Dietary Manganese, Plasma Markers of Inflammation, and the Development of Type 2 Diabetes in Postmenopausal Women: Findings From the Women's Health Initiative

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OBJECTIVE

To examine the association between manganese intake and the risk of type 2 diabetes in postmenopausal women and determine whether this association is mediated by circulating markers of inflammation.

RESEARCH DESIGN AND METHODS

We included 84,285 postmenopausal women without a history of diabetes from the national Women's Health Initiative Observational Study (WHI-OS). Replication analysis was then conducted among 62,338 women who participated in the WHI-Clinical Trial (WHI-CT). Additionally, data from a case-control study of 3,749 women nested in the WHI-OS with information on biomarkers of inflammation and endothelial dysfunction were examined using mediation analysis to determine the relative contributions of these known biomarkers by which manganese affects type 2 diabetes risk.

RESULTS

Compared with the lowest quintile of energy-adjusted dietary manganese, WHI-OS participants in the highest quintile had a 30% lower risk of type 2 diabetes (hazard ratio [HR] 0.70 [95% CI 0.65, 0.76]). A consistent association was also confirmed in the WHI-CT (HR 0.79 [95% CI 0.73, 0.85]). In the nested case-control study, higher energy-adjusted dietary manganese was associated with lower circulating levels of inflammatory biomarkers that significantly mediated the association between dietary manganese and type 2 diabetes risk. Specifically, 19% and 12% of type 2 diabetes risk due to manganese were mediated through interleukin 6 and hs-CRP, respectively.

CONCLUSIONS

Higher intake of manganese was directly associated with a lower type 2 diabetes risk independent of known risk factors. This association may be partially mediated by inflammatory biomarkers.

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Manganese is an essential element mainly obtained from nuts, grains, fruits, green vegetables, and caffeinated drinks (1,2). Manganese plays significant roles in multiple physiological functions, including glucose and lipid metabolism and insulin production and secretion (3). Manganese deficiency leads to impaired glucose tolerance and increased risk of metabolic syndrome through impaired glucose and lipid metabolism (3,4). Manganese deficiency also leads to mitochondrial oxidative stress by increasing the production of reactive oxygen species (ROS) (4), a group of physiological products that contribute to inflammation and endothelial dysfunction (5–8).

A mouse study demonstrated that a manganese-rich diet can downregulate ROS generation (9), while in vitro and animal studies demonstrated reduced endothelial dysfunction followed by manganese supplementation (10). Another study reported that manganese supplementation reduced levels of inflammatory biomarkers in blood in rats (11). These studies have provided a physiological basis for examining manganese in diabetes prevention. To our knowledge, there is paucity of research on the association between dietary manganese and type 2 diabetes. A study of two Chinese prospective cohorts suggested that greater dietary manganese intake was associated with lower risk of type 2 diabetes (12).

Association between dietary manganese and type 2 diabetes in a large cohort of postmenopausal women has not yet been investigated. Postmenopausal women are associated with a higher risk of type 2 diabetes compared with premenopausal women (13), and pathogenesis of menopausal symptoms has been reported as the result of oxidative stress and ROS generation (14). In the current study, we sought to prospectively investigate the association between dietary manganese and type 2 diabetes risk in a large cohort of postmenopausal women enrolled in the Women's Health Initiative (WHI) and to explore potential explanations for this association using dietary antioxidant quality, dietary mineral intakes, and inflammatory biomarkers.

RESEARCH DESIGN AND METHODS

Study Population

The WHI Observational Study (WHI-OS) included 93,676 women aged 50–79 enrolled during 1993–1998. Detailed study procedures have been previously described (15). WHI-OS participants were monitored for an average of 10.8 years until 30 September 2010. Participants were selected at baseline based on the following criteria: *1*) no history of diabetes, *2*) no baseline antidiabetic medication use, *3*) complete information of food frequency questionnaires at baseline, and *4*) plausible baseline total energy intake (between 600 and 5,000 kcal/day). Among the participants enrolled in the WHI-OS, 84,285 met the criteria (Supplementary Fig. 1). Missing values for covariates were imputed using multivariate imputation by chained equations (16). Participants enrolled in the WHI Clinical Trial (WHI-CT) were included for a replication analysis. WHI-CT consisted of three overlapping components: Hormone Therapy Trial, Dietary Modification Trial, and Calcium and Vitamin D Trial. Among the 68,132 participants enrolled in the WHI-CT, 62,338 met the selection criteria (Supplementary Fig. 2).

