Dietary Protein Sources, Mediating Biomarkers, and Incidence of Type 2 Diabetes: Findings From the Women's Health Initiative and the UK Biobank

Jie Li,[1,2] Andrea J. Glenn,[3–5] Qingling Yang,[1] Ding Ding,[1] Lingling Zheng,[1] Wei Bao,[6] Jeannette Beasley,[7] Erin LeBlanc,[8] Kenneth Lo,[9] JoAnn E. Manson,[10] Lawrence Philips,[11] Lesley Tinker,[12] and Simin Liu[1,2,13]

Diabetes Care 2022;45:1742–1753 | https://doi.org/10.2337/dc22-0368

OBJECTIVE

Whether and how dietary protein intake is linked to type 2 diabetes (T2D) remains unclear. The aim of this study was to investigate the associations of protein intake with development of T2D and the potential mediating roles of T2D biomarkers.

RESEARCH DESIGN AND METHODS

We included 108,681 postmenopausal women without T2D at baseline from the Women's Health Initiative (WHI) (primary cohort) and 34,616 adults without T2D from the U.K. Biobank (UKB) (replication cohort). Cox proportional hazard models were used for estimation of protein-T2D associations. Mediation analysis was performed to assess the mediating roles of biomarkers in case-control studies nested in the WHI.

RESULTS

In the WHI, 15,842 incident T2D cases were identified during a median follow-up of 15.8 years. Intake of animal protein was associated with increased T2D risk (hazard ratio in comparing the highest to the lowest quintile = 1.31 [95% CI 1.24–1.37]) and plant protein with decreased risk (0.82 [0.78–0.86]). Intakes of red meat, processed meat, poultry, and eggs were associated with increased T2D risk and whole grains with decreased risk. Findings from the UKB were similar. These findings were materially attenuated after additional adjustment for BMI. Substituting 5% energy from plant protein for animal protein was associated with 21% decreased T2D risk (0.79 [0.74–0.84]), which was mediated by levels of hs-CRP, interleukin-6, leptin, and SHBG.

CONCLUSIONS

Findings from these two large prospective cohorts support the notion that substituting plant protein for animal protein may decrease T2D risk mainly by reducing obesity-related inflammation.

Dietary modification or medical nutrition therapy remains the cornerstone in preventing and managing type 2 diabetes (T2D) (1,2). Although much is known about the effects of fats versus carbohydrates on risk of T2D (3), fewer studies have directly investigated the role of dietary protein in the development of T2D (4). While

[1]Global Health Research Center, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China
[2]Department of Epidemiology and Center for Global Cardiometabolic Health, School of Public Health, Brown University, Providence, RI
[3]Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada
[4]Toronto 3D Knowledge Synthesis and Clinical Trials Unit, Clinical Nutrition and Risk Factor Modification Centre, St. Michael's Hospital, Toronto, Ontario, Canada
[5]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA
[6]Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei, China
[7]Division of General Internal Medicine and Clinical Innovation, New York University Langone Health, New York, NY
[8]Kaiser Permanente Center for Health Research, Portland, OR
[9]Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong Special Administrative Region, China
[10]Brigham and Women's Hospital, Harvard Medical School, and Harvard T.H. Chan School of Public Health, Boston, MA
[11]Division of Endocrinology, Emory University, Atlanta, GA
[12]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA
[13]Departments of Surgery and Medicine, The Warren Alpert Medical School, Brown University, Providence, RI

Corresponding authors: Jie Li, jie_li@brown.edu, and Simin Liu, simin_liu@brown.edu

Received 21 February 2022 and accepted 3 May 2022

This article contains supplementary material online at https://doi.org/10.2337/figshare.19860148.

This article is featured in a podcast available at diabetesjournals.org/journals/pages/diabetes-core-update-podcasts.

J.L. and A.J.G. contributed equally to this work.

recent work has shown a positive association between intake of total and animal proteins with risk of T2D, findings relating plant protein intake to T2D risk remain inconsistent (5–8). Moreover, few prospective studies (9,10) have assessed the relation of protein food sources intake to T2D risk and the beneficial associations between substituting different protein sources and T2D risk.

Although substituting plant protein for animal protein was associated with decreased risk of T2D, none have attempted to evaluate the mediating roles of known biochemical intermediaries, limiting our understanding of mechanistic pathways by which dietary proteins may affect T2D risk. Obesity (11), inflammation (12), endothelial dysfunction (13), sex steroids and sex hormone binding globulin (SHBG) (14–17), and telomere attrition (18,19) are well-characterized T2D risk factors or biomarkers, which are also associated with some protein sources intake (20–23). Therefore, these biomarkers may represent critical mediators for the beneficial association between substituting plant protein for animal protein and T2D risk.

To fill such gaps in the knowledge base for T2D prevention related to dietary modification, we conducted analyses for *1*) investigation of the roles of different animal and plant protein sources in relation to incident T2D among participants enrolled in the Women's Health Initiative (WHI) and the U.K. Biobank (UKB), *2*) evaluation of the beneficial association between substituting different protein sources with T2D risk in the WHI and the UKB, and *3*) examination of the extent to which known T2D-related biomarkers could explain the association of substituting plant protein for animal protein in case-control studies nested in the WHI.

RESEARCH DESIGN AND METHODS

Study Populations

We included the WHI as the primary analytical cohort and the UKB as the replication cohort. Between 1993 and 1998, 161,808 postmenopausal women aged 50–79 years from 40 clinical centers in the U.S. were recruited into the WHI Observational Study (OS) and clinical trials (CT). The study design was previously described in detail (24). We excluded participants who were assigned to the treatment arms in the WHI CT or had self-reported diabetes at baseline, missing dietary information, or implausible daily energy intake ($<$600 or $>$5,000 kcal/day) from a validated food-frequency questionnaire (FFQ) (25). Finally, a total of 108,681 participants with a median follow-up duration of 15.8 years until 28 February 2020 were available for the analysis. In the UKB, we included 34,616 participants free of diabetes at baseline who then were followed up for a median of 11.4 years. Details of the UKB cohort and related methods can be found in Supplementary Methods.

Measurement of Dietary Protein Sources

In the WHI, dietary data were collected for each participant at enrollment with a validated self-administered FFQ at baseline (25). Daily nutrient intake was calculated with use of the University of Minnesota Nutrient Data System for Research (25). The energy-adjusted correlation coefficients between the FFQ and food records ranged from 0.2 to 0.7 for the estimated intakes of 30 nutrients, with 0.4 for protein (25). Food items in the FFQ were summarized into 32 food groups based on the MyPyramid Equivalents Database (MPED) 2.0 (26,27), among which were the dietary protein sources of interest. Based on a study in 10 countries in the European Prospective Investigation into Cancer and Nutrition (EPIC) (28), we modified the EPIC protein source list to include red meat, processed meat, poultry, high–*n*-3 seafood, low–*n*-3 seafood, cheese, yogurt, milk, and eggs as major animal protein sources and legumes, nuts, and whole grains as major plant protein sources. Specific food items in each protein source are listed in Supplementary Table 1.

Ascertainment of Incident T2D Cases

In the WHI, incident T2D cases were identified during annual follow-up with self-administered questionnaires. Participants were asked if "a doctor prescribed pills or insulin shots for diabetes for the first time" since their last medical update. Validation studies of self-reported diabetes with use of medical records and biomarkers indicated high accuracy and reliability (12,29).

Covariates

Demographic characteristics and lifestyle factors for each participant collected at baseline were included in our models as covariates, including age, race/ethnicity, smoking status, alcohol intake, total energy intake, education, family income, physical activity, region of residence, family history of diabetes, use of menopausal hormone therapy, multivitamin use, and antihypertension medicine and score of a modified Alternate Healthy Eating Index (AHEI)-2010 (30) with removal of red/processed meat, long-chain (*n*-3) fats including eicosapentaenoic acid and docosahexaenoic acid, nuts and legumes, and whole grains. The AHEI-2010 modified score ranged from 0 to 70 indicating a lower- to a higher-quality modified diet. Detailed descriptions of the validity and reproducibility of baseline measurements have previously been published (31). The proportions of missing data for covariates were $<$4%; mean or mode imputation was used for continuous and categorical covariates, respectively.

Nested Case-Control Studies Within the WHI

We completed a series of case-control studies nested in the WHI to investigate the associations between biomarkers and T2D risk (12,13,16,18). Using these nested case-control studies, we further examined the potential roles of specific T2D-related biomarkers that could mediate the protein-T2D associations. Details of these case-control studies (12,15,18) and quality control of specific biomarker measurements (24) have previously been published. After exclusion of participants with missing dietary data, 3,464 participants with five sets of biomarkers were included in the mediation analysis. These included *1*) biomarkers for inflammation, including tumor necrosis factor-α receptor 2 (TNFα-R2), interleukin-6 (IL-6), and hs-CRP; *2*) biomarkers for endothelial dysfunction (i.e., vascular cell adhesion molecule 1 [VCAM-1], E-selectin, and soluble intercellular adhesion molecule-1 [SICAM-1]); *3*) sex steroids, including estradiol, testosterone, and SHBG; *4*) leptin and soluble leptin receptor; and *5*) leukocyte telomere length for aging.

