

Relations of Dietary Magnesium Intake to Biomarkers of Inflammation and Endothelial Dysfunction in an Ethnically Diverse Cohort of Postmenopausal Women

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OBJECTIVE — Although magnesium may favorably affect metabolic outcomes, few studies have investigated the role of magnesium intake in systemic inflammation and endothelial dysfunction in humans.

RESEARCH DESIGN AND METHODS — Among 3,713 postmenopausal women aged 50–79 years in the Women's Health Initiative Observational Study and free of cardiovascular disease, cancer, and diabetes at baseline, we measured plasma concentrations of high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), tumor necrosis factor- α receptor 2 (TNF- α -R2), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and E-selectin. Magnesium intake was assessed using a semiquantitative food frequency questionnaire.

RESULTS — After adjustment for age, ethnicity, clinical center, time of blood draw, smoking, alcohol, physical activity, energy intake, BMI, and diabetes status, magnesium intake was inversely associated with hs-CRP (P for linear trend = 0.003), IL-6 ($P < 0.0001$), TNF- α -R2 ($P = 0.0006$), and sVCAM-1 ($P = 0.06$). Similar findings remained after further adjustment for dietary fiber, fruit, vegetables, folate, and saturated and *trans* fat intake. Multivariable-adjusted geometric means across increasing quintiles of magnesium intake were 3.08, 2.63, 2.31, 2.53, and 2.16 mg/l for hs-CRP ($P = 0.005$); 2.91, 2.63, 2.45, 2.27, and 2.26 pg/ml for IL-6 ($P = 0.0005$); and 707, 681, 673, 671, and 656 ng/ml for sVCAM-1 ($P = 0.04$). An increase of 100 mg/day magnesium was inversely associated with hs-CRP (-0.23 mg/l \pm 0.07; $P = 0.002$), IL-6 (-0.14 ± 0.05 pg/ml; $P = 0.004$), TNF- α -R2 (-0.04 ± 0.02 pg/ml; $P = 0.06$), and sVCAM-1 (-0.04 ± 0.02 ng/ml; $P = 0.07$). No significant ethnic differences were observed.

CONCLUSIONS — High magnesium intake is associated with lower concentrations of certain markers of systemic inflammation and endothelial dysfunction in postmenopausal women.

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Magnesium is a biologically active mineral that acts as a cofactor in hundreds of enzymatic reactions in the human body. In observational studies, magnesium intake has been inversely associated with metabolic disease outcomes including hypertension (1), type 2 diabetes (2), cardiovascular disease (3), and colorectal cancer (4). The biological mechanism underlying these relations is not entirely clear, although experimental data in animals indicate that dietary magnesium deficiency may promote an inflammatory response (5), ultimately leading to endothelial dysfunction and metabolic disease. Available data in humans also suggest that low magnesium intake may be related to dyslipidemia (1) and insulin resistance (2). Although magnesium intake has been inversely associated with high-sensitivity C-reactive protein (hs-CRP) (1,6) in white women, its associations with interleukin-6 (IL-6) and tumor necrosis factor (TNF)- α (6,7) have been inconsistent. In addition, evidence regarding the relation of magnesium intake to endothelial dysfunction as measured by elevated concentrations of adhesion molecules is limited (6,8). Studies assessing dietary magnesium intake in relation to inflammation and endothelial markers in minority populations are lacking, and it is not known whether there are ethnic differences modifying this relation.

We therefore examined comprehensively the association between dietary magnesium intake and circulating concentrations of biomarkers of systemic inflammation (hs-CRP, IL-6, and TNF- α receptor 2 [TNF- α -R2]) and endothelial dysfunction (soluble intercellular adhesion molecule-1 [sICAM-1], soluble vascular cell adhesion molecule-1 [sVCAM-1], and E-selectin) in an ethnically diverse cohort of postmenopausal women aged 50–79 years enrolled in the Women's Health Initiative Observational Study (WHI-OS), including whites, African American, Hispanics, and Asians/Pacific Islanders.

RESEARCH DESIGN AND METHODS

The WHI-OS is an ongoing national health study designed to examine demographic, lifestyle, dietary, and biological factors on health outcomes among postmenopausal women. Details on design and recruitment have been published elsewhere (9,10). In brief, a total of 93,676 postmenopausal women aged 50–79 years were enrolled in the WHI-OS between September 1994 and December 1998 from 40 clinical centers nationwide. Eligible participants completed baseline demographic and dietary questionnaires, underwent physical examinations, and provided fasting blood samples. All WHI study procedures were approved by human subject review at each clinical center, and all women provided informed consent before participating.

For the current study, we included all participants from a case-control study of type 2 diabetes nested within the WHI-OS ($n = 3,713$), because all were free of cardiovascular disease, type 2 diabetes, and cancers at baseline. Details of this diabetes ancillary study have been published elsewhere (11,12).

Baseline measurements

Certified WHI trained staff measured height, weight, hip circumference, and blood pressure at the baseline visit. BMI (measured as weight in kilograms divided by the square of height in meters) was calculated. Standardized questionnaires including information on age, ethnicity, education, income, occupation, medical and family history, smoking status, alcohol use, recreational physical activity, and medication and supplement use were administered. A semiquantitative food frequency questionnaire (FFQ) used previously among similar populations (13,14) was used to assess food and nutrient intake in the last 3 months. The nutrient database for the WHI FFQ was derived from the University of Minnesota Nutrition Coding Center (Minneapolis, MN) nutrient database (15). All dietary nutrient variables were adjusted for total energy intake using the residual method (16). The energy-adjusted correlation coefficient for dietary magnesium intake comparing the FFQ with 8 days of dietary intake (four 24-h recalls and a 4-day food record) among the WHI postmenopausal female population was reported to be 0.7, indicating that the WHI FFQ reasonably captures short-term intake of dietary magnesium in this population (17).

