinolate intake in the model. This caused the positive association of cruciferous vegetables to be substantially attenuated toward the null (HR: 0.99; 95% CI: 0.89, 1.10;

TABLE 2
HR (95% CI) of T2D according to quintiles of total glucosinolate intake[1]

	Quintiles of total glucosinolates intake					
	1 (low)	2	3	4	5 (high)	P_{trend}
NHS						
Median intake, mg/d	4.16	7.20	10.4	14.3	22.0	
Cases/person-years, *n*/*n*	1338/336,911	1455/337,012	1556/336,744	1566/336,744	1671/336,754	
Rate per 100,000 person-years	397	432	462	465	496	
Model 1[2]	1	1.09 (1.01, 1.17)	1.15 (1.07, 1.24)	1.15 (1.07, 1.24)	1.21 (1.13, 1.30)	<0.001
Model 2[3]	1	1.09 (1.01, 1.17)	1.13 (1.05, 1.22)	1.12 (1.04, 1.20)	1.11 (1.04, 1.20)	0.02
Model 3[4]	1	1.12 (1.04, 1.21)	1.19 (1.10, 1.28)	1.21 (1.12, 1.31)	1.27 (1.17, 1.37)	<0.001
NHS II						
Median intake, mg/d	2.71	5.10	8.00	12.5	20.9	
Cases/person-years, *n*/*n*	1133/356,097	1064/356,212	924/356,789	1026/356,530	1291/356,197	
Rate per 100,000 person-years	318	299	259	288	362	
Model 1[2]	1	0.90 (0.83, 0.98)	0.77 (0.71, 0.84)	0.83 (0.76, 0.91)	0.99 (0.92, 1.08)	0.21
Model 2[3]	1	1.03 (0.95, 1.12)	0.95 (0.87, 1.04)	1.01 (0.93, 1.11)	1.05 (0.97, 1.14)	0.21
Model 3[4]	1	1.05 (0.96, 1.14)	0.98 (0.89, 1.07)	1.06 (0.97, 1.16)	1.12 (1.02, 1.22)	0.008
HPFS						
Median intake, mg/d	3.25	6.76	10.5	15.0	24.2	
Cases/person-years, *n*/*n*	722/167,256	684/167,605	675/167,650	697/167,608	765/167,584	
Rate per 100,000 person-years	432	408	403	416	456	
Model 1[2]	1	0.93 (0.84, 1.04)	0.92 (0.82, 1.02)	0.94 (0.84, 1.04)	1.01 (0.91, 1.12)	0.44
Model 2[3]	1	0.98 (0.88, 1.09)	0.98 (0.88, 1.09)	0.98 (0.88, 1.09)	1.04 (0.94, 1.16)	0.36
Model 3[4]	1	1.01 (0.90, 1.12)	1.02 (0.92, 1.14)	1.05 (0.94, 1.17)	1.15 (1.03, 1.28)	0.01
Pooled[5]						
Model 1[2]	1	0.99 (0.94, 1.04)	0.96 (0.91, 1.01)	0.99 (0.94, 1.04)	1.09 (1.04, 1.14)	<0.001
Model 2[3]	1	1.04 (0.99, 1.10)	1.03 (0.98, 1.09)	1.05 (1.00, 1.10)	1.08 (1.02, 1.13)	0.007
Model 3[4]	1	1.07 (1.02, 1.13)	1.08 (1.03, 1.14)	1.12 (1.07, 1.18)	1.19 (1.13, 1.25)	<0.001

[1] AHEI, alternative healthy eating index; HPFS, Health Professionals Follow-up Study; MET, metabolic equivalent of task; NHS, Nurses' Health Study; T2D, type 2 diabetes.

[2] Estimates are calculated using Cox proportional hazards models. Model 1, adjusted for age (years).