Nested Case-Control Study

In addition to analyzing data from the main WHI-OS and CT, we used data from a case-control study nested within WHI-OS to explore whether the association between dietary manganese and type 2 diabetes was mediated by inflammatory processes or endothelial dysfunction. Among all participants from the WHI-OS, 3,781 were included in the nested case-control study designed to examine the role of inflammatory biomarkers and endothelial dysfunction in the risk of type 2 diabetes. Control subjects were matched to case subjects by age ($\pm$2.5 years), racial/ethnic group (white/Caucasian, black/African, Hispanic/Latino, and Asian/Pacific Islander), clinical center (geographic location), time of blood draw ($\pm$0.10 h), and the length of follow-up. We included these participants to assess the role of insulin resistance, β-cell function, inflammatory biomarkers, and endothelial dysfunction in the association between dietary manganese and type 2 diabetes. Among these participants, 3,749 (2,041 control subjects and 1,708 case subjects with type 2 diabetes) with complete data for fasting glucose, fasting insulin, HOMA-insulin resistance (IR), HOMA-β, vascular cell adhesion molecule 1 (VCAM1), tumor necrosis factor (TNF) receptor 2 (TNFR2), interleukin 6 (IL6), and hs-CRP were included in the analysis (Supplementary Fig. 1). Details of the study design have been published elsewhere (5,6). The prospective cohort and the nested case-control study were both reviewed and approved by human subjects review committees at each participating institution, and signed informed consent was obtained from all women enrolled.

Identification of Diabetes at Baseline and Follow-up Period

Diabetes was defined as a physician diagnosis of treated diabetes. Treated diabetes at baseline and during follow-up was identified by a self-administered questionnaire at each semiannual contact, "Since the date given on the front of this form, has a doctor prescribed any of the following pills or treatments?" where the choices were "pills for diabetes" and "insulin shots for diabetes." This identification method has been used in prior publications by the WHI investigators (5,17,18). A confirmation study on the WHI type 2 diabetes questionnaire showed that the prevalence and incidence of type 2 diabetes cases were consistent with medication inventories of oral pills and insulin shots for 77% of WHI-OS and for 79% of WHI-CT participants (19).

Measurement of Dietary Variables

At baseline, all WHI participants completed a semiquantitative food frequency questionnaire (FFQ), which was extensively validated using biomarkers and dietary records (20). The main section of the WHI FFQ includes 122 food groups reported in a semiquantitative manner in servings/day. Responses to questionnaire items ranged from "Never or less than once per month" to a certain number of servings per day depending on the food group. Nutrient analysis for the WHI was conducted at the Fred Hutchinson Cancer Research Center Nutrition Assessment Shared Resource using the Nutrition Data System for Research (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN). This study included the following dietary nutrients for analysis: manganese (mg/day), vitamin A (IU/day), vitamin C (mg/day), vitamin E (IU/day), zinc (mg/day), selenium (μg/day), calcium (mg/day), iron (mg/day), magnesium (mg/day), and alcohol (mg/day). Dietary manganese was used as the main exposure in the current study. A variable for dietary antioxidant quality score (DAQS)

was calculated (21,22) based on the dietary reference intakes of five dietary nutrients for women who were 51–70 years old—vitamin A (8 IU/day), vitamin C (75 mg/day), vitamin E (15 IU/day), zinc (8 mg/day), and selenium (55 μg/day) (23). Manganese supplementation information, assessed by in-person interviews and a computerized inventory method (24), was included for a sensitivity analysis, from which we computed the total manganese intake from diet and supplemental intake as exposure.

Other Covariates

At enrollment, all WHI-OS participants completed self-administered questionnaires that included demographic and lifestyle information. Race/ethnicity was self-reported as white (not of Hispanic origin), African American, Hispanic, American Indian or Alaskan Native, Asian/Pacific Islander, or unknown/none of the above. Region of residence in the United States at baseline was reported in four categories (Northeast, South, Midwest, or West). Family income was reported in nine categories (<$10,000, $10,000–$19,999, $20,000–$34,999, $35,000–$49,999, $50,000–$74,999, $75,000–$99,999, $100,000–$149,999, ≥$150,000, or "don't know"). Education was reported in 11 categories ("Didn't go to school," "Grade school (1–4 years)," "Grade school (5–8 years)," "Some high school (9–11 years)," "High school diploma or GED," "Vocational or training school," "Some college or Associate Degree," "College graduate or Baccalaureate Degree," "Some postgraduate or professional," "Master's Degree," or "Doctoral Degree"). Family history of diabetes was self-reported as the incidence of diabetes in a parent or sibling. During the initial screening visit, anthropometric measurements including weight and height, systolic and diastolic blood pressures, and fasting blood samples were collected. BMI was calculated as the ratio of weight (kg) to the square of height (m). Smoking status was self-reported as past smoker, current smoker, or never. Alcohol consumption (g/day) was calculated along with other dietary factors using the WHI FFQ results. Baseline use of antihypertensive medication was reported as medication therapeutic class codes.

WHI-CT participants also had covariates regarding their treatment status for each arm of the clinical trial. Hormone Therapy Trial treatment was categorized into five categories ("Not randomized," "Estrogen-alone intervention," "Estrogen-alone control," "Estrogen + progestin intervention," or "Estrogen + progestin control"). Dietary Modification Trial treatment was categorized to three categories ("Not randomized," "Intervention," or "Control"). Calcium and Vitamin D Trial treatment was categorized to three categories ("Not randomized," "Intervention," or "Control").

