Supplemental Material

­­­Title: 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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Supplemental Figure 1 Selection of study population for the main analysis

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Supplemental Figure 2 Selection of study population for the replication analysis

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\* Out of 62,452 Women’s Health Initiative Clinical Trial participants selected for the replication analysis, 24,256 participated in the Hormone Therapy (HT) Trial, 45,414 participated in the Dietary Modification (DM) Trial, and 33,458 participated in the Calcium-Vitamin D (CaD) Trial.

† 16,924 Women’s Health Initiative Clinical Trial participants were not randomized to the DM. Among the DM participants, 18,182 received dietary intervention; 27,232 were controls.

Supplemental Table 1 Baseline characteristics according to quintiles of energy-adjusted dietary manganese among 62,338 postmenopausal women aged 50-79 enrolled in the Women’s Health Initiative Clinical Trial

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Energy-adjusted dietary manganese** |  |  |  |  |  |
|  | **Quintile 1** | **Quintile 2** | **Quintile 3** | **Quintile 4** | **Quintile 5** | **P value||** |
| N | 12450 | 12472 | 12464 | 12471 | 12481 |  |
| Person-year | 134076 | 140066 | 142261 | 144812 | 146167 |  |
| Participants with type 2 diabetes (%) | 1913 (15.4 %) | 1528 (12.3 %) | 1327 (10.6 %) | 1198 (9.6 %) | 1105 (8.9 %) |  |
| Manganese intake (mg/day) \* | 2.17 ± 0.94 | 2.4 ± 0.85 | 2.89 ± 0.82 | 3.48 ± 0.83 | 4.8 ± 1.22 |  |
| Age (years) | 62 ± 7 | 62 ± 7 | 63 ± 7 | 63 ± 7 | 63 ± 7 | <0.001 |
|  |  |  |  |  |  |  |
| Race/ethnicity |  |  |  |  |  | <0.001 |
| White | 8968 (72 %) | 9994 (80.1 %) | 10563 (84.7 %) | 10914 (87.5 %) | 11350 (90.9 %) |  |
| Black | 2083 (16.7 %) | 1396 (11.2 %) | 954 (7.7 %) | 805 (6.5 %) | 561 (4.5 %) |  |
| Hispanic | 1020 (8.2 %) | 557 (4.5 %) | 404 (3.2 %) | 278 (2.2 %) | 196 (1.6 %) |  |
|  |  |  |  |  |  |  |
| Region |  |  |  |  |  | <0.001 |
| Northeast | 2744 (22 %) | 2896 (23.2 %) | 3005 (24.1 %) | 2937 (23.6 %) | 2776 (22.2 %) |  |
| South | 3787 (30.4 %) | 3284 (26.3 %) | 2968 (23.8 %) | 2847 (22.8 %) | 2827 (22.7 %) |  |
| Midwest | 2773 (22.3 %) | 2685 (21.5 %) | 2657 (21.3 %) | 2777 (22.3 %) | 2889 (23.1 %) |  |
| West | 3146 (25.3 %) | 3607 (28.9 %) | 3834 (30.8 %) | 3910 (31.4 %) | 3989 (32 %) |  |
|  |  |  |  |  |  |  |
| Income (US dollars) |  |  |  |  |  | <0.001 |
| <20,000 | 2678 (21.5 %) | 2135 (17.1 %) | 1884 (15.1 %) | 1780 (14.3 %) | 1715 (13.7 %) |  |
| 20,000-49,999 | 5704 (45.8 %) | 5666 (45.4 %) | 5691 (45.7 %) | 5636 (45.2 %) | 5642 (45.2 %) |  |
| 50,000-99,999 | 2998 (24.1 %) | 3475 (27.9 %) | 3606 (28.9 %) | 3635 (29.1 %) | 3696 (29.6 %) |  |
| >100,000 | 699 (5.6 %) | 893 (7.2 %) | 1018 (8.2 %) | 1120 (9 %) | 1173 (9.4 %) |  |
|  |  |  |  |  |  |  |
| Highest education |  |  |  |  |  | <0.001 |
| High school | 3782 (30.4 %) | 3101 (24.9 %) | 2834 (22.7 %) | 2519 (20.2 %) | 2290 (18.3 %) |  |
| Vocational/training school | 1522 (12.2 %) | 1414 (11.3 %) | 1228 (9.9 %) | 1250 (10 %) | 1197 (9.6 %) |  |