Statistical Analyses

In the WHI, each participant's follow-up time was defined as the duration between entry to the cohort and the date

of occurrence of T2D or censor (death, lost to follow-up, or end of follow-up)—whichever occurred first. Cox proportional hazards model was used to evaluate the associations of energy-adjusted dietary protein and its food sources with risk of incident T2D. Dietary protein sources were entered into the models as quintiles, with the lowest quintile serving as the reference group.

Covariates were adjusted for in multivariable models. In model 1, we adjusted for age, study group indicator (OS, CT), self-identified race and ethnicity (White, Black, Hispanic, Asian), region of residence at baseline (Northeast, South, Midwest, West), family income (<$20,000, $20,000–49,999, $50,000–99,999, >$100,000), education (<high school, high school, college, postgraduate), and family history of diabetes (yes/no); in model 2, we further included smoking status (never, past, current smoker), alcohol intake (continuous), physical activity (continuous), hormone replacement therapy (never, past, current user), multivitamin use (yes/no), antihypertensive medication use (never, previously, currently treated), and total energy intake (continuous); in model 3 (full model), we additionally adjusted for modified AHEI-2010 score (continuous) and intake of other dietary protein sources; in model 4 (sensitivity model), we further adjusted for BMI and waist-to-hip ratio (WHR) given that BMI could be both a confounder and mediator in protein-T2D associations. We also included restricted cubic spline term for dietary protein and food sources with three knots at 10^{th}, 50^{th}, and 90^{th} centiles in model 3 to explore potential nonlinear relations of specific dietary protein sources to T2D risk. The nonlinearity *P* value was estimated with a likelihood ratio test.

We used the leave-one-out model to evaluate the association between isocaloric substitution of plant protein for animal protein and T2D risk. We simultaneously included the percentage of energy from carbohydrate, fat, and plant protein and total energy intake with covariates in a multivariable model. The coefficient of plant protein from the model can be interpreted as the estimated effect of substituting a specific percentage of energy from plant protein for the same percentage of energy from animal protein.

In the mediation analysis, we examined whether the beneficial associations of substituting plant protein for animal protein with decreased risk of T2D were mediated by well-characterized T2D-related biochemical intermediaries. We constructed two regression models, a logistic model, to regress the outcome (T2D) on the exposure (protein intake) and the mediator (biomarker), and a weighted linear model, to regress the mediator on the exposure, with adjustment for potential confounders. We integrated these two regressions to obtain the estimates for direct and indirect effects using the regression-based approach proposed by VanderWeele (24,32,33). The proportion of mediating effect was estimated on an odds ratio (OR) scale; 95% CIs were obtained via bootstrapping. All analyses were performed with R (version 4.0.3). Two-sided *P* values and 95% CIs were calculated for statistical inference.

RESULTS

Associations of Dietary Protein Food Sources Intake With Risk of Incident T2D

In the WHI, among the 108,681 postmenopausal women, mean age 63 years at baseline, 15,842 (14.6%) participants developed T2D during a median follow-up period of 15.8 years (Table 1). The postmenopausal women with higher energy-adjusted intake of total protein were more likely to be White, educated, physically active, and have a higher BMI and income, while they were less likely to be a smoker or drinker, compared with those with lower energy-adjusted intake of total protein (Table 1). In the fully adjusted model, participants with higher energy-adjusted intake of animal protein had increased risk of incident T2D (adjusted hazard ratio [HR] in comparing the highest with the lowest quintile: 1.31 [95% CI 1.24–1.37], *P* for trend <0.001). In contrast, participants with greater intake of energy-adjusted plant protein had lower risk of incident T2D (0.82 [0.78–0.86], *P* for trend <0.001) (Table 2).

Among the 12 dietary protein sources, intake of red meat, processed meat, poultry, eggs, and low–n-3 seafood was associated with increased T2D risk in the fully adjusted model. Adjusted HRs of T2D for participants in the highest (vs. the lowest) quintile of these protein sources intake was 1.15 (95% CI 1.09–1.22) for red meat, 1.14 (1.08–1.20) for processed meat, 1.09 (1.03–1.15) for poultry, 1.21 (1.14–1.27) for eggs, and 1.12 (1.06–1.18) for low–n-3 seafood. In contrast, intake of whole grains and nuts was inversely associated with T2D risk, with HRs of 0.84 (0.79–0.88) for whole grains and 0.90 (0.86–0.95) for nuts in comparing the two extreme quintiles. Intakes of high–n-3 seafood, cheese, milk, yogurt, and legumes had neutral associations with T2D risk in the fully adjusted model (Table 2). Results of additional restricted cubic spline analyses in the WHI showed nonlinear relations for intake of plant protein, processed meat, high–*n*-3 seafood, and nuts to T2D risk (all *P* for overall <0.01 and all *P* for nonlinearity <0.05) and linear relations for intake of total protein, animal protein, red meat, poultry, eggs, low–*n*-3 seafood, and whole grains with T2D risk (all *P* for overall <0.001 and all *P* for nonlinearity >0.05) (Fig. 1).

We further conducted a replication analysis in the UKB of 14,902 men and 19,714 women followed for a median of 11.4 years, during which 663 incident T2D cases were identified (Supplementary Table 3). The positive associations of intake of total protein, red meat, processed meat, eggs, and poultry and the inverse associations of whole grain intake with risk of incident T2D observed in the WHI were confirmed in the UKB. However, the intake of low–n-3 seafood and nuts had no association with risk of incident T2D in the UKB (Supplementary Table 4).

Associations Between Substituting Different Protein Sources and T2D Risk

In the WHI, substituting 5% energy from plant protein for 5% of energy from animal protein was associated with a 21% reduced risk of T2D (HR 0.79 [95% CI 0.74–0.84]). Specifically for protein sources, replacing 1 serving/day red meat (0.87 [0.85–0.90]), processed meat (0.79 [0.75–0.84]), eggs (0.78 [0.75–0.81]), or poultry (0.87 [0.84–0.91]) with plant protein sources, particularly whole grains, was associated with 13–26% decreased risk of T2D (Table 3).

We also examined the associations between replacing red meat, processed meat, eggs, and poultry with other animal protein sources and risk of T2D. Substituting milk, yogurt, or cheese for red meat, processed meat, eggs, or

Table 1—Baseline characteristics according to quintiles of daily intake of total dietary protein sources among 108,681 postmenopausal women in the WHI