[3] Model 2, further adjusted for ethnicity (Caucasian, African American, Asian, and other ethnicity), family history of diabetes (yes or no), smoking status [never, former, current (1–14, 15–24, or ≥25 cigarettes/d), or missing], alcohol intake (0, 0.1–4.9, 5.0–14.9, and ≥15.0 g/d in women, 0, 0.1–4.9, 5.0–29.9, and ≥30.0 g/d in men, or missing), physical activity (<3, 3.0–8.9, 9.0–17.9, 18.0–26.9, or ≥27.0 MET h/wk, or missing), menopausal status and postmenopausal hormone use [premenopause, postmenopause (never, former, or current hormone use), or missing, for women], oral contraceptive use (yes, no, or missing, for NHS II), multivitamin use (yes or no), hypertension (yes or no), hypercholesterolemia (yes or no), BMI [(kg/m^2) <23, 23–24.9, 25–29.9, 30–34.9, ≥35, or missing], and total energy intake based on model 1.

[4] Model 3, further adjusted for modified AHEI score (in quintiles), based on model 2.

[5] Results from each cohort were pooled using the fixed-effects model.

$P_{trend} = 0.93$), suggesting that glucosinolates contributed to the observed positive association for cruciferous vegetables. In a secondary analysis, we evaluated each individual cruciferous vegetable (**Supplemental Table 3**). The strongest associations were observed for Brussels sprouts (HR: 1.18; 95% CI: 1.11, 1.26; $P_{trend} < 0.001$) and cabbage (HR: 1.15; 95% CI: 1.09, 1.22; $P_{trend} < 0.001$) for a comparison of the highest (≥1 serving/wk) with the lowest intake category (never or almost never). Higher consumption of other cruciferous vegetables also tended to be associated with a higher T2D risk, although only cauliflower reached statistical significance (HR: 1.05; 95% CI:1.00, 1.10; $P_{trend} < 0.001$).

DISCUSSION

Contrary to our study hypothesis, in the 3 cohorts of US men and women, glucosinolate intake was associated with a modest elevation of T2D risk in a dose-response manner, independent of other dietary and nondietary risk factors for T2D. The results were similar for specific subgroups of glucosinolates, and the positive association persisted across subgroups of participants with various diabetes risk profiles. Consumption of cruciferous vegetables, particularly cabbage and Brussels sprouts, was significantly associated with an increased risk of T2D, and this association was statistically accounted for by glucosinolate intake.

To our knowledge, the current study is the first prospective observational study that has examined the association between glucosinolate intake and risk of T2D. A meta-analysis of prospective studies reported that consumption of total vegetables was not significantly associated with T2D risk, although increased consumption of green leafy vegetables was linked to a reduced risk of T2D (24). In a prospective study in Japanese adults, Kurotani et al. (25) found that high consumption of cruciferous vegetables was associated, albeit not statistically significantly, with a reduced risk of T2D in men. Several clinical trials have reported conflicting results for the effect of supplementation of glucosinolates or food sources of glucosinolates on metabolic traits (26–29). In a 4-wk clinical trial among T2D patients, consumption of broccoli sprouts resulted in a significant decrease in serum insulin concentration and homoeostasis model assessment of insulin resistance, but not overall insulin sensitivity as measured

TABLE 3
Stratified HR (95% CI) of T2D according to quintiles of total glucosinolate intake by various characteristics of participants[1]