For participants included in the nested case-control study, fasting blood samples collected at baseline were processed locally, frozen, and shipped to a central repository, where the samples were stored at −70°C. Blood samples of case subjects and control subjects were treated identically, shipped in the same batch, and assayed in the same order to reduce systemic bias and interassay variation. Fasting glucose, fasting insulin, VCAM1, TNFR2, IL6, and hs-CRP were assayed from blood samples. Details on biomarker assays have previously been published (5,6). The HOMA-IR and the HOMA-β were computed from the equations described by Matthews et al. (25).

Statistical Analysis

Each participant's follow-up time was determined as the duration from the date of the baseline visit to the date of self-reported treated diabetes status or censoring (death from other causes besides type 2 diabetes or the end of enrollment in WHI until 30 September 2010), whichever occurred first. Energy-adjusted dietary manganese intake was calculated using a linear regression model to remove potential bias in estimated manganese intake due to total energy intake (26). A P value of <0.05 was considered as statistically significant. All statistical analyses were done using R 3.6.1 software.

Cox Regression Analysis for Dietary Manganese and Type 2 Diabetes

For both cohorts, Cox proportional hazards regression models were used to estimate the hazard ratios (HRs) and 95% CIs of type 2 diabetes risk based on the quintile of energy-adjusted dietary manganese (lowest quintile as the reference). The first model was adjusted for age and race/ethnicity. The second model was additionally adjusted for total energy intake, and the third model was additionally adjusted for smoking status, alcohol intake, systolic blood pressure, physical activity, family diabetes history, hormone replacement therapy status, and baseline use of antihypertensive medication. The fourth model was additionally adjusted for BMI. The replication analysis with the WHI-CT cohort included an additional model that further adjusted for the clinical trial treatment status (Hormone Therapy Trial treatment, Diet Modification Trial treatment, and Calcium-Vitamin D Trial treatment).

Sensitivity analysis was conducted in the following order: *1*) the association between the quintile of energy-adjusted dietary manganese and type 2 diabetes risk was assessed in the WHI-OS participants without any missing covariates (n = 75,753), *2*) the association by race/ethnicity between the quintile of energy-adjusted dietary manganese and type 2 diabetes risk in whites (n = 72,226), blacks (n = 5,713), and Hispanics (n = 2,859), *3*) the association between the quintile of energy-unadjusted dietary manganese and type 2 diabetes risk was assessed in the WHI-OS, *4*) the association between the quintile of total manganese from dietary and supplemental sources and type 2 diabetes risk was assessed in the WHI-OS population, and *5*) the association between the quintile of energy-adjusted dietary manganese and type 2 diabetes risk was assessed separately for the WHI-CT participants who were not randomized to the Diet Modification Trial, those who were given dietary intervention, and those who were Diet Modification Trial control subjects.

Stratification and Interaction Analysis

Participants were stratified based on BMI (<25, 25–30, or >30 kg/m^2), DAQS (0–3, 4 or 5), dietary calcium (tertile 1, 2, or 3), dietary iron (tertile 1, 2, or 3), and dietary magnesium (tertile 1, 2, or 3); these dietary minerals were selected for their ability to reduce manganese bioavailability (27–29). Interaction analysis was conducted to investigate the interaction between energy-adjusted dietary manganese and BMI, DAQS, and dietary minerals in the development of type 2 diabetes. The quintile of energy-adjusted dietary manganese, a potential confounder (BMI, DAQS, dietary calcium, dietary iron, or dietary magnesium) categorized as above, and the corresponding interaction

term with the quintile of energy-adjusted dietary manganese were included for the interaction analysis. All stratification and interaction analysis models were adjusted for age, race/ethnicity, daily energy intake, region of residence at baseline, family income, education, smoking status, alcohol intake, systolic blood pressure, physical activity, family diabetes history, hormone replacement therapy status, use of antihypertensive medication, and BMI.

Mediation Analysis

Linear regression was used to determine biomarkers that were significantly associated with energy-adjusted dietary manganese intake. To address the nonlinear association between dietary manganese and the associated biomarkers, energy-adjusted dietary manganese was dichotomized to a binary variable (lowest quintile vs. other quintiles). Logistic regression models using the binary dietary manganese variable as the exposure, biomarker as the mediator, and type 2 diabetes as the outcome were examined. A linear regression model using the binary manganese variable as the exposure and a biomarker as outcome was also examined. These two types of regression models were integrated for mediation analysis to evaluate the causal role of dietary manganese in relation to the biomarker-type 2 diabetes pathway (30,31). All models were adjusted for age, race/ethnicity, daily energy intake, region of residence at baseline, family income, education, smoking status, alcohol intake, systolic blood pressure, physical activity, family diabetes history, hormone replacement therapy status, use of antihypertensive medication, and BMI. Proportion of mediating effects was calculated in two scales, odds ratio [OR] scale and risk difference scale. The 95% CIs of the proportions mediated were obtained using bootstrapping.