		Quintiles of energy-adjusted total protein				
	Total	1	2	3	4	5
N	108,681	21,737	21,736	21,736	21,736	21,736
Follow-up duration (person-years)	1,654,022	321,986	328,762	334,337	336,959	331,979
Incident diabetes	15,842 (14.6)	3,039 (14.0)	2,985 (13.7)	3,108 (14.3)	3,193 (14.7)	3,517 (16.2)
Age at baseline (years)	63 ± 7	63 ± 7	63 ± 7	64 ± 7	63 ± 7	63 ± 7
Self-identified ethnicity or race						
White	92,209 (84.8)	17,248 (79.3)	18,045 (83.0)	18,605 (85.6)	19,097 (87.9)	19,214 (88.4)
Black	8,018 (7.4)	2,595 (11.9)	1,789 (8.2)	1,442 (6.6)	1,123 (5.2)	1,069 (4.9)
Hispanic	3,768 (3.5)	876 (4.0)	805 (3.7)	720 (3.3)	667 (3.1)	700 (3.2)
Asian	2,818 (2.6)	597 (2.7)	721 (3.3)	591 (2.7)	494 (2.3)	415 (1.9)
Region of residence at baseline						
Northeast	25,126 (23.1)	5,212 (24.0)	4,944 (22.7)	4,951 (22.8)	5,196 (23.9)	4,823 (22.2)
South	27,803 (25.6)	6,088 (28.0)	5,700 (26.2)	5,501 (25.3)	5,208 (24.0)	5,306 (24.4)
Midwest	23,823 (21.9)	4,197 (19.3)	4,345 (20.0)	4,647 (21.4)	5,015 (23.1)	5,619 (25.9)
West	31,929 (29.4)	6,240 (28.7)	6,747 (31.0)	6,637 (30.5)	6,317 (29.1)	5,988 (27.5)
Education, *n* (%)						
<High school	4,878 (4.5)	1,343 (6.2)	1,071 (4.9)	954 (4.4)	763 (3.5)	747 (3.4)
High school	28,030 (25.8)	6,212 (28.6)	5,930 (27.3)	5,571 (25.6)	5,401 (24.8)	4,916 (22.6)
College	42,650 (39.2)	8,236 (37.9)	8,479 (39)	8,555 (39.4)	8,751 (40.3)	8,629 (39.7)
Postgraduate	33,123 (30.5)	5,946 (27.4)	6,256 (28.8)	6,656 (30.6)	6,821 (31.4)	7,444 (34.2)
BMI (kg/m^2)	27.3 ± 5.7	27.3 ± 5.8	26.8 ± 5.5	27.1 ± 5.5	27.4 ± 5.7	28.1 ± 5.9
Waist circumference (cm)	84.9 ± 13.3	85.1 ± 13.5	83.8 ± 12.9	84.2 ± 12.8	84.9 ± 13.2	86.5 ± 13.7
Recreational physical activity (MET h/week)	13.4 ± 13.9	11.8 ± 13.7	13.0 ± 13.7	13.4 ± 13.7	13.9 ± 14	14.5 ± 14.2
Alcohol intake (g/day)	5.63 ± 11.0	8.3 ± 16.6	5.8 ± 10.2	5.1 ± 8.9	4.7 ± 8.3	4.3 ± 7.9
Smoking status						
Never	55,871 (51.4)	10,798 (49.7)	11,311 (52.0)	11,297 (52.0)	11,269 (51.8)	11,196 (51.5)
Past	45,870 (42.2)	9,048 (41.6)	8,982 (41.3)	9,144 (42.1)	9,256 (42.6)	9,440 (43.4)
Current	6,940 (6.4)	1,891 (8.7)	1,443 (6.6)	1,295 (6.0)	1,211 (5.6)	1,100 (5.1)
Income (USD)						
<20,000	15,167 (14.0)	3,844 (17.7)	3,289 (15.1)	2,902 (13.4)	2,620 (12.1)	2,512 (11.6)
20,000–49,999	52,139 (48.0)	10,728 (49.4)	10,517 (48.4)	10,434 (48.0)	10,319 (47.5)	10,141 (46.7)
50,000–99,999	30,433 (28.0)	5,406 (24.9)	5,835 (26.8)	6,209 (28.6)	6,435 (29.6)	6,548 (30.1)
>100,000	10,942 (10.1)	1,759 (8.1)	2,095 (9.6)	2,191 (10.1)	2,362 (10.9)	2,535 (11.7)
Family history of diabetes	32,612 (30.0)	6,397 (29.4)	6,334 (29.1)	6,489 (29.9)	6,505 (29.9)	6,887 (31.7)
Hormone replacement therapy						
Never	35,252 (32.4)	7,556 (34.8)	7,109 (32.7)	6,827 (31.4)	6,848 (31.5)	6,912 (31.8)
Past	23,027 (21.2)	4,790 (22.0)	4,541 (20.9)	4,651 (21.4)	4,476 (20.6)	4,569 (21.0)
Current	50,402 (46.4)	9,391 (43.2)	10,086 (46.4)	10,258 (47.2)	10,412 (47.9)	10,255 (47.2)
Antihypertension medication						
Never treated	75,334 (69.3)	14,877 (68.4)	15,252 (70.2)	15,167 (69.8)	15,131 (69.6)	14,907 (68.6)
Previously treated	8,270 (7.6)	1,779 (8.2)	1,640 (7.5)	1,556 (7.2)	1,537 (7.1)	1,758 (8.1)
Currently treated	25,077 (23.1)	5,081 (23.4)	4,844 (22.3)	5,013 (23.1)	5,068 (23.3)	5,071 (23.3)
Total energy intake (kcal/day)	1,610 ± 619	1,803 ± 706	1,453 ± 562	1,455 ± 542	1,531 ± 538	1,796 ± 626
Total carbohydrate (g/day)	203 ± 78.6	230.7 ± 89.5	188.0 ± 72.0	186.7 ± 70.2	193.5 ± 70	218.6 ± 79.3
Total fat (g/day)	57.5 ± 31.2	69.8 ± 37.6	52.6 ± 27.8	51.2 ± 26.6	52.8 ± 26.7	61.0 ± 31.7
Saturated fat (g/day)	19.3 ± 11.3	23.2 ± 13.7	17.5 ± 10.1	17.1 ± 9.6	17.7 ± 9.7	20.8 ± 11.6
Monounsaturated fat (g/day)	21.8 ± 12.1	26.6 ± 14.5	19.9 ± 10.8	19.4 ± 10.3	19.9 ± 10.5	23.0 ± 12.4
Polyunsaturated fat (g/day)	11.9 ± 6.7	14.9 ± 8.5	11.1 ± 6.0	10.6 ± 5.6	10.8 ± 5.6	12.1 ± 6.2
Total protein (g/day)	67.1 ± 27.8	49.9 ± 8.0	60.8 ± 1.8	66.5 ± 1.6	72.7 ± 2.1	85.7 ± 9.2
Animal protein (g/day)	46.7 ± 22.7	36.0 ± 19.1	35.7 ± 16.8	41.4 ± 16.2	49.7 ± 16.2	70.8 ± 23.7

Continued on p. 1746

Table 1—Continued

	Total	Quintiles of energy-adjusted total protein				
		1	2	3	4	5
Plant protein (g/day)	20.3 ± 8.7	21.5 ± 9.4	18.8 ± 8.2	19.0 ± 8.0	19.8 ± 8.1	22.3 ± 9.1
Dietary protein sources (g/day)						
Red meat	41.1 ± 36.5	31.5 ± 27.8	31.4 ± 26.1	36.1 ± 28.1	43.5 ± 32.9	63.1 ± 51.4
Processed meat	11.2 ± 14.0	10.7 ± 14.3	9.3 ± 12	10.1 ± 12.3	11.3 ± 13.2	14.8 ± 17
Eggs	15.5 ± 18.4	15.9 ± 17.3	13.7 ± 15.3	14.1 ± 15.4	15.2 ± 17.5	18.8 ± 24.3
Poultry	25.6 ± 22.0	16.5 ± 15.9	18 ± 15.7	22.5 ± 16.8	29 ± 19.6	42.1 ± 28.7
High–*n*-3 seafood	9.4 ± 11.8	5.63 ± 7.35	6.76 ± 7.84	8.46 ± 9.08	10.81 ± 11.34	15.53 ± 17.7
Low–*n*-3 seafood	7.9 ± 8.8	6.71 ± 7.55	6.56 ± 6.88	7.26 ± 7.14	8.21 ± 8.25	10.99 ± 12.19
Cheese	111.0 ± 104	95.4 ± 91	89.8 ± 83	98.8 ± 85.1	114.4 ± 97.3	155.8 ± 140.7
Milk	235.9 ± 233.9	171.7 ± 161.7	172.9 ± 158.1	205.4 ± 178.8	252.3 ± 214.4	377 ± 339.7
Yogurt	41.0 ± 68.7	27.3 ± 55.4	32.9 ± 59.6	39.2 ± 63.9	44.6 ± 67.5	60.8 ± 87.5
Whole grains	34.4 ± 28.2	33.08 ± 29.9	31.32 ± 26.41	32.72 ± 26.38	35.07 ± 27.08	39.95 ± 30.12
Legumes	20.3 ± 26.4	18.4 ± 25.3	18.4 ± 23.9	19.2 ± 24.4	20.7 ± 26.4	24.7 ± 30.7
Nuts	10.1 ± 16.0	12.2 ± 19.1	9.6 ± 14.9	9.1 ± 14.8	9.2 ± 14.6	10.2 ± 15.9
Modified AHEI-2010 score	35.5 ± 8.0	33.3 ± 8.3	35.8 ± 8	36.3 ± 7.7	36.4 ± 7.7	35.8 ± 7.7

Data for continuous variables are presented as mean ± SD. Data for categorical variables are presented as *n* (%).

poultry was associated with decreased risk of T2D. For example, replacing 1 serving/day eggs with 1 serving/day yogurt (HR 0.82 [95% CI 0.79–0.85]), milk (0.82 [0.79–0.86]), or cheese (0.83 [0.80–0.86]) was associated with a 17–18% lower risk of T2D. Substituting high–n-3 seafood for processed meat or eggs was also associated with decreased risk of T2D. The HRs of T2D were 0.88 (95% CI 0.81–0.97) for substituting 1 serving/day high–n-3 seafood for processed meat and 0.87 (0.80–0.94) for substituting 1 serving/day high–n-3 seafood for eggs in the WHI. These findings were consistent with the results of substitution analysis in the UKB (Table 3).

In sensitivity analysis, additional adjustment for BMI and WHR attenuated associations of intakes of dietary protein and food sources with T2D risk, leading most associations toward neutral (model 4 in Table 2 and Supplementary Table 4). Similarly, additional adjustment for BMI and WHR also materially attenuated the beneficial associations of substituting different protein and food sources with T2D risk (Supplementary Table 5).

Mediating Roles of T2D-Related Biomarkers in the Associations Between Substituting Plant Protein for Animal Protein and T2D Risk

We included 1,445 incident T2D case and 2,019 control subjects in the case-control studies nested within the WHI. Compared with the control subjects, the T2D case subjects had higher BMI, waist circumference, HOMA of insulin resistance, inflammation factors (hs-CRP, IL-6, and TNFα-R2), endothelial dysfunction markers (E-selectin, SICAM-1, and VCAM-1), and leptin but had lower SHBG (Supplementary Tables 6 and 7).

The observed association of substituting plant protein for animal protein with decreased risk of T2D was mainly mediated by inflammation factors, leptin, endothelial dysfunction, and SHBG. Specifically, hs-CRP mediated 77% of the total relation of substituting 5% energy from plant protein for 5% energy from animal protein to decreased risk of T2D; IL-6 accounted for 47% of the total relation. We also constructed an inflammation score by summing up inflammation markers above the median value (34). The constructed inflammation score mediated 45% of the total relation. Leptin, a major hormone secreted by adipocytes, mediated 66% of the total relation. SHBG accounted for 46% of the total relation. E-selectin mediated 30% of the total relation (Table 4). After additional adjustment for BMI, almost all mediating effects due to biomarkers were eliminated (Supplementary Table 8).

CONCLUSIONS

In two large prospective cohorts of the WHI and the UKB, we found that intake of animal protein sources from red meat, processed meat, poultry, or eggs was directly associated with increased T2D risk, while plant protein sources from whole grains or nuts were associated with decreased risk. Substituting plant protein sources for animal protein sources showed benefits in reducing T2D risk, which was mainly mediated by inflammation, followed by endothelial dysfunction and SHBG.