	Quintiles of total glucosinolates intake						
	1 (low)	2	3	4	5 (high)	P_{trend}	$P_{interaction}$[2]
Race/ethnicity							0.71
Caucasian	1	1.07 (1.01, 1.12)	1.07 (1.02, 1.13)	1.11 (1.05, 1.17)	1.19 (1.13, 1.25)	<0.001	
Other	1	1.00 (0.78, 1.27)	1.02 (0.80, 1.31)	1.26 (0.99, 1.59)	1.21 (0.95, 1.52)	0.05	
Age, y							0.08
<65	1	1.07 (0.99, 1.17)	1.10 (1.01, 1.20)	1.16 (1.07, 1.26)	1.28 (1.17, 1.39)	<0.001	
≥65	1	1.09 (0.99, 1.19)	1.16 (1.06, 1.27)	1.15 (1.05, 1.25)	1.18 (1.08, 1.29)	0.002	
BMI, kg/m^2							0.18
<30	1	1.06 (0.98, 1.14)	1.10 (1.02, 1.19)	1.15 (1.07, 1.24)	1.23 (1.14, 1.33)	<0.001	
≥30	1	1.07 (1.00, 1.14)	1.06 (0.99, 1.13)	1.09 (1.02, 1.17)	1.15 (1.07, 1.23)	<0.001	
Modified AHEI score							0.86
<Median level	1	1.08 (1.01, 1.16)	1.05 (0.97, 1.13)	1.05 (0.97, 1.14)	1.18 (1.08, 1.29)	0.001	
≥Median level	1	1.06 (0.98, 1.14)	1.10 (1.03, 1.19)	1.16 (1.08, 1.24)	1.20 (1.12, 1.29)	<0.001	
Physical activity							0.99
<Median level	1	1.04 (0.98, 1.11)	1.02 (0.96, 1.09)	1.10 (1.03, 1.17)	1.18 (1.11, 1.26)	<0.001	
≥Median level	1	1.12 (1.03, 1.23)	1.17 (1.07, 1.28)	1.17 (1.08, 1.28)	1.23 (1.13, 1.34)	<0.001	
Smoking status							0.40
Never	1	1.06 (1.00, 1.11)	1.07 (1.01, 1.12)	1.11 (1.06, 1.18)	1.18 (1.12, 1.24)	<0.001	
Ever	1	1.10 (0.94, 1.29)	1.10 (0.94, 1.29)	1.13 (0.95, 1.33)	1.28 (1.08, 1.51)	0.009	
Alcohol consumption							0.32
Never	1	1.11 (1.03, 1.19)	1.08 (1.01, 1.16)	1.18 (1.10, 1.27)	1.22 (1.13, 1.31)	<0.001	
Ever	1	1.02 (0.95, 1.10)	1.06 (0.99, 1.14)	1.07 (0.99, 1.14)	1.16 (1.08, 1.24)	<0.001	

[1]Estimates are calculated using Cox proportional hazards models, adjusted for age, race/ethnicity (Caucasian, African American, Asian, and other race/ethnicity), family history of diabetes (yes or no), smoking status [never, former, current (1–14, 15–24, or ≥25 cigarettes/d), or missing], alcohol intake (0, 0.1–4.9, 5.0–14.9, and ≥15.0 g/d in women; 0, 0.1–4.9, 5.0–29.9, and ≥30.0 g/d in men; or missing), physical activity (<3, 3.0–8.9, 9.0–17.9, 18.0–26.9, or ≥27.0 MET h/wk, or missing), menopausal status and postmenopausal hormone use [premenopause, postmenopause (never, former, or current hormone use), or missing, for women], oral contraceptive use (yes, no, or missing, for NHS II), multivitamin use (yes or no), hypertension (yes or no), hypercholesterolemia (yes or no), BMI [(kg/m^2) <23, 23–24.9, 25–29.9, 30–34.9, ≥35, or missing], total energy intake, and the modified AHEI score (quintiles). AHEI, alternative healthy eating index; MET, metabolic equivalent of task; T2D, type 2 diabetes.

[2]$P_{interaction}$ was calculated using likelihood-ratio test.

by the fasting glucose to insulin ratio (26). Two trials reported no measurable changes in markers of endothelial function and inflammation upon consumption of a glucosinolate-rich diet (27, 28). Moreover, in a study among healthy nonsmoking participants, broccoli intake for 6 d induced significant activity of cytochrome P450 1A2, a phase I enzyme implicated in the generation of reactive oxygen species (ROS) (29).

Somewhat conflicting results regarding the effects of ITCs on oxidative stress and insulin resistance have been observed in experimental research. Emerging evidence has suggested that sulforaphane exerts protective effects against oxidative stress by inducing phase II enzymes such as glutathione-S-transferase, glutathione reductase, and NAD(P)H:quinone oxidoreductase, which play a pivotal role in the defense against oxidation (30). ITCs might also exert anti-inflammatory effects by inhibiting nuclear factor κB (31). In contrast, some studies have demonstrated detrimental effects of glucosinolate intake or ITCs on oxidative stress. In a rat model inoculated with human gut microbiota, a diet rich in glucosinolates significantly increased concentrations of both cytochrome P450 and glutathione-S-transferase (32). Similar findings were shown in another rat model, in which a supplementation of glucosinolates induced significant activity of cytochrome P450 and other phase I enzymes, and increased ROS in rat liver (33). In vitro experiments have also revealed that ITCs rapidly undergo conjugation with reduced glutathione (GSH), and ITC-GSH conjugates are quickly exported, causing a depletion of GSH, which may facilitate subsequent ROS generation and oxidative damage (34).