RESULTS

WHI-OS

Baseline Characteristics

Table 1 presents the baseline characteristics of WHI-OS participants according to the quintile of energy-adjusted dietary manganese. Participants with higher dietary manganese had a lower proportion of participants with type 2 diabetes, higher proportion of current smokers, lower BMI, and lower systolic blood pressure. These participants also had higher proportion of whites and more alcohol consumption.

Cox Regression Analysis on Dietary Manganese and Type 2 Diabetes

Throughout a total of 914,422 person-years, 6,799 participants developed type 2 diabetes in the WHI-OS. After adjusting for age and race/ethnicity (Table 2, model 1), the estimated HR of type 2 diabetes for participants in the highest quintile of energy-adjusted dietary manganese intake compared with the lowest quintile was 0.46 (95% CI 0.43, 0.50). This association was consistent after further adjusting for total energy intake (Table 2, model 2). Adjusting for region of residence at baseline, family income, education, smoking status, alcohol intake, systolic blood pressure, physical activity, family diabetes history, hormone replacement therapy status, and baseline use of antihypertensive medication in addition to the model 2 covariates resulted in an HR of 0.62 (95% CI 0.57, 0.67) (Table 2, model 3). Further adjustment for BMI led to an HR of 0.70 (95% CI 0.65, 0.76) (Table 2, model 4).

Sensitivity Analysis

Sensitivity analysis on the WHI-OS participants without any missing covariates (n = 75,753) resulted in no change in the associations (Supplementary Table 3, model 4). Stratification of WHI-OS participants by race/ethnicity resulted in a similar association among whites (HR 0.72 [95% CI 0.65, 0.78]) and blacks (HR 0.67 [95% CI 0.51, 0.89]). However, the association was weaker among Hispanics (HR 0.84 [95% 0.53, 1.33]) (Supplementary Table 4). Sensitivity analysis on the WHI-OS participants according to the quintile of dietary manganese intake (unadjusted to total energy intake) resulted in a stronger association (HR 0.63 [95% CI 0.57, 0.70]) (Supplementary Table 5, model A). Sensitivity analysis on the WHI-OS participants according to the quintile of total manganese intake from dietary and supplemental sources resulted in a similar HR of 0.63 (95% CI 0.57, 0.69) (Supplementary Table 5, model B).

Subgroup Analysis

The adjusted HR for women with a DAQS of 5 across quintiles of energy-adjusted dietary manganese (HR 0.83 [95% CI 0.72, 0.96]) was higher compared with the adjusted HR for women with a DAQS of 0–3 (HR 0.64 [95% CI 0.53, 0.76]). After stratification by tertiles of dietary calcium, dietary iron, and dietary magnesium, there were weaker associations for the groups in the highest tertile of intake of these minerals (Table 3). Stratification by dietary magnesium resulted in the greatest change in the HR of the highest quintile of energy-adjusted dietary manganese from 0.58 (95% CI 0.42, 0.79) to 0.76 (95% CI 0.66, 0.87) when examining increasing tertiles of magnesium. We observed no significant interactions of energy-adjusted dietary manganese with BMI because there was no noticeable change in the association between dietary manganese and type 2 diabetes when the subgroup with BMI <25 kg/m^2 (HR 0.73 [95% CI 0.61, 0.87]) was compared with BMI >30 kg/m^2 (HR 0.74 [95% CI 0.65, 0.83]).

WHI-CT

Baseline Characteristics

Supplementary Table 1 presents the baseline characteristics of WHI-CT participants. Similar trends among covariates were found as those in WHI-OS participants.

Cox Regression Analysis on Dietary Manganese and Type 2 Diabetes

Analysis on the association between dietary manganese and type 2 diabetes using the WHI-CT cohort resulted in a consistent HR of 0.79 (95% CI 0.73, 0.85) (Supplementary Table 2, model 5) as the association found using the WHI-OS cohort.

Sensitivity Analysis

Stratification of WHI-CT participants by the Diet Modification Trial treatment status resulted in the weakest association between dietary manganese and type 2 diabetes in participants with dietary intervention (HR 0.84 [95% CI 0.73, 0.98]). The strongest association (HR 0.76, [95% CI 0.68, 0.86]) was found for the control participants in the Diet Modification Trial (Supplementary Table 6).