An accumulating body of evidence now indicates that red meat (9,35), processed meat (9,36), or eggs (10) may increase T2D risk, whereas whole grains (37,38) and dairy products (39) may decrease risk. In the current study, the inverse associations of milk or yogurt intake with T2D risk in the WHI became null after adjustment for the other dietary protein sources. A previous study in the WHI reported that a diet high in low-fat dairy products, but not high-fat dairy products, was associated with lower T2D risk in postmenopausal women, indicating that fat in dairy products may distort the dairy-T2D relation (40). Our analysis in the UKB, however, indicates that milk intake appeared to be associated with increased T2D risk. Further work is warranted to clarify these inconsistent findings, particularly in diverse populations where different T2D risk and dietary practices may be associated with dairy intake.

In both the WHI and the UKB, intake of poultry was associated with increased T2D risk, which appeared to be in contrast with null findings of a recent meta-analysis of 28 prospective studies regarding poultry and fish intake (41). In contrast, in the China Kadoorie Biobank

Table 2—Adjusted HRs with 95% CIs of T2D according to quintiles of daily intake of dietary protein and food sources among 108,681 postmenopausal women in the WHI

	Quintiles of dietary protein and food sources					
	1	2	3	4	5	*P* for trend
N	21,737	21,736	21,736	21,736	21,736	
Total protein						
Daily intake, g/day	49.9 ± 8.0	60.8 ± 1.8	66.5 ± 1.6	72.7 ± 2.1	85.7 ± 9.2	
Cases/person-years	3,039/321,986	2,985/328,762	3,108/334,337	3,193/336,959	3,517/331,979	
Model 1	Ref	1.01 (0.96–1.06)	1.05 (1.00–1.11)	1.09 (1.04–1.15)	1.25 (1.19–1.31)	<0.001
Model 2	Ref	1.05 (0.99–1.10)	1.09 (1.03–1.14)	1.11 (1.06–1.17)	1.21 (1.16–1.28)	<0.001
Model 3	Ref	1.06 (1.00–1.11)	1.10 (1.05–1.16)	1.13 (1.07–1.19)	1.24 (1.18–1.30)	<0.001
Model 4	Ref	1.06 (1.00–1.12)	1.10 (1.04–1.18)	1.12 (1.04–1.20)	1.21 (1.10–1.33)	<0.001
Animal protein						
Daily intake, g/day	28.3 ± 8.0	40.0 ± 2.0	46.1 ± 1.7	52.6 ± 2.2	66.6 ± 10.2	
Cases/person-years	2,911/329,625	3,017/330,174	3,064/332,403	3,192/334,318	3,658/327,503	
Model 1	Ref	1.06 (1.01–1.12)	1.09 (1.03–1.14)	1.14 (1.09–1.20)	1.35 (1.29–1.42)	<0.001
Model 2	Ref	1.09 (1.04–1.15)	1.11 (1.06–1.17)	1.16 (1.10–1.22)	1.31 (1.25–1.37)	<0.001
Model 3	Ref	1.09 (1.03–1.14)	1.11 (1.05–1.17)	1.15 (1.09–1.21)	1.31 (1.24–1.37)	<0.001
Model 4	Ref	1.06 (1.00–1.13)	1.06 (1.00–1.14)	1.08 (1.00–1.17)	1.20 (1.08–1.32)	0.008
Plant protein						
Daily intake, g/day	13.5 ± 2.8	17.5 ± 0.7	19.7 ± 0.6	22.3 ± 0.9	28.5 ± 5.0	
Cases/person-years	3,628/313,107	3,230/326,656	3,081/332,941	3,021/338,691	2,882/342,627	
Model 1	Ref	0.88 (0.84–0.92)	0.84 (0.80–0.88)	0.82 (0.78–0.86)	0.77 (0.73–0.81)	<0.001
Model 2	Ref	0.92 (0.87–0.96)	0.87 (0.83–0.91)	0.84 (0.80–0.89)	0.78 (0.75–0.83)	<0.001
Model 3	Ref	0.93 (0.88–0.97)	0.89 (0.84–0.93)	0.87 (0.83–0.92)	0.82 (0.78–0.86)	<0.001
Model 4	Ref	1.00 (0.95–1.05)	1.00 (0.94–1.05)	1.02 (0.96–1.09)	1.04 (0.96–1.13)	0.34
Red meat						
Daily intake, g/day	6.7 ± 3.8	18.6 ± 3.3	31.3 ± 4.3	50.5 ± 7.2	98.5 ± 37.4	
Cases/person-years	2,679/331,140	2,997/331,851	3,097/332,529	3,310/331,514	3,759/326,988	
Model 1	Ref	1.13 (1.07–1.19)	1.18 (1.12–1.24)	1.27 (1.21–1.34)	1.43 (1.36–1.50)	<0.001
Model 2	Ref	1.09 (1.03–1.15)	1.12 (1.06–1.18)	1.17 (1.11–1.24)	1.27 (1.20–1.34)	<0.001
Model 3	Ref	1.05 (1.00–1.11)	1.06 (1.01–1.12)	1.10 (1.04–1.16)	1.15 (1.09–1.22)	<0.001
Model 4	Ref	1.03 (0.97–1.08)	1.00 (0.94–1.06)	1.00 (0.94–1.06)	0.97 (0.89–1.06)	0.59
Processed meat						
Daily intake, g/day	0.5 ± 0.6	3.2 ± 0.9	6.6 ± 1.2	12.7 ± 2.6	33.3 ± 16.9	
Cases/person-years	2,687/335,320	2,908/333,408	3,163/335,418	3,434/326,240	3,650/323,637	
Model 1	Ref	1.09 (1.03–1.14)	1.17 (1.11–1.23)	1.29 (1.23–1.36)	1.37 (1.30–1.44)	<0.001
Model 2	Ref	1.06 (1.00–1.12)	1.11 (1.06–1.17)	1.20 (1.13–1.26)	1.23 (1.16–1.30)	<0.001
Model 3	Ref	1.03 (0.97–1.08)	1.06 (1.00–1.12)	1.12 (1.06–1.18)	1.14 (1.08–1.20)	<0.001
Model 4	Ref	1.02 (0.97–1.07)	1.01 (0.96–1.07)	1.04 (0.98–1.10)	0.99 (0.92–1.07)	0.60
Poultry						
Daily intake, g/day	4.7 ± 2.5	11.7 ± 1.8	19.9 ± 3.4	31.9 ± 5.6	59.9 ± 21.9	
Cases/person-years	2,751/322,365	3,110/330,299	3,166/333,001	3,315/337,557	3,500/330,800	
Model 1	Ref	1.10 (1.05–1.16)	1.12 (1.06–1.18)	1.16 (1.10–1.22)	1.21 (1.15–1.27)	<0.001
Model 2	Ref	1.07 (1.02–1.13)	1.07 (1.02–1.13)	1.10 (1.04–1.16)	1.11 (1.05–1.17)	<0.001
Model 3	Ref	1.04 (0.99–1.10)	1.03 (0.98–1.09)	1.06 (1.01–1.12)	1.09 (1.03–1.15)	0.003
Model 4	Ref	1.03 (0.98–1.09)	1.00 (0.95–1.06)	1.01 (0.94–1.07)	0.97 (0.89–1.06)	0.72
Eggs						
Daily intake, g/day	1.5 ± 1.1	5.6 ± 1.3	9.8 ± 1.4	17.8 ± 3.5	42.8 ± 24.3	
Cases/person-years	2,844/336,357	2,872/336,758	3,146/336,639	3,224/330,310	3,756/313,959	
Model 1	Ref	1.00 (0.95–1.06)	1.09 (1.04–1.15)	1.15 (1.09–1.21)	1.38 (1.31–1.45)	<0.001
Model 2	Ref	0.99 (0.94–1.04)	1.05 (1.00–1.10)	1.11 (1.05–1.16)	1.27 (1.20–1.34)	<0.001
Model 3	Ref	0.97 (0.92–1.02)	1.01 (0.96–1.07)	1.07 (1.01–1.13)	1.21 (1.14–1.27)	<0.001
Model 4	Ref	0.94 (0.89–0.99)	0.95 (0.91–1.01)	1.00 (0.94–1.05)	1.03 (0.96–1.11)	0.29
High–*n*-3 seafood						
Daily intake, g/day	0.3 ± 0.3	2.6 ± 0.9	6.1 ± 1	11.1 ± 1.8	27.1 ± 15.5	
Cases/person-years	3,289/311,838	3,184/321,288	3,093/334,076	3,129/343,799	3,147/343,021	
Model 1	Ref	0.98 (0.93–1.03)	0.94 (0.90–0.99)	0.95 (0.90–1.00)	0.98 (0.94–1.04)	0.26
Model 2	Ref	0.99 (0.94–1.04)	0.96 (0.91–1.01)	0.97 (0.92–1.02)	1.01 (0.96–1.06)	0.86
Model 3	Ref	0.99 (0.94–1.04)	0.96 (0.91–1.01)	0.97 (0.92–1.02)	1.01 (0.96–1.07)	0.87
Model 4	Ref	0.99 (0.95–1.04)	0.98 (0.93–1.03)	0.99 (0.93–1.05)	1.02 (0.95–1.11)	0.95