| College | 4627 (37.2 %) | 4938 (39.6 %) | 5059 (40.6 %) | 5018 (40.2 %) | 5012 (40.2 %) |  |
| Post-graduate | 2519 (20.2 %) | 3019 (24.2 %) | 3343 (26.8 %) | 3684 (29.5 %) | 3982 (31.9 %) |  |
|  |  |  |  |  |  |  |
| BMI (kg/m2) | 30.3 ± 6.3 | 29.1 ± 5.8 | 28.4 ± 5.6 | 28 ± 5.4 | 27.5 ± 5.3 | <0.001 |
| Systolic blood pressure (mmHg) | 128.7 ± 17.1 | 127.8 ± 17.2 | 127.4 ± 17.3 | 127.1 ± 17.3 | 126.4 ± 17.4 | <0.001 |
| Physical Activity (METs-hr/week) | 8.1 ± 11.2 | 9.5 ± 11.6 | 10.7 ± 12.3 | 11.8 ± 12.9 | 13.4 ± 13.9 | <0.001 |
| Total energy intake (kcal/day) | 1916 ± 820 | 1585 ± 642 | 1622 ± 617 | 1686 ± 611 | 1875 ± 638 | 0.27 |
| Alcohol consumption (g/day) | 3.3 ± 8.8 | 3.3 ± 6.9 | 4.1 ± 7.1 | 5.1 ± 8 | 8.9 ± 14.5 | <0.001 |
|  |  |  |  |  |  |  |
| Smoking status |  |  |  |  |  | <0.001 |
| Never smoked | 6719 (54 %) | 6593 (52.9 %) | 6392 (51.3 %) | 6237 (50 %) | 5835 (46.8 %) |  |
| Past smoker | 4535 (36.4 %) | 4858 (39 %) | 5183 (41.6 %) | 5299 (42.5 %) | 5709 (45.7 %) |  |
| Current smoker | 1196 (9.6 %) | 1021 (8.2 %) | 889 (7.1 %) | 935 (7.5 %) | 937 (7.5 %) |  |
|  |  |  |  |  |  |  |
| Hormone therapy usage at baseline |  |  |  |  |  | <0.001 |
| Never used hormones | 4876 (39.2 %) | 4540 (36.4 %) | 4393 (35.2 %) | 4394 (35.2 %) | 4734 (37.9 %) |  |
| Past user | 3135 (25.2 %) | 3094 (24.8 %) | 3116 (25 %) | 3215 (25.8 %) | 3378 (27.1 %) |  |
| Current user | 4439 (35.7 %) | 4838 (38.8 %) | 4955 (39.8 %) | 4862 (39 %) | 4369 (35 %) |  |
|  |  |  |  |  |  |  |
| Diabetes family history |  |  |  |  |  | <0.001 |
| Yes | 4236 (34 %) | 4000 (32.1 %) | 3938 (31.6 %) | 3700 (29.7 %) | 3678 (29.5 %) |  |
| No | 7437 (59.7 %) | 7889 (63.3 %) | 7948 (63.8 %) | 8264 (66.3 %) | 8285 (66.4 %) |  |
|  |  |  |  |  |  |  |
| Use of antihypertensive medication |  |  |  |  |  | <0.001 |
| Yes | 1448 (11.6 %) | 1298 (10.4 %) | 1249 (10 %) | 1159 (9.3 %) | 965 (7.7 %) |  |
| No | 11002 (88.4 %) | 11174 (89.6 %) | 11215 (90 %) | 11312 (90.7 %) | 11516 (92.3 %) |  |
|  |  |  |  |  |  |  |
| CAD arm† |  |  |  |  |  | <0.001 |
| Not randomized | 6008 (48.3 %) | 5878 (47.1 %) | 5656 (45.4 %) | 5689 (45.6 %) | 5649 (45.3 %) |  |
| Intervention | 3262 (26.2 %) | 3251 (26.1 %) | 3434 (27.6 %) | 3412 (27.4 %) | 3401 (27.2 %) |  |
| Control | 3180 (25.5 %) | 3343 (26.8 %) | 3374 (27.1 %) | 3370 (27 %) | 3431 (27.5 %) |  |
|  |  |  |  |  |  |  |
| DM arm‡ |  |  |  |  |  | <0.001 |
| Not randomized | 2750 (22.1 %) | 2732 (21.9 %) | 3044 (24.4 %) | 3620 (29 %) | 4778 (38.3 %) |  |
| Intervention | 3907 (31.4 %) | 3861 (31 %) | 3782 (30.3 %) | 3543 (28.4 %) | 3089 (24.7 %) |  |
| Control | 5793 (46.5 %) | 5879 (47.1 %) | 5638 (45.2 %) | 5308 (42.6 %) | 4614 (37 %) |  |
|  |  |  |  |  |  |  |
| HRT arm§ |  |  |  |  |  | <0.001 |
| Not randomized | 7851 (63.1 %) | 8168 (65.5 %) | 7946 (63.8 %) | 7543 (60.5 %) | 6574 (52.7 %) |  |
| E-only intervention | 1012 (8.1 %) | 875 (7 %) | 878 (7 %) | 854 (6.8 %) | 939 (7.5 %) |  |
| E-only control | 1022 (8.2 %) | 872 (7 %) | 847 (6.8 %) | 906 (7.3 %) | 1007 (8.1 %) |  |
| E+P intervention | 1301 (10.4 %) | 1327 (10.6 %) | 1426 (11.4 %) | 1619 (13 %) | 2024 (16.2 %) |  |
| E+P control | 1264 (10.2 %) | 1230 (9.9 %) | 1367 (11 %) | 1549 (12.4 %) | 1937 (15.5 %) |  |