Nested Case-Control Study

Supplementary Table 7 presents the associations between the quintile of manganese intake and the estimated relative level of circulating biomarkers at baseline in the nested case-control study. After adjusting for covariates, estimated levels of fasting glucose (P for trend <0.001) and fasting insulin (P for trend <0.001) were lower for higher manganese intake. Insulin resistance, indicated by HOMA-IR (P for trend <0.001), was also lower for higher energy-adjusted manganese

Table 1—Baseline characteristics according to quintiles of energy-adjusted dietary manganese among 84,285 postmenopausal women aged 50–79 enrolled in the WHI-OS

	Energy-adjusted dietary manganese					
	Quintile 1 $n = 16,857$	Quintile 2 $n = 16,857$	Quintile 3 $n = 16,857$	Quintile 4 $n = 16,857$	Quintile 5 $n = 16,857$	*P* value†
Person-years	169,939	179,177	184,970	188,577	191,759	
Participants with type 2 diabetes	1,972 (11.7)	1,518 (9)	1,275 (7.6)	1,061 (6.3)	973 (5.8)	
Manganese intake (mg/day)*	2.17 ± 0.93	2.49 ± 0.82	2.99 ± 0.78	3.64 ± 0.78	5.11 ± 1.25	
Age (years)	63 ± 8	64 ± 7	64 ± 7	64 ± 7	64 ± 7	<0.001
Race/ethnicity						<0.001
White	12,935 (76.7)	14,114 (83.7)	14,686 (87.1)	15,110 (89.6)	15,381 (91.2)	
Black	2,164 (12.8)	1,273 (7.6)	923 (5.5)	714 (4.2)	639 (3.8)	
Hispanic	1,167 (6.9)	655 (3.9)	450 (2.7)	339 (2)	248 (1.5)	
Region (U.S.)						<0.001
Northeast	3,527 (20.9)	3,867 (22.9)	4,040 (24)	4,004 (23.8)	3,913 (23.2)	
South	4,782 (28.4)	4,202 (24.9)	4,174 (24.8)	4,188 (24.8)	4,182 (24.8)	
Midwest	4,141 (24.6)	3,803 (22.6)	3,551 (21.1)	3,587 (21.3)	3,634 (21.6)	
West	4,407 (26.1)	4,985 (29.6)	5,092 (30.2)	5,078 (30.1)	5,128 (30.4)	
Income (U.S. dollars)						<0.001
<20,000	3,546 (21)	2,546 (15.1)	2,176 (12.9)	1,909 (11.3)	1,773 (10.5)	
20,000–49,999	7,529 (44.7)	7,437 (44.1)	7,111 (42.2)	6,874 (40.8)	6,729 (39.9)	
50,000–99,999	4,047 (24)	4,676 (27.7)	5,082 (30.1)	5,443 (32.3)	5,610 (33.3)	
>100,000	1,156 (6.9)	1,673 (9.9)	2,007 (11.9)	2,159 (12.8)	2,351 (13.9)	
Highest education						
High school	5,078 (30.1)	3,812 (22.6)	3,182 (18.9)	2,727 (16.2)	2,255 (13.4)	
Vocational/training school	1,984 (11.8)	1,758 (10.4)	1,610 (9.6)	1,368 (8.1)	1,313 (7.8)	
College	6,202 (36.8)	6,619 (39.3)	6,580 (39)	6,704 (39.8)	6,424 (38.1)	
Postgraduate	3,593 (21.3)	4,668 (27.7)	5,485 (32.5)	6,058 (35.9)	6,865 (40.7)	
BMI (kg/m^2)	29 ± 6.6	27.3 ± 5.6	26.7 ± 5.3	26.2 ± 5.1	25.8 ± 5	<0.001
Systolic blood pressure (mmHg)	128.2 ± 17.8	127.1 ± 18.1	126.3 ± 17.7	125.6 ± 17.7	125.1 ± 17.7	<0.001
Physical activity (METs-h/week)	9.5 ± 12.2	12.6 ± 13.7	14.4 ± 14.1	16 ± 14.8	17.5 ± 15.6	<0.001
Total energy intake (kcal/day)	1,740 ± 735	1,442 ± 556	1,445 ± 523	1,511 ± 508	1,711 ± 545	0.46
Alcohol consumption (g/day)	3.9 ± 10	4 ± 8.1	4.7 ± 8.1	6 ± 9.4	10.6 ± 17.1	<0.001
Smoking status						<0.001
Never smoked	8,910 (52.9)	8,887 (52.7)	8,752 (51.9)	8,399 (49.8)	7,753 (46)	
Past smoker	6,374 (37.8)	6,879 (40.8)	7,244 (43)	7,669 (45.5)	8,303 (49.3)	
Current smoker	1,573 (9.3)	1,091 (6.5)	861 (5.1)	789 (4.7)	801 (4.8)	
Hormone replacement therapy status						<0.001
Never used hormones	5,622 (33.4)	5,059 (30)	4,839 (28.7)	4,662 (27.7)	4,681 (27.8)	
Past user	3,661 (21.7)	3,602 (21.4)	3,536 (21)	3,391 (20.1)	3,497 (20.7)	
Current user	7,574 (44.9)	8,196 (48.6)	8,482 (50.3)	8,804 (52.2)	8,679 (51.5)	
Diabetes family history						<0.001
Yes	5,511 (32.7)	5,158 (30.6)	4,915 (29.2)	4,795 (28.4)	4,718 (28)	
No	10,444 (62)	10,961 (65)	11,291 (67)	11,427 (67.8)	11,507 (68.3)	
Use of antihypertensive medication						<0.001
Yes	1,984 (11.8)	1,780 (10.6)	1,638 (9.7)	1,485 (8.8)	1,294 (7.7)	
No	14,873 (88.2)	15,077 (89.4)	15,219 (90.3)	15,372 (91.2)	15,563 (92.3)	