Continued on p. 1748

Table 2—Continued

	Quintiles of dietary protein and food sources					
	1	2	3	4	5	*P* for trend
Low–*n*-3 seafood						
Daily intake, g/day	0.8 ± 0.7	3.2 ± 0.7	5.7 ± 0.9	9.4 ± 1.3	20.6 ± 11.8	
Cases/person-years	2,807/322,442	3,067/332,773	3,071/335,496	3,300/337,318	3,597/325,993	
Model 1	Ref	1.08 (1.03–1.14)	1.07 (1.02–1.13)	1.14 (1.08–1.20)	1.25 (1.19–1.31)	<0.001
Model 2	Ref	1.08 (1.02–1.13)	1.05 (1.00–1.10)	1.10 (1.05–1.16)	1.17 (1.11–1.24)	<0.001
Model 3	Ref	1.07 (1.01–1.12)	1.03 (0.98–1.08)	1.07 (1.02–1.13)	1.12 (1.06–1.18)	<0.001
Model 4	Ref	1.06 (1.01–1.12)	1.01 (0.96–1.06)	1.03 (0.97–1.09)	0.99 (0.93–1.07)	0.75
Cheese						
Daily intake, g/day	18 ± 10.7	49.1 ± 8.4	81.9 ± 10.8	131.9 ± 19.4	273.3 ± 117.3	
Cases/person-years	2,968/308,884	3,089/328,020	3,115/337,099	3,344/339,742	3,326/340,278	
Model 1	Ref	1.04 (0.99–1.10)	1.05 (1.00–1.11)	1.15 (1.09–1.21)	1.14 (1.09–1.20)	<0.001
Model 2	Ref	1.02 (0.96–1.07)	1.02 (0.96–1.07)	1.09 (1.03–1.14)	1.04 (0.99–1.11)	0.02
Model 3	Ref	1.00 (0.95–1.06)	1.00 (0.95–1.05)	1.06 (1.00–1.12)	1.02 (0.97–1.09)	0.11
Model 4	Ref	0.99 (0.94–1.04)	0.98 (0.92–1.03)	1.04 (0.98–1.10)	1.00 (0.92–1.10)	0.41
Milk						
Daily intake, g/day	35.1 ± 17.8	96.9 ± 18.8	167.2 ± 23.5	276.9 ± 40	603.3 ± 264.2	
Cases/person-years	3,179/320,510	3,202/329,166	3,109/333,262	3,211/334,186	3,141/336,897	
Model 1	Ref	1.01 (0.96–1.06)	0.99 (0.94–1.04)	1.04 (0.99–1.09)	1.04 (0.98–1.09)	0.09
Model 2	Ref	0.97 (0.92–1.02)	0.93 (0.89–0.98)	0.95 (0.90–1.00)	0.91 (0.86–0.96)	0.002
Model 3	Ref	0.98 (0.93–1.03)	0.96 (0.91–1.01)	0.99 (0.94–1.04)	0.98 (0.93–1.04)	0.78
Model 4	Ref	0.98 (0.93–1.03)	0.96 (0.91–1.02)	0.98 (0.92–1.04)	0.98 (0.90–1.07)	0.51
Yogurt						
Daily intake, g/day	0 ± 0	0.4 ± 1.1	12.3 ± 4.7	42.4 ± 16.2	149.8 ± 85.5	
Cases/person-years	3,249/314,010	3,248/316,639	3,231/334,222	3,162/341,702	2,952/347,449	
Model 1	Ref	0.99 (0.94–1.04)	0.97 (0.92–1.02)	0.95 (0.90–1.00)	0.89 (0.85–0.94)	<0.001
Model 2	Ref	1.00 (0.95–1.05)	0.99 (0.94–1.04)	0.97 (0.92–1.01)	0.91 (0.86–0.95)	<0.001
Model 3	Ref	1.00 (0.95–1.05)	1.02 (0.97–1.07)	1.00 (0.96–1.06)	0.98 (0.93–1.03)	0.63
Model 4	Ref	0.99 (0.95–1.04)	1.01 (0.96–1.06)	1.00 (0.95–1.05)	0.98 (0.90–1.06)	0.95
Whole grains						
Daily intake, g/day	6.3 ± 3.2	17.2 ± 3.1	28.2 ± 3.3	41.9 ± 4.9	78.5 ± 28.2	
Cases/person-years	3,372/308,523	3,193/326,973	3,160/334,239	3,139/340,953	2,978/343,334	
Model 1	Ref	0.92 (0.88–0.97)	0.92 (0.87–0.96)	0.90 (0.86–0.95)	0.84 (0.80–0.89)	<0.001
Model 2	Ref	0.91 (0.86–0.95)	0.90 (0.85–0.94)	0.86 (0.82–0.90)	0.77 (0.73–0.81)	<0.001
Model 3	Ref	0.93 (0.88–0.97)	0.93 (0.88–0.97)	0.90 (0.86–0.95)	0.84 (0.79–0.88)	<0.001
Model 4	Ref	0.95 (0.90–1.00)	0.96 (0.90–1.01)	0.95 (0.89–1.02)	0.92 (0.83–1.02)	0.17
Legumes						
Daily intake, g/day	1.2 ± 1.3	6.3 ± 1.6	12.7 ± 2.1	22.4 ± 3.9	58.9 ± 36.6	
Cases/person-years	3,191/319,639	3,158/326,702	3,142/331,320	3,161/338,060	3,190/338,301	
Model 1	Ref	0.96 (0.91–1.00)	0.96 (0.92–1.01)	0.96 (0.91–1.01)	0.97 (0.92–1.02)	0.25
Model 2	Ref	0.95 (0.91–1.00)	0.95 (0.91–1.00)	0.94 (0.89–0.99)	0.93 (0.88–0.98)	0.007
Model 3	Ref	0.96 (0.91–1.01)	0.97 (0.92–1.02)	0.96 (0.92–1.01)	0.98 (0.93–1.04)	0.60
Model 4	Ref	0.97 (0.92–1.02)	0.98 (0.93–1.03)	0.96 (0.91–1.02)	0.95 (0.88–1.02)	0.17
Nuts						
Daily intake, g/day	0 ± 0	1.7 ± 0.5	4.3 ± 0.8	9.9 ± 3.1	34.5 ± 21.6	
Cases/person-years	3,315/317,036	3,099/328,827	3,266/336,047	3,097/337,094	3,065/335,019	
Model 1	Ref	0.91 (0.87–0.96)	0.95 (0.90–1.00)	0.90 (0.86–0.95)	0.90 (0.85–0.94)	<0.001
Model 2	Ref	0.91 (0.86–0.95)	0.93 (0.89–0.98)	0.88 (0.83–0.92)	0.85 (0.81–0.90)	<0.001
Model 3	Ref	0.91 (0.87–0.95)	0.94 (0.89–0.98)	0.89 (0.85–0.94)	0.90 (0.86–0.95)	<0.001
Model 4	Ref	0.92 (0.87–0.96)	0.95 (0.90–1.00)	0.91 (0.86–0.96)	0.90 (0.83–0.97)	0.002

Data for daily intakes of protein and food sources are shown as mean ± SD. Model 1: adjustment for age, study group indicator, self-identified race/ethnicity, region of residence at baseline, family income, education, and family history of diabetes. Model 2: model 1 adjustments plus smoking status, alcohol intake, physical activity, hormone replacement therapy, multivitamin use, antihypertensive medication use, and total energy intake. Model 3 (full model): model 2 adjustments plus modified AHEI-2010 score and intake of other dietary protein sources. Model 4 (sensitivity model): model 3 adjustments plus BMI and WHR. Ref, reference.

(CKD) study of ∼0.5 million Chinese adults, published after the aforementioned meta-analysis, findings showed no association between poultry intake and T2D and a positive association between total fish intake and T2D (35). In our study, we found that intake of low–n-3 seafood, but not high–n-3 seafood, was associated with increased risk of T2D, suggesting that the association between seafood intake and T2D risk may be dependent on n-3 content in seafood,