Continuous variables are presented as mean ± SD.

Categorical variables are presented as mean (%). Categorical variables may not add up to 100% due to missing or unknown information.

\* Energy-unadjusted dietary manganese intake estimated from WHI FFQ

† Calcium and Vitamin D Trial arm

‡ Diet Modification Trial arm

§ Hormone Therapy Trial arm (E: estrogen, P: progestin)

|| Chi-square test was used to compare proportions/frequencies for categorical variables. One-way ANOVA was used to compare means for continuous variables.

Supplemental Table 2 Hazard ratios with 95% CIs of type 2 diabetes according to quintiles of energy-adjusted dietary manganese among 62,338 postmenopausal women aged 50-79 enrolled in the Women’s Health Initiative Clinical Trial

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Energy-adjusted dietary manganese** |  |  |  |  |  |
| **Model** | **Quintile 1** | **Quintile 2** | **Quintile 3** | **Quintile 4** | **Quintile 5** | **P for trend** |
| Model 1\* | 1.00 | 0.80 (0.74, 0.85) | 0.70 (0.65, 0.75) | 0.63 (0.58, 0.67) | 0.58 (0.54, 0.63) | <0.001 |
| Model 2† | 1.00 | 0.82 (0.77, 0.88) | 0.72 (0.67, 0.77) | 0.64 (0.59, 0.69) | 0.59 (0.54, 0.63) | <0.001 |
| Model 3‡ | 1.00 | 0.87 (0.81, 0.93) | 0.79 (0.74, 0.85) | 0.73 (0.68, 0.79) | 0.72 (0.67, 0.78) | <0.001 |
| Model 4§ | 1.00 | 0.89 (0.83, 0.96) | 0.84 (0.78, 0.90) | 0.78 (0.73, 0.85) | 0.79 (0.73, 0.85) | <0.001 |
| Model 5¶ | 1.00 | 0.89 (0.83, 0.96) | 0.84 (0.78, 0.90) | 0.78 (0.73, 0.85) | 0.79 (0.73, 0.85) | <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 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, use of antihypertensive medication, and BMI.