Continuous variables are presented as mean ± SD. Categorical variables are presented as *n* (%). Categorical variables may not add up to 100% due to missing or unknown information. *Energy-unadjusted dietary manganese intake estimated from WHI FFQ. †The χ^2 test was used to compare proportions/frequencies for categorical variables. One-way ANOVA was used to compare means for continuous variables.

intake, but β-cell function, indicated by HOMA-β (*P* for trend = 0.20), was not significantly associated with manganese intake. Change in estimated relative level of VCAM1, an indicator for endothelial dysfunction, was associated with dietary manganese (*P* for trend = 0.04). Covariate-adjusted estimated levels of the inflammatory biomarkers TNFR2 (*P* for trend = 0.002), IL6 (*P* for trend <0.001), and hs-CRP (*P* for trend <0.001) were also lower for higher energy-adjusted manganese intake.

Biomarkers that were significantly associated with dietary manganese were further examined via mediation analysis (Table 4). Fasting glucose levels significantly mediated the dietary manganese-type 2 diabetes OR (0.69 [95% CI 0.55, 0.82]), accounting for 91% of the proportion mediated. Fasting insulin mediated the dietary manganese-type 2 diabetes OR (0.87 [95% CI 0.82, 0.92]), accounting for 52% of the proportion mediated. HOMA-IR significantly mediated the dietary manganese-type 2 diabetes OR (0.77 [95% CI 0.70, 0.84]), accounting for 84% of the proportion mediated. IL6 and hs-CRP also mediated the dietary manganese-type 2 diabetes OR, accounting

Table 2—HRs with 95% CIs of type 2 diabetes according to quintiles of energy-adjusted dietary manganese among 84,285 postmenopausal women aged 50–79 enrolled in the WHI-OS

	Energy-adjusted dietary manganese					
Model	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for trend
Model 1*	1.00	0.75 (0.70, 0.81)	0.62 (0.58, 0.67)	0.51 (0.47, 0.55)	0.46 (0.43, 0.50)	<0.001
Model 2†	1.00	0.80 (0.74, 0.85)	0.66 (0.61, 0.70)	0.53 (0.50, 0.58)	0.47 (0.43, 0.50)	<0.001
Model 3‡	1.00	0.88 (0.82, 0.94)	0.77 (0.72, 0.83)	0.66 (0.61, 0.71)	0.62 (0.57, 0.67)	<0.001
Model 4§	1.00	0.93 (0.87, 1.00)	0.84 (0.78, 0.90)	0.73 (0.68, 0.79)	0.70 (0.65, 0.76)	<0.001

*Model 1 was adjusted for age and race/ethnicity. †Model 2 was adjusted for age, race/ethnicity, and total energy intake. ‡Model 3 was adjusted for age, race/ethnicity, total energy intake, region of residence at baseline, family income, education, smoking status, alcohol intake, systolic blood pressure, physical activity, family diabetes history, hormone usage, and the use of antihypertensive medication. §Model 4 was adjusted for age, race/ethnicity, total energy intake, region of residence at baseline, family income, education, smoking status, alcohol intake, systolic blood pressure, physical activity, family diabetes history, hormone usage, the use of antihypertensive medication, and BMI.

for 19% and 12% for IL6 and hs-CRP, respectively.

CONCLUSIONS

In a large study of postmenopausal women, participants with the highest compared with the lowest amount of dietary manganese had 30% lower risk of type 2 diabetes. This association was independent of identified risk factors such as BMI, but dietary manganese had significant interactions with dietary quality in antioxidant and essential minerals. A similar association was observable in the replication analysis of WHI-CT participants, although dietary intervention seemed to weaken the association. In the nested casecontrol study, higher energy-adjusted

Table 3—HRs with 95% CIs of type 2 diabetes according to quintiles of energy-adjusted dietary manganese among 84,285 postmenopausal women aged 50–79 stratified to BMI, DAQS, and dietary minerals