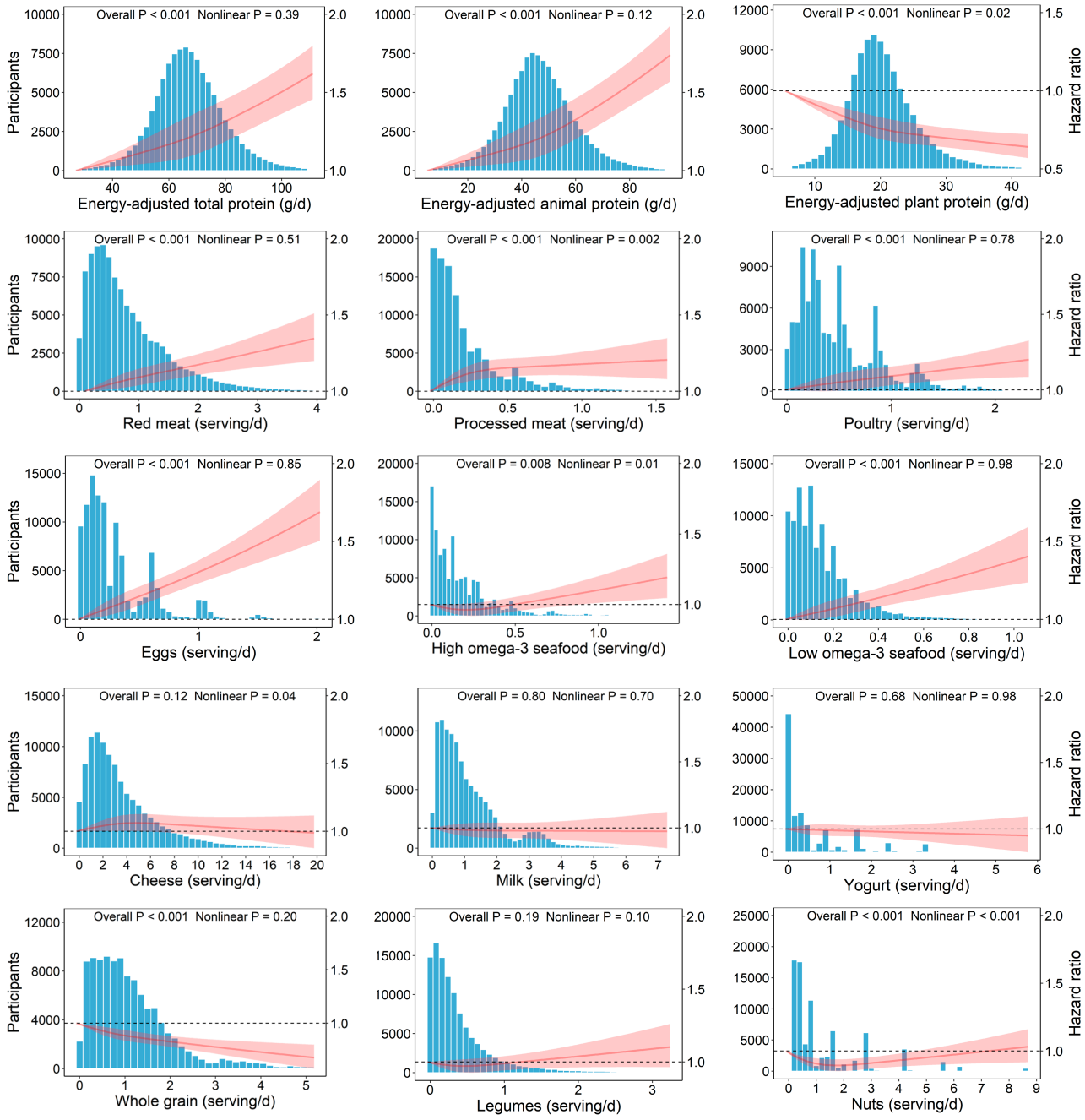

Figure 1—Associations of dietary protein and food sources with risk of incident T2D among 108,681 postmenopausal women in the WHI. Cox proportional hazards models were used for analysis, including restricted cubic spline term for each protein source, with adjustment for age, study group indicator, self-identified race/ethnicity, region of residence, family income, education, family history of diabetes, smoking status, alcohol intake, physical activity, hormone replacement therapy, multivitamin use, antihypertensive medication use, total energy intake, modified AHEI-2010 score, and intake of other dietary protein sources. d, day; serving/d, servings per day.

which may explain the discrepancy in reported associations with fish intake.

Additionally, our substitution analysis confirmed the reduction of T2D risk associated with replacing animal protein sources, such as red meat, processed meat, poultry, or eggs, with plant protein sources, milk, yogurt, cheese, or high–*n*-3 seafood. These findings are consistent with the previous studies showing beneficial associations of protein sources substitution with T2D risk, although which focused mainly on the replacement of red meat with other foods (42–44). Our findings are also consistent with previous research showing that adherence to a plant-based dietary pattern may lower T2D risk (45) and cardiovascular disease (46) and support the 2021 dietary guidelines for improving cardiovascular health from the American Heart Association (47). These observed protein-T2D associations were materially attenuated if we additionally adjusted for BMI and WHR. Dietary data collected with an FFQ reflect one's long-term nutritional status. In our study, dietary intake and BMI were assessed at baseline simultaneously. The possibilities of BMI being a cause and effect of the measured dietary intake exist.

Table 3—Associations of substitution of different protein and food sources with risk of incident T2D in the WHI and the UKB

Substituted protein	Equivalent amount of substituted protein	WHI	UKB
5% of energy from animal protein	Plant protein	0.79 (0.74–0.84)	NA
1 serving/day red meat	Whole grains	0.87 (0.85–0.90)	0.80 (0.71–0.89)
	Nuts	0.92 (0.90–0.94)	0.81 (0.71–0.91)
	Legumes	0.95 (0.91–0.99)	0.91 (0.75–1.10)
	Yogurt	0.92 (0.89–0.94)	0.80 (0.71–0.90)
	Milk	0.92 (0.90–0.95)	0.97 (0.83–1.15)
	Cheese	0.92 (0.90–0.95)	0.78 (0.65–0.92)
	High–*n*-3 seafood	0.97 (0.90–1.04)	0.82 (0.66–1.02)
	Low–*n*-3 seafood	1.15 (1.06–1.25)	0.82 (0.68–0.98)
1 serving/day processed meat	Whole grains	0.79 (0.75–0.84)	0.70 (0.59–0.83)
	Nuts	0.84 (0.79–0.89)	0.71 (0.59–0.85)
	Legumes	0.86 (0.81–0.92)	0.80 (0.62–1.02)
	Yogurt	0.83 (0.79–0.88)	0.70 (0.59–0.84)
	Milk	0.84 (0.79–0.89)	0.86 (0.70–1.06)
	Cheese	0.84 (0.80–0.89)	0.68 (0.55–0.85)
	High–*n*-3 seafood	0.88 (0.81–0.97)	0.72 (0.56–0.93)
	Low–*n*-3 seafood	1.05 (0.95–1.16)	0.72 (0.57–0.91)
1 serving/day eggs	Whole grains	0.78 (0.75–0.81)	0.84 (0.73–0.96)
	Nuts	0.82 (0.79–0.86)	0.85 (0.73–0.99)
	Legumes	0.85 (0.81–0.89)	0.96 (0.77–1.19)
	Yogurt	0.82 (0.79–0.85)	0.84 (0.73–0.97)
	Milk	0.82 (0.79–0.86)	1.03 (0.86–1.23)
	Cheese	0.83 (0.80–0.86)	0.82 (0.67–1.00)
	High–*n*-3 seafood	0.87 (0.80–0.94)	0.86 (0.67–1.10)
	Low–*n*-3 seafood	1.03 (0.94–1.13)	0.86 (0.70–1.07)
1 serving/day poultry	Whole grains	0.87 (0.84–0.91)	0.84 (0.75–0.93)
	Nuts	0.92 (0.89–0.96)	0.84 (0.75–0.96)
	Legumes	0.95 (0.91–1.00)	0.95 (0.78–1.16)
	Yogurt	0.92 (0.88–0.96)	0.84 (0.74–0.94)
	Milk	0.93 (0.89–0.96)	1.02 (0.87–1.20)
	Cheese	0.93 (0.89–0.96)	0.81 (0.69–0.96)
	High–*n*-3 seafood	0.97 (0.89–1.06)	0.86 (0.69–1.07)
	Low–*n*-3 seafood	1.16 (1.05–1.27)	0.86 (0.71–1.04)

Data are HR (95% CI). One serving size was defined as 50 g/day for red meat, processed meat, eggs, high–*n*-3 seafood, low–*n*-3 seafood, and legumes; 30 g/day for cheese and whole grains; 200 g/day for milk; 70 g/day for yogurt; and 10 g/day for nuts. In the WHI, the substitution analysis models included adjustment for age, study group indicator, self-identified race/ethnicity, region of residence, family income, education, and family history of diabetes, smoking status, alcohol intake, physical activity, hormone replacement therapy, multivitamin use, antihypertensive medication use, total energy intake, modified AHEI-2010 score, and intake of other protein sources. In the UKB, the substitution analyses included adjustment for age, sex, residence area, Townsend deprivation index at recruitment, smoking status, drinking status, physical activity, family history of diabetes, multivitamin use, antihypertensive medication treatment, total energy intake, and other protein sources. NA: In the UKB, only total protein was calculated and released. The variables of animal protein and plant protein intake were not available in the UKB database; therefore, the according substitution analysis cannot be conducted.

Therefore, BMI could be both a confounder and mediator in the protein-T2D relation; hence, the additional adjustment for BMI and WHR that attenuated the protein-T2D association.

The mechanisms underlying the observed beneficial association between substituting plant protein sources for animal protein sources and T2D risk remain to be determined. Previous studies have implicated red meat intake in accumulation of abdominal fat (48,49) and higher serum ferritin, amino acids, and lipid metabolites (42). In mediation analysis, we examined well-characterized T2D-related biomarkers, including inflammation factors, endothelial dysfunction, sex steroids and SHBG, leptin and its soluble receptor, and cellular aging (24). Our isocaloric substitution analysis indicated T2D risk reduction to be associated with replacing 5% energy from animal protein with plant protein, which was mediated mainly by inflammation factors, followed by leptin, endothelial dysfunction biomarkers, and SHBG. Recent advances in gut microbiota also support the mediating role of inflammation in the dietary protein–T2D association. Dietary factors can modulate the gut microbial composition and then alter intestinal permeability, leading to lipopolysaccharides leakage and inflammatory activation through toll-like receptors (50). In addition, intake of red meat, which is particularly high in L-carnitine, was associated with elevated levels of inflammatory trimethylamine *N*-oxide, which may increase risk of cardiometabolic diseases (51).