¶ Model 5 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, use of antihypertensive medication, BMI, Hormone Therapy Trial treatment, Diet Modification Trial treatment, and Calcium-Vitamin D Trial treatment.

Supplemental Table 3 Hazard ratios with 95% CIs of type 2 diabetes according to quintiles of energy-adjusted dietary manganese among 75,753 postmenopausal women aged 50-79 without missing covariates enrolled in the Women’s Health Initiative Observational Study

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Model** | **Energy-adjusted dietary manganese** |  |  |  |  | **P for trend** |
| **Quintile 1** | **Quintile 2** | **Quintile 3** | **Quintile 4** | **Quintile 5** |
| Model 1\* | 1.00 | 0.74 (0.69, 0.79) | 0.62 (0.58, 0.67) | 0.50 (0.46, 0.54) | 0.46 (0.42, 0.50) | <0.001 |
| Model 2† | 1.00 | 0.78 (0.73, 0.84) | 0.66 (0.61, 0.71) | 0.53 (0.49, 0.57) | 0.46 (0.43, 0.50) | <0.001 |
| Model 3‡ | 1.00 | 0.86 (0.80, 0.92) | 0.77 (0.71, 0.83) | 0.65 (0.60, 0.70) | 0.61 (0.56, 0.67) | <0.001 |
| Model 4§ | 1.00 | 0.92 (0.85, 0.99) | 0.84 (0.78, 0.91) | 0.72 (0.66, 0.78) | 0.70 (0.64, 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, region of residence at baseline, family income, education, total energy intake, smoking status, alcohol intake, systolic blood pressure, physical activity, family diabetes history, hormone usage, and 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, use of antihypertensive medication, and BMI.

Supplemental Table 4 Hazard ratios with 95% CIs of type 2 diabetes according to quintiles of energy-adjusted dietary manganese among 84,285 postmenopausal women aged 50-79 stratified by race/ethnicity

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Energy-adjusted dietary manganese** |  |  |  |  |  |
| **Race/ethnicity** | **Quintile 1** | **Quintile 2** | **Quintile 3** | **Quintile 4** | **Quintile 5** | **P for trend** |
| White (N = 72,226) | 1.00 | 0.94 (0.86, 1.01) | 0.84 (0.77, 0.91) | 0.73 (0.67, 0.8) | 0.72 (0.65, 0.78) | <0.001 |
| Black (N = 5,713) | 1.00 | 0.98 (0.82, 1.17) | 0.94 (0.76, 1.16) | 0.83 (0.65, 1.05) | 0.67 (0.51, 0.89) | 0.005 |
| Hispanic (N = 2,859) | 1.00 | 0.69 (0.51, 0.94) | 0.67 (0.47, 0.96) | 0.62 (0.40, 0.95) | 0.84 (0.53, 1.33) | 0.03 |

All models were adjusted for age, 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, use of antihypertensive medication, and BMI.

Supplemental Table 5 Hazard ratios with 95% CIs of type 2 diabetes according to quintiles of dietary manganese and total manganese intake among 84,285 postmenopausal women aged 50-79 enrolled in the Women’s Health Initiative Observational Study

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Energy-unadjusted dietary manganese** |  |  |  |  |  |
| **Model** | **Quintile 1** | **Quintile 2** | **Quintile 3** | **Quintile 4** | **Quintile 5** | **P for trend** |
| Model A | 1.00 | 0.87 (0.81, 0.93) | 0.75 (0.70, 0.82) | 0.71 (0.66, 0.78) | 0.63 (0.57, 0.70) | <0.001 |
|  | **Total manganese intake**\* |  |  |  |  |  |
| **Quintile 1** | **Quintile 2** | **Quintile 3** | **Quintile 4** | **Quintile 5** |
| Model B | 1.00 | 0.85 (0.79, 0.92) | 0.77 (0.71, 0.83) | 0.70 (0.65, 0.76) | 0.63 (0.57, 0.69) | <0.001 |

Both 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 usage, use of antihypertensive medication, and BMI.