	Energy-adjusted dietary manganese					
Biomarkers	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P* for trend
BMI						
<25 kg/m^2 (n = 35,772)	1.00	0.96 (0.80, 1.14)	0.77 (0.64, 0.92)	0.72 (0.60, 0.86)	0.73 (0.61, 0.87)	<0.001
25–30 kg/m^2 (n = 28,904)	1.00	0.89 (0.79, 1.01)	0.77 (0.67, 0.87)	0.73 (0.64, 0.84)	0.69 (0.60, 0.79)	<0.001
>30 kg/m^2 (n = 19,609)	1.00	0.94 (0.85, 1.04)	0.95 (0.85, 1.05)	0.77 (0.69, 0.87)	0.74 (0.65, 0.83)	<0.001
Interaction with energy-adjusted dietary manganese						0.68
DAQS						
0–3 (n = 26,350)	1.00	0.90 (0.81, 1.01)	0.76 (0.67, 0.87)	0.69 (0.60, 0.80)	0.64 (0.53, 0.76)	<0.001
4 (n = 37,043)	1.00	0.90 (0.81, 1.01)	0.81 (0.72, 0.92)	0.75 (0.66, 0.84)	0.65 (0.58, 0.74)	<0.001
5 (n = 20,892)	1.00	1.03 (0.89, 1.19)	0.97 (0.83, 1.12)	0.74 (0.63, 0.87)	0.83 (0.72, 0.96)	<0.001
Interaction with energy-adjusted dietary manganese						0.02
Dietary calcium						
Tertile 1 (n = 28,095)	1.00	0.89 (0.80, 0.99)	0.79 (0.70, 0.90)	0.68 (0.59, 0.78)	0.66 (0.56, 0.77)	<0.001
Tertile 2 (n = 28,095)	1.00	0.96 (0.85, 1.09)	0.89 (0.78, 1.02)	0.74 (0.65, 0.85)	0.64 (0.55, 0.74)	<0.001
Tertile 3 (n = 28,095)	1.00	0.97 (0.85, 1.10)	0.85 (0.74, 0.97)	0.80 (0.70, 0.91)	0.82 (0.72, 0.93)	<0.001
Interaction with energy-adjusted dietary manganese						0.03
Dietary iron						
Tertile 1 (n = 28,095)	1.00	0.90 (0.81, 1.01)	0.79 (0.70, 0.89)	0.67 (0.58, 0.79)	0.71 (0.56, 0.91)	<0.001
Tertile 2 (n = 28,095)	1.00	0.88 (0.78, 1.00)	0.78 (0.69, 0.89)	0.66 (0.57, 0.76)	0.60 (0.51, 0.70)	<0.001
Tertile 3 (n = 28,095)	1.00	1.03 (0.90, 1.18)	0.94 (0.82, 1.07)	0.83 (0.73, 0.96)	0.77 (0.68, 0.87)	<0.001
Interaction with energy-adjusted dietary manganese						0.04
Dietary magnesium						
Tertile 1 (n = 28,095)	1.00	0.88 (0.80, 0.98)	0.81 (0.72, 0.91)	0.63 (0.53, 0.74)	0.58 (0.42, 0.79)	<0.001
Tertile 2 (n = 28,095)	1.00	0.99 (0.86, 1.13)	0.86 (0.74, 0.99)	0.78 (0.66, 0.91)	0.70 (0.58, 0.85)	<0.001
Tertile 3 (n = 28,095)	1.00	0.96 (0.83, 1.13)	0.87 (0.75, 1.01)	0.79 (0.68, 0.92)	0.76 (0.66, 0.87)	<0.001
Interaction with energy-adjusted dietary manganese						0.06

Stratification analysis models were adjusted for age, race/ethnicity, total energy intake, region of residence at baseline, family income, education, smoking status, alcohol intake, systolic blood pressure, physical activity, family diabetes history, hormone use, use of antihypertensive medication, and BMI. Interaction analysis models were adjusted for age, race/ethnicity, total energy intake, region of residence at baseline, family income, education, smoking status, alcohol intake, systolic blood pressure, physical activity, family diabetes history, hormone use, use of antihypertensive medication, BMI, and the corresponding interaction terms (BMI, DAQS, dietary calcium, dietary iron, and dietary magnesium).

Table 4—Effect of dietary manganese on type 2 diabetes risk with mediation of established biomarkers

Biomarkers	Effect mediated (95% CIs)	Effect not mediated (95% CIs)	Proportion mediated on OR scale (%)	Proportion mediated on risk difference (%)
Fasting glucose	0.69 (0.55, 0.82)	0.96 (0.78, 1.18)	91	89
Fasting insulin	0.87 (0.82, 0.92)	0.88 (0.74, 1.04)	52	49
HOMA-IR	0.77 (0.70, 0.84)	0.95 (0.79, 1.15)	84	82
VCAM1	1.00 (0.99, 1.00)	0.78 (0.66, 0.92)	1	1
TNFR2	0.99 (0.98, 1.00)	0.78 (0.66, 0.93)	2	2
IL6	0.95 (0.93, 0.98)	0.82 (0.69, 0.96)	19	17
hs-CRP	0.97 (0.95, 0.99)	0.80 (0.68, 0.95)	12	10

Higher manganese intake (four highest quintiles of energy-adjusted dietary manganese) was compared with the first quintile as reference. Effects are shown in ORs with 95% CIs. CIs were calculated using bootstrapping. Models were adjusted for age, race/ethnicity, total energy intake, region of residence at baseline, family income, education, smoking status, alcohol intake, systolic blood pressure, physical activity, family diabetes history, hormone use, use of antihypertensive medication, and BMI.

dietary manganese intake was associated with lower levels of diabetes-associated biomarkers and inflammatory markers. According to our mediation analysis, several biomarkers, including fasting glucose, fasting insulin, HOMA-IR, IL6, and hs-CRP, significantly mediated the association between dietary manganese and type 2 diabetes.