The mediating effects of these biomarkers were decreased after additional adjustment for BMI in the mediation analyses. This is consistent with other reports indicating the mediating and confounding roles of BMI in the relations of inflammation and leptin to metabolic diseases (52,53). At a population level, BMI is a reliable measure that captures the biological actions of adipose tissues, including inflammatory processes and leptin's action. Therefore, it is no surprise that the proportion mediated by inflammation and leptin diminished with additional adjustment for BMI in our study.

The current study has several strengths, including analysis of a large prospective national cohort of multiethnic postmenopausal women with long-term follow-up, with replication in a large independent cohort of men and women; a comprehensive investigation of 12 major sources of dietary protein, with possible substitution combinations—which is beneficial for identifying optimal T2D dietary intervention strategies; and nested case-control studies in the WHI allowing for the comprehensive examination of the potential mechanistic mediators that may explain the specific protein-T2D relations observed. Our findings revealed novel mechanisms underlying the beneficial association between substituting plant protein sources for animal protein sources and decreased risk of T2D, which is helpful for the development of a mechanism-based dietary intervention strategy for the prevention and management of T2D.

Nevertheless, there are limitations that should be considered in interpreting our findings. First, self-reported dietary data are prone to measurement error (both

Table 4—Mediating effects of biomarkers on the association of isocaloric substitution of plant protein for animal protein with risk of T2D among postmenopausal women enrolled in nested case-control studies in the WHI

	Substituting 5% energy from plant protein for 5% energy from animal protein → T2D				
Mediators	*N*	Total effect	Indirect effect	Direct effect	Proportion mediated
BMI	3,151	0.78 (0.55–1.07)	0.73 (0.65–0.81)	1.06 (0.74–1.52)	NA
WHR	3,175	0.80 (0.57–1.14)	0.83 (0.72–0.95)	0.97 (0.69–1.39)	65
Inflammation factors					
TNFα-R2	2,771	0.82 (0.59–1.15)	0.97 (0.90–1.02)	0.85 (0.61–1.21)	10
IL-6	2,780	0.81 (0.57–1.15)	0.87 (0.79–0.95)	0.93 (0.65–1.34)	47
hs-CRP	2,786	0.77 (0.52–1.09)	0.80 (0.72–0.89)	0.97 (0.66–1.35)	77
Inflammation score	2,769	0.79 (0.56–1.12)	0.88 (0.79–0.97)	0.91 (0.64–1.27)	45
Endothelial dysfunction					
VCAM-1	2,778	0.84 (0.60–1.19)	1.01 (0.98–1.05)	0.82 (0.59–1.17)	NA
E-selectin	2,772	0.88 (0.62–1.26)	0.92 (0.83–1.02)	0.95 (0.68–1.37)	30
SICAM-1	2,752	0.89 (0.65–1.24)	1.04 (0.95–1.14)	0.85 (0.61–1.22)	NA
Sex steroids and SHBG					
Estradiol	1,402	0.81 (0.52–1.22)	0.99 (0.96–1.01)	0.82 (0.53–1.23)	2
Testosterone	1,402	0.80 (0.51–1.22)	0.98 (0.93–1.03)	0.81 (0.52–1.24)	4
SHBG	1,402	0.81 (0.50–1.23)	0.85 (0.74–0.98)	0.94 (0.59–1.46)	46
Leptin and leptin receptor					
Leptin	1,403	0.78 (0.51–1.20)	0.80 (0.70–0.89)	0.98 (0.65–1.48)	66
Soluble leptin receptor	1,403	0.81 (0.54–1.23)	0.97 (0.92–1.01)	0.84 (0.55–1.27)	7
Cellular aging: leukocyte telomere length	3,101	0.80 (0.58–1.09)	1.00 (0.98–1.01)	0.80 (0.58–1.10)	1

Data are OR (95% CI) or % unless otherwise indicated. NA: proportion mediated was not calculated when the point estimate of the direct effect was in a direction oppositive to that of the indirect effect. The covariates adjusted for in the mediation analyses included age, ethnicity, education, smoking, drinking, and physical activity. We calculated inflammation score by summing up inflammation markers above the median value.

random and systematic) (54), even with extensive validation effort of FFQ with use of biomarkers in WHI and multiple records used in the UKB to reduce errors. One has to assume that participants' dietary habits did not change materially during the follow-up; otherwise, the occurrence of such changes could introduce bias. Second, the observed beneficial associations of food substitution with T2D were simulated based on statistical modeling, which may be different from interventions with real foods in an experimental setting. Third, although our analyses included adjustment for known confounding factors, some residual confounding could still exist. For example, WHI dietary data were collected in the 1990s, while UKB's were from the 2010s, which may lead to higher exposure to ultraprocessed foods (UPFs) in the more recent UKB as sales of UPFs have been increasing globally (55–57). Given lack of adjustment for UPFs in our analyses, there may have been differences in the estimated associations between the WHI and the UKB. Finally, the majority of both the WHI and UKB participants were White, which may limit the generalizability of our findings to other populations, although a relatively homogenous population does enhance the internal validity of the relations observed.

In conclusion, in these two large prospective cohort studies, intake of animal protein sources, such as red meat, processed meat, poultry, and eggs, was associated with increased T2D risk, whereas intake of plant protein sources, especially whole grains and nuts, was associated with decreased risk. Substituting the unfavorable animal protein sources as determined in this study with plant protein sources, milk, yogurt, cheese, or high–*n*-3 seafood was associated with lower risk of T2D. The beneficial association of isocaloric substitution of plant protein for animal protein was mechanistically mediated mainly by obesity-related inflammation. These findings support the recommendation that dietary protein sources should be given attention for the prevention of T2D.

Acknowledgments. The authors appreciate all WHI and UKB participants and all staff for their contribution to these studies.

Funding. J.L. was supported by the National Natural Science Foundation of China (81673156, 82073528, and 81302417). S.L. was partly supported by the National Institutes of Health (NIH) grants R01DK125403, R01ES031391, and R01ES029082. J.B. was supported by NIH grant R01DK127916. A.J.G. was supported by the Canadian Institute of Health Research, CIHR Fellowship. The WHI program is funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts 75N92021D00001, 75N92021D00002, 75N92021D00003, 75N92021D00004, and 75N92021D00005.

The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the manuscript.