\*Total manganese intake was calculated by adding energy-unadjusted dietary manganese and supplemental manganese intake

Supplemental Table 6 Hazard ratios with 95% CIs of type 2 diabetes according to quintiles of energy-adjusted dietary manganese among postmenopausal women enrolled in the Women’s Health Initiative Clinical Trial stratified by the Diet Modification Trial status (not randomized, dietary intervention, or clinical trial control)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Energy-adjusted dietary manganese** |  |  |  |  |  |
| **Population** | **Quintile 1** | **Quintile 2** | **Quintile 3** | **Quintile 4** | **Quintile 5** | **P for trend** |
| Not randomized (N = 16,924) | 1.00 | 0.81 (0.69, 0.94) | 0.86 (0.74, 1.00) | 0.80 (0.69, 0.92) | 0.77 (0.67, 0.89) | <0.001 |
| Dietary intervention (N = 18,182) | 1.00 | 0.97 (0.86, 1.11) | 0.85 (0.75, 0.98) | 0.80 (0.70, 0.92) | 0.84 (0.73, 0.98) | <0.001 |
| Clinical trial control (N = 27,232) | 1.00 | 0.88 (0.80, 0.98) | 0.81 (0.73, 0.90) | 0.78 (0.69, 0.87) | 0.76 (0.68, 0.86) | <0.001 |

All three 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 usage, use of antihypertensive medication, BMI, Hormone Therapy Trial treatment, Diet Modification Trial treatment, and Calcium-Vitamin D Trial treatment.

Supplemental Table 7 Adjusted estimation of relative levels of biomarkers according to quintiles of energy-adjusted dietary manganese among 3,749 postmenopausal women enrolled in the nested case-control study

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Energy-adjusted dietary manganese** |  |  |  |  |  |
| **Biomarkers** | **Quintile 1** | **Quintile 2** | **Quintile 3** | **Quintile 4** | **Quintile 5** | **P for trend** |
| **Fasting glucose** | 1.00 | 0.98 (0.95, 1.00) | 0.97 (0.95, 1.00) | 0.96 (0.94, 0.99) | 0.94 (0.92, 0.97) | <0.001 |
| **Fasting insulin** | 1.00 | 0.93 (0.88, 0.99) | 0.89 (0.84, 0.95) | 0.89 (0.84, 0.94) | 0.84 (0.79, 0.89) | <0.001 |
| **HOMA-IR** | 1.00 | 0.91 (0.85, 0.98) | 0.87 (0.81, 0.93) | 0.86 (0.80, 0.92) | 0.79 (0.74, 0.85) | <0.001 |
| **HOMA-Beta** | 1.00 | 0.98 (0.92, 1.05) | 0.96 (0.90, 1.02) | 0.97 (0.90, 1.03) | 0.96 (0.89, 1.02) | 0.20 |
| **VCAM1**\* | 1.00 | 1.00 (0.97, 1.04) | 1.00 (0.96, 1.03) | 1.01 (0.97, 1.04) | 0.96 (0.93, 0.99) | 0.04 |
| **TNFR2**† | 1.00 | 1.00 (0.97, 1.03) | 0.99 (0.96, 1.02) | 0.98 (0.95, 1.02) | 0.95 (0.92, 0.98) | 0.002 |
| **IL6**‡ | 1.00 | 0.93 (0.86, 1.01) | 0.92 (0.85, 0.99) | 0.86 (0.79, 0.93) | 0.85 (0.78, 0.92) | <0.001 |
| **hsCRP**§ | 1.00 | 0.96 (0.86, 1.07) | 0.91 (0.82, 1.02) | 0.90 (0.81, 1.01) | 0.81 (0.73, 0.91) | <0.001 |

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 usage, use of antihypertensive medication, and BMI.

\*VCAM1: Vascular cell adhesion molecule 1

† TNFR2: Tumor necrosis factor receptor 2

‡ IL6: interleukin 6

§ hsCRP: high-sensitivity C-reactive protein