Our results are consistent with prior studies examining the relationship between manganese and type 2 diabetes. A prospective study of two Chinese cohorts with ages ranging from 20 to 74 years showed that higher manganese intake was associated with lower type 2 diabetes risk (12). Du et al. (12) showed similar findings with HRs comparing the highest tertile of dietary manganese intake to the lowest tertile, HR of 0.52 (95% CI 0.32, 0.81) and 0.61 (95% CI 0.32, 0.81) in multivariable models of the two prospective cohorts.

Stratified analysis based on BMI categories resulted in no observable change in the associations across the quintiles of dietary manganese in the WHI-OS cohort. The subpopulation with DAQS of 0–3 showed lower HRs across the quintiles of energy-adjusted manganese intake than that with DAQS of 5. The subgroups in the lowest tertiles of calcium, iron, or magnesium tended to have lower HRs across the quintiles of energy-adjusted manganese intake compared with the subgroups in the highest tertiles of the corresponding minerals. Interactions between dietary manganese and dietary minerals were consistent with the effects of calcium, iron, and magnesium on manganese bioavailability (27–29).

Our findings on diabetes-associated biomarkers were consistent with previous findings examining associations between these biomarkers and diabetes risk (4–6,32,33). However, different results were reported in a cohort study of elderly men. Unlike our findings, Kresovich et al. (34) reported no significant association of dietary manganese with TNF receptor or C-reactive protein and a direct association between manganese intake and interleukins, including IL6, in the study population. In addition, Kresovich et al. (34) included relatively small number male participants ($n = 633$). Sex differences in manganese metabolism in diabetes need to be evaluated in future studies.

In a subcohort of WHI-OS, findings from our mediation analysis showed that the association of manganese with type 2 diabetes was significantly mediated by fasting glucose and insulin levels and insulin resistance indicated by HOMA-IR. As diabetes is clinically defined by a hyperglycemic state that induces insulin resistance and abnormal secretion of insulin, such mediation supports a potential role of manganese in type 2 diabetes risk. Our mediation analysis in the same subcohort also suggested that the association of manganese with type 2 diabetes may be partly mediated by inflammatory biomarkers. Our findings were consistent with animal studies showing a suppression of inflammatory processes, followed by mineral supplementation and injection (11,35).

This study has several strengths. The prospective design and large sample size with high follow-up rate reduce the possibility of bias due to participants lost to follow-up. Nutrient intake estimates made with the WHI FFQ were likely to reflect usual dietary intake (20). Our findings are relevant for a population with an increased risk of type 2 diabetes compared with the premenopausal counterpart (13). Also, we used mediation analysis (31) to quantify the effects of type 2 diabetes-associated biomarkers (5,6). This method may help explain the association between dietary manganese and type 2 diabetes risk observed in prospective cohorts by quantifying the magnitude and relative contributions of various pathways.

Meanwhile, our study had several limitations. Diabetes status in the current study was self-reported, but validation studies concluded that self-report of "treated diabetes" was sufficiently consistent with medication inventory to be used in epidemiologic studies (19,36). Physician diagnosis of treated diabetes may have missed some subtypes of diabetes (37) such as pancreatic diabetes or latent autoimmune diabetes in adults. Our primary exposure was energy-adjusted dietary manganese, which did not include supplemental manganese. However, our sensitivity analysis results suggest that both energy-unadjusted dietary manganese and total manganese intake are consistently, if not more strongly, associated with lower type 2 diabetes risk. In addition, our study only included postmenopausal women; thus, our findings cannot be applied to younger women or men. Moreover, dietary manganese intake was assessed only once at baseline from 1993 to 1998, so we were not able to capture the time-varying association of dietary manganese with risk of type 2 diabetes. Finally, we were not able to measure the level of manganese in blood, which would verify our findings using an alternative method of measurement.

In conclusion, dietary manganese was associated with a lower risk of type 2 diabetes among postmenopausal women, and this association was partly mediated by fasting glucose, fasting insulin, insulin resistance, and inflammatory biomarkers. However, potential physiological mechanisms explaining the association of manganese intake with diabetes development requires further research among demographically diverse populations. Consumption of food groups rich in manganese could potentially be targets for intervention against type 2 diabetes risk in postmenopausal women.

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