The pro-oxidant activity has been proposed to underlie the potentially anticarcinogenic role of ITCs because the variation of the intracellular redox status triggers apoptosis and other defensive mechanisms (35). Interesting results have been observed regarding the effects of ITCs on β-cell survival and function. Sulforaphane protects β-cells by repressing the nuclear factor κB pathway or other Nrf2-mediated pathways (11, 36); whereas an in vitro study has shown that sulforaphane acutely stimulated basal insulin secretion of β-cells mediated by ROS, although prolonged sulforaphane exposure led to suppressed glucose-stimulated insulin secretion, possibly by reducing ROS levels (37).

The evidence discussed above illustrates the complicated biological functions associated with these potent phytochemicals. The exact biological mechanisms underlying the putative effects of glucosinolates on T2D risk deserve more elucidation. It is well established that the bioavailability of ITCs depends on the activity of plant myrosinase and the metabolic potential of human gut microbiota (38). Transportation and storage of cruciferous vegetables, chewing intensity, cooking temperature and duration, and the composition of meals containing cruciferous vegetables can all affect the activity of plant myrosinase (6, 39). Because these factors dictate the bioavailability of ITCs, in feeding studies, typical between-individual variability of ITC production upon

TABLE 4
HR (95% CI) of T2D according to consumption levels of total cruciferous vegetables[1]

	Consumption level					
	<1 serving/wk	1–3 servings/wk	4–6 servings/wk	≥1 serving/d	Every 2 servings/wk	P_{trend}
NHS						
Cases/person-years, *n*/*n*	565/157,801	4622/1,047,731	1877/371,908	522/106,782		
Rate per 100,000 person-years	358	441	505	489		
Multivariable adjusted HR[2]	1	1.14 (1.04, 1.25)	1.23 (1.11, 1.36)	1.22 (1.07, 1.38)	1.03 (1.01, 1.05)	<0.001
NHS II						
Cases/person-years, *n*/*n*	1081/368,502	2878/1,011,882	1021/295,511	458/105,931		
Rate per 100,000 person-years	293	284	346	432		
Multivariable adjusted HR[2]	1	1.00 (0.93, 1.07)	1.10 (1.00, 1.20)	1.10 (0.98, 1.24)	1.02 (1.00, 1.04)	0.02
HPFS						
Cases/person-years, *n*/*n*	435/104,112	1996/484,210	771/177,970	341/71,412		
Rate per 100,000 person-years	418	412	433	478		
Multivariable adjusted HR	1	0.98 (0.88, 1.09)	1.04 (0.92, 1.18)	1.17 (1.00, 1.36)	1.03 (1.01, 1.06)	0.02
Pooled results[3]	1	1.03 (0.98, 1.09)	1.13 (1.06, 1.20)	1.16 (1.07, 1.25)	1.03 (1.01, 1.04)	<0.001

[1]Total cruciferous vegetables included broccoli, cabbage, cauliflower, Brussels sprouts, kale, and mustard and chard greens. HPFS, Health Professionals Follow-up Study; MET, metabolic equivalent of task; NHS, Nurses' Health Study; T2D, type 2 diabetes.