Duality of Interest. A.J.G. has received consulting fees from Solo GI Nutrition and has received an honorarium from the Soy Nutrition Institute. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. J.L. and S.L. designed the research. J.L., A.J.G., Q.Y., D.D. and L.Z. conducted statistical analysis. J.L. and S.L. led the writing of the manuscript. 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. J.L. and S.L. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Hu FB, Manson JE, Stampfer MJ, et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med 2001;345:790–797
2. Khazrai YM, Defeudis G, Pozzilli P. Effect of diet on type 2 diabetes mellitus: a review. Diabetes Metab Res Rev 2014;30(Suppl. 1):24–33
3. Ludwig DS, Willett WC, Volek JS, Neuhouser ML. Dietary fat: From foe to friend? Science 2018;362: 764–770
4. Sluijs I, Beulens JW, van der A DL, Spijkerman AM, Grobbee DE, van der Schouw YT. Dietary intake of total, animal, and vegetable protein and risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL study. Diabetes Care 2010;33:43–48
5. Malik VS, Li Y, Tobias DK, Pan A, Hu FB. Dietary protein intake and risk of type 2 diabetes in US men and women. Am J Epidemiol 2016;183:715–728
6. Viguiliouk E, Glenn AJ, Nishi SK, et al. Associations between dietary pulses alone or with other legumes and cardiometabolic disease outcomes: an umbrella review and updated systematic review and meta-analysis of prospective cohort studies. Adv Nutr 2019;10(Suppl. 4): S308–S319
7. Pearce M, Fanidi A, Bishop TRP, et al.; EPIC-InterAct Consortium. Associations of total legume, pulse, and soy consumption with incident type 2 diabetes: federated meta-analysis of 27 studies from diverse world regions. J Nutr 2021;151: 1231–1240
8. Zhao LG, Zhang QL, Liu XL, Wu H, Zheng JL, Xiang YB. Dietary protein intake and risk of type 2 diabetes: a dose-response meta-analysis of prospective studies. Eur J Nutr 2019;58:1351–1367
9. Pan A, Sun Q, Bernstein AM, Manson JE, Willett WC, Hu FB. Changes in red meat consumption and subsequent risk of type 2 diabetes mellitus: three cohorts of US men and women. JAMA Intern Med 2013;173:1328–1335
10. Drouin-Chartier JP, Schwab AL, Chen S, et al. Egg consumption and risk of type 2 diabetes: findings from 3 large US cohort studies of men and women and a systematic review and meta-analysis of prospective cohort studies. Am J Clin Nutr 2020;112:619–630
11. Ford ES, Williamson DF, Liu S. Weight change and diabetes incidence: findings from a national cohort of US adults. Am J Epidemiol 1997;146:214–222
12. Liu S, Tinker L, Song Y, et al. A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. Arch Intern Med 2007;167:1676–1685
13. Song Y, Manson JE, Tinker L, et al. Circulating levels of endothelial adhesion molecules and risk of diabetes in an ethnically diverse cohort of women. Diabetes 2007;56:1898–1904
14. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2006;295:1288–1299
15. Ding EL, Song Y, Manson JE, et al. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. N Engl J Med 2009;361:1152–1163
16. Chen BH, Brennan K, Goto A, et al. Sex hormone-binding globulin and risk of clinical diabetes in American black, Hispanic, and Asian/ Pacific Islander postmenopausal women. Clin Chem 2012;58:1457–1466
17. Liu S, Sun Q. Sex differences, endogenous sex-hormone hormones, sex-hormone binding globulin, and exogenous disruptors in diabetes and related metabolic outcomes. J Diabetes 2018;10:428–441
18. You NC, Chen BH, Song Y, et al. A prospective study of leukocyte telomere length and risk of type 2 diabetes in postmenopausal women. Diabetes 2012;61:2998–3004
19. Shen Q, Zhao X, Yu L, et al. Association of leukocyte telomere length with type 2 diabetes in mainland Chinese populations. J Clin Endocrinol Metab 2012;97:1371–1374
20. Azadbakht L, Kimiagar M, Mehrabi Y, Esmaillzadeh A, Hu FB, Willett WC. Soy consumption, markers of inflammation, and endothelial function: a cross-over study in postmenopausal women with the metabolic syndrome. Diabetes Care 2007;30:967–973
21. Barak F, Falahi E, Keshteli AH, Yazdannik A, Saneei P, Esmaillzadeh A. Red meat intake, insulin resistance, and markers of endothelial function among Iranian women. Mol Nutr Food Res 2015;59:315–322
22. Brinkman MT, Baglietto L, Krishnan K, et al. Consumption of animal products, their nutrient components and postmenopausal circulating steroid hormone concentrations. Eur J Clin Nutr 2010;64:176–183
23. Zhou M, Zhu L, Cui X, et al. Influence of diet on leukocyte telomere length, markers of inflammation and oxidative stress in individuals with varied glucose tolerance: a Chinese population study. Nutr J 2016;15:39
24. Song Y, Huang YT, Song Y, et al. Birthweight, mediating biomarkers and the development of type 2 diabetes later in life: a prospective study of multi-ethnic women. Diabetologia 2015;58:1220–1230
25. Patterson RE, Kristal AR, Tinker LF, Carter RA, Bolton MP, Agurs-Collins T. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. Ann Epidemiol 1999;9: 178–187
26. Bowman SA, Friday JE, Moshfegh AJ. MyPyramid Equivalents Database, 2.0 for USDA Survey Foods, 2003-2004. U.S. Department of Agriculture, 2008. Accessed 13 June 2022. Available from https://www.ars.usda.gov/ARSUserFiles/80400530/pdf/mped/mped2_doc.pdf
27. For the WHI FFQ: My Pyramid Equivalents Database 2.0 (MPED 2.0). Accessed 13 June 2022. Available from https://www.whi.org/doc/f60_MPEDs_v2_ReadMe_inv-FINAL.pdf
28. Halkjaer J, Olsen A, Bjerregaard LJ, et al. Intake of total, animal and plant proteins, and their food sources in 10 countries in the European Prospective Investigation into Cancer and Nutrition. Eur J Clin Nutr 2009;63(Suppl. 4):S16–S36
29. Margolis KL, Lihong Qi, Brzyski R, et al.; Women Health Initiative Investigators. Validity of diabetes self-reports in the Women's Health Initiative: comparison with medication inventories and fasting glucose measurements. Clin Trials 2008;5:240–247
30. Chiuve SE, Fung TT, Rimm EB, et al. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr 2012;142:1009–1018
31. Langer RD, White E, Lewis CE, Kotchen JM, Hendrix SL, Trevisan M. The Women's Health Initiative Observational Study: baseline characteristics of participants and reliability of baseline measures. Ann Epidemiol 2003;13(Suppl.):S107–S121
32. VanderWeele TJ. Mediation analysis: a practitioner's guide. Annu Rev Public Health 2016;37:17–32
33. Gong JH, Lo K, Liu Q, et al. Dietary manganese, plasma markers of inflammation, and the development of type 2 diabetes in postmenopausal women: findings from the Women's Health Initiative. Diabetes Care 2020;43:1344–1351
34. Duncan BB, Schmidt MI, Pankow JS, et al.; Atherosclerosis Risk in Communities Study. Low-grade systemic inflammation and the development of type 2 diabetes: the Atherosclerosis Risk in Communities study. Diabetes 2003;52:1799–1805
35. Du H, Guo Y, Bennett DA, et al.; China Kadoorie Biobank collaborative group. Red meat, poultry and fish consumption and risk of diabetes: a 9 year prospective cohort study of the China Kadoorie Biobank. Diabetologia 2020;63:767–779
36. Ericson U, Sonestedt E, Gullberg B, et al. High intakes of protein and processed meat associate with increased incidence of type 2 diabetes. Br J Nutr 2013;109:1143–1153
37. Aune D, Norat T, Romundstad P, Vatten LJ. Whole grain and refined grain consumption and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies. Eur J Epidemiol 2013;28:845–858
38. Parker ED, Liu S, Van Horn L, et al. The association of whole grain consumption with incident type 2 diabetes: the Women's Health Initiative Observational Study. Ann Epidemiol 2013;23:321–327
39. Gijsbers L, Ding EL, Malik VS, de Goede J, Geleijnse JM, Soedamah-Muthu SS. Consumption of dairy foods and diabetes incidence: a dose-response meta-analysis of observational studies. Am J Clin Nutr 2016;103:1111–1124
40. Margolis KL, Wei F, de Boer IH, et al.; Women's Health Initiative Investigators. A diet high in low-fat dairy products lowers diabetes risk in postmenopausal women. J Nutr 2011;141:1969–1974
41. Yang X, Li Y, Wang C, et al. Meat and fish intake and type 2 diabetes: dose-response meta-analysis of prospective cohort studies. Diabetes Metab 2020;46:345–352
42. Ibsen DB, Steur M, Imamura F, et al. Replacement of red and processed meat with other food sources of protein and the risk of type 2 diabetes in European Populations: the EPIC-InterAct Study. Diabetes Care 2020;43:2660–2667
43. Würtz AML, Jakobsen MU, Bertoia ML, et al. Replacing the consumption of red meat with other major dietary protein sources and risk of type 2 diabetes mellitus: a prospective cohort study. Am J Clin Nutr 2021;113:612–621
44. Ibsen DB, Jakobsen MU, Halkjær J, et al. Replacing red meat with other nonmeat food sources of protein is associated with a reduced risk of type 2 diabetes in a Danish cohort of middle-aged adults. J Nutr 2021;151:1241–1248
45. Chen Z, Drouin-Chartier JP, Li Y, et al. Changes in plant-based diet indices and subsequent risk of type 2 diabetes in women and men: three U.S. prospective cohorts. Diabetes Care 2021;44:663–671
46. Glenn AJ, Lo K, Jenkins DJA, et al. Relationship between a plant-based dietary portfolio and risk of cardiovascular disease: findings from the Women's Health Initiative

prospective cohort study. J Am Heart Assoc 2021;10:e021515
47. Lichtenstein AH, Appel LJ, Vadiveloo M, et al. 2021 dietary guidance to improve cardiovascular health: a scientific statement from the American Heart Association. Circulation 2021;144:e472–e487
48. Mazidi M, Kengne AP, George ES, Siervo M. The association of red meat intake with inflammation and circulating intermediate biomarkers of type 2 diabetes is mediated by central adiposity. Br J Nutr 2021;125:1043–1050
49. Chen Z, Franco OH, Lamballais S, et al. Associations of specific dietary protein with longitudinal insulin resistance, prediabetes and type 2 diabetes: The Rotterdam Study. Clin Nutr 2020;39:242–249
50. de Vos WM, Tilg H, Van Hul M, Cani PD. Gut microbiome and health: mechanistic insights. Gut 2022;71:1020–1032
51. Abbasi J. TMAO and heart disease: the new red meat risk? JAMA 2019;321:2149–2151
52. Duncan BB, Schmidt MI, Chambless LE, Folsom AR, Carpenter M, Heiss G. Fibrinogen, other putative markers of inflammation, and weight gain in middle-aged adults–the ARIC study. Atherosclerosis Risk in Communities. Obes Res 2000;8:279–286
53. Schmidt MI, Duncan BB, Vigo A, et al.; ARIC Investigators. Leptin and incident type 2 diabetes: risk or protection? Diabetologia 2006;49:2086–2096
54. Tinker LF, Sarto GE, Howard BV, et al. Biomarker-calibrated dietary energy and protein intake associations with diabetes risk among postmenopausal women from the Women's Health Initiative. Am J Clin Nutr 2011;94:1600–1606
55. Levy RB, Rauber F, Chang K, et al. Ultra-processed food consumption and type 2 diabetes incidence: a prospective cohort study. Clin Nutr 2021;40:3608–3614
56. Llavero-Valero M, Escalada-San Martín J, Martínez-González MA, Basterra-Gortari FJ, de la Fuente-Arrillaga C, Bes-Rastrollo M. Ultra-processed foods and type-2 diabetes risk in the SUN project: a prospective cohort study. Clin Nutr 2021;40:2817–2824
57. Srour B, Fezeu LK, Kesse-Guyot E, et al. Ultraprocessed food consumption and risk of type 2 diabetes among participants of the NutriNet-Santé prospective cohort. JAMA Intern Med 2020;180:283–291