[2]Estimates are calculated using Cox proportional hazards models, adjusted for age, race/ethnicity (Caucasian, African American, Asian, and other race/ethnicity), family history of diabetes (yes or no), smoking status [never, former, current (1–14, 15–24, or ≥25 cigarettes/d), or missing], alcohol intake (0, 0.1–4.9, 5.0–14.9, and ≥15.0 g/d in women; 0, 0.1–4.9, 5.0–29.9, and ≥30.0 g/d in men; or missing), physical activity (<3, 3.0–8.9, 9.0–17.9, 18.0–26.9, ≥27.0 MET h/wk, or missing), menopausal status and postmenopausal hormone use [premenopause, postmenopause (never, former, or current hormone use), or missing, for women], oral contraceptive use (yes, no, or missing, for NHS II), multivitamin use (yes or no), hypertension (yes or no), hypercholesterolemia (yes or no), BMI [(kg/m^2) <23, 23–24.9, 25–29.9, 30–34.9, ≥35, or missing], total energy intake, and the modified alternate healthy eating index score (quintiles).

[3]Results from each cohort were pooled using the fixed-effects model.

ingestion of the same amount of glucosinolates was observed (39). Moreover, ITCs are not the only metabolites that can be derived from glucosinolates. Metabolites such as nitriles, epithionitriles, and thiocyanates could be produced upon the consumption of glucosinolates (9). In comparison with ITCs, nitriles are much less potent at inducing phase II enzymes and have the potential to induce cytotoxicity and genotoxicity (40). Furthermore, some glucosinolate metabolites, such as thiocyanate and goitrin, were shown to inhibit iodine utilization by the thyroid gland and subsequently interfered with the synthesis of thyroid hormones (41).

The strengths of the current study include the prospective design, the large sample size, high follow-up rates, long duration of follow-up, and repeated assessments of dietary and lifestyle variables. In addition, the consistency of results across all 3 independent cohorts indicates that our findings are unlikely to be due to chance. Our results also need to be interpreted in the context of several limitations.

First, our study populations consisted mostly of working health professionals with European ancestry. Although the homogeneity of educational attainment and socioeconomic status helped minimize potential residual confounding, the generalizability of our findings to other populations is limited.

Second, because diet was self-reported through FFQs, some measurement errors in the assessment of food consumption were inevitable. However, the FFQs used in these studies have been validated against multiple diet records with reasonable reproducibility and validity. Because of the prospective design, misclassification of glucosinolate intake was independent of the outcome ascertainment and was therefore more likely to be nondifferential, which would tend to attenuate true associations toward the null. Moreover, the use of cumulative average intakes of multiple repeated measurements could reduce potential random measurement errors and would accommodate dietary changes over time.

Third, human diets are extremely complicated and consist of numerous nutrients and nonnutrient constituents that may have additive or synergistic effects on human health. Although the statistically significant positive association for glucosinolates and cruciferous vegetables persisted after adjustment for nutrients that the vegetables are rich in, such as β-carotene, flavonoids, vitamin C, vitamin E, and fiber in our study, we could not rule out the impact of potential synergistic effects of glucosinolates and other dietary factors on T2D risk.

Fourth, we did not inquire about cooking methods for cruciferous vegetables, which is a potential limitation of our study. In epidemiologic studies conducted among free-living individuals, estimated consumption of cruciferous vegetables or glucosinolates without considering cooking, transportation, or storage conditions was still reasonably accurately associated with urinary excretion of ITCs, suggesting that despite the measurement errors, the estimated intake of glucosinolates could still largely differentiate individuals with different ITC levels (42, 43). We also found a positive association between glucosinolate intake and urinary ITC excretion in a small pilot study in the NHS. Finally, although we were able to carefully adjust for a wide range of established and potential risk factors for T2D, the possibility of residual or unmeasured confounding could not completely be ruled out because of the observational nature of this study.

In summary, data from 3 large prospective cohort studies consistently showed modest, positive associations of dietary glucosinolate intake with risk of developing T2D. A higher consumption of cruciferous vegetables was also associated with a slightly elevated risk of T2D. Further studies, especially ones based on

objective ITC biomarkers, are needed to replicate these findings and facilitate a further understanding of this relation.

The authors' responsibilities were as follows—QS, WCW, and FBH: obtained funding from the National Institutes of Health and were involved in data collection; QS and FBH: designed the study; LM, GL, and QS: provided statistical expertise; LM and QS: analyzed the data and wrote the first draft of the manuscript; and all authors: contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content, and approved the final version of the manuscript. The authors declare no conflicts of interest.

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