Blood collection and assessment of biomarkers

Fasting blood specimens were collected from all participants at baseline according to a standardized protocol. Aliquots of serum, plasma, and buffy coat were frozen and shipped on dry ice to a central repository and stored at -70°C for future assays in outside laboratories. hs-CRP was measured on a chemistry analyzer (Hitachi 911; Roche Diagnostics, Indianapolis, IN) using an immunoturbidimetric assay with reagents and calibrators (Denka Seiken, Niigata, Japan). IL-6 was measured by an ultrasensitive enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). TNF- α -R2, sICAM-1, sVCAM-1, and E-selectin were measured by an enzyme-linked immunosorbent assay (R&D Systems). The coefficients of variation for each analyte were 1.61% for hs-CRP, 7.6% for IL-6, 3.5% for TNF- α -R2 (11), 6.7% for sICAM-1, 8.9% for sVCAM-1, and 6.5% for E-selectin (12).

Statistical analysis

The distributions of hs-CRP, IL-6, TNF- α -R2, sICAM-1, sVCAM-1, and E-selectin were highly skewed, and thus log transformations were performed to achieve normal distributions. Multiple linear regression models (using PROC GLM) were used to compute geometric means of inflammatory and endothelial biomarkers across quintiles of dietary magnesium after adjustment for potential confounding variables. Geometric means were calculated by regressing the natural logarithmic values of the plasma concentration of inflammatory and endothelial biomarkers on dietary magnesium intake and taking the antilog of the resulting mean logarithmic value. Tests of linear trend across increasing quintiles of dietary magnesium intake were conducted by using the median value of each quintile as a continuous variable in the model. Regression coefficients for the change in inflammatory and endothelial biomarkers for an increase of 100 mg/day dietary magnesium stratified by ethnicity were also calculated using linear regression models. All multivariable models were adjusted for matching factors (age, ethnicity [white, African American, Hispanic/Latino, and Asian/Pacific Islander], clinical center, and time of blood draw), smoking status (never, past, and current), alcohol (never, past, and current drinkers), physical activity measured by expenditure of energy from recreational activity (continuous), total energy intake (continuous), BMI (contin-

uous), and type 2 diabetes case-control status (case and control subjects). To specifically address the independent association of magnesium intake, the final multivariate models were further adjusted for dietary fiber intake and other dietary factors such as fruit (continuous), vegetable (continuous), folate (continuous), *trans* fat (continuous), and saturated fat (continuous) intake. Variance inflation factors were examined for all models to check for multicollinearity issues. To further assess potential effect modification, subgroup analyses with prespecified factors including ethnicity (white, African American, Hispanic/Latino, and Asian/Pacific Islander), BMI (<25 and ≥ 25 kg/m^2), smoking status (never smokers and ever smokers [current and past smokers]), alcohol intake (nondrinkers and current drinkers), and dietary fiber intake (low fiber intake [<21 g/day] and high fiber intake [≥ 21 g/day]) were conducted. The Wald test was used to assess the significance of multiplicative interaction terms. To account for measurement error, we further conducted a deattenuation analysis using a regression coefficient relating the dietary magnesium measurement from the baseline FFQ to 8 days of food records obtained from 113 women in the WHI (17,18).

All P values were two-tailed, and $P < 0.05$ was considered to indicate statistical significance unless otherwise specified. All statistical analyses were conducted using SAS software (version 9.1; SAS Institute, Cary, NC).

RESULTS

Characteristics of participants are summarized in Table 1. On average, African Americans had a higher BMI, higher smoking rate, higher dietary fat intake, and lower intake of dietary fiber and total magnesium than women in other ethnic groups. Asian/Pacific Islanders had lower concentrations of hs-CRP, IL-6, TNF- α -R2, sICAM-1, and E-selectin than did other ethnic groups, whereas African Americans had higher concentrations of hs-CRP and IL-6.

Higher dietary magnesium intake was significantly associated with lower concentrations of inflammatory biomarkers including hs-CRP (P for linear trend = 0.003), IL-6 (P for linear trend < 0.0001), and TNF- α -R2 (P for linear trend = 0.0006) (Table 2). Further adjustment for dietary fiber intake attenuated but did not alter the linear trends for hs-CRP (P for linear trend = 0.009), IL-6 (P for linear trend = 0.0005), and TNF-

Table 1—Baseline characteristics according to ethnicity among postmenopausal women in the WHI-OS

	Race/ethnicity			
	White	African American	Hispanic/Latino	Asian/Pacific Islander
<i>n</i>	1,922	1,123	423	245
Mean age (years)	64 ± 6.9	61.0 ± 6.7	60 ± 6.8	64 ± 7.6
Mean BMI (kg/m ²)	29 ± 6.7	31 ± 7.1	29 ± 5.8	25 ± 4.5
Current smoker (%)	6	12	5	4
Current drinker (%)	69	51	51	40
Total energy expenditure from recreational physical activity (METs/week)	12 ± 13.3	11 ± 13.5	11 ± 14.9	14 ± 14.4
Current postmenopausal hormone use (%)	43	31	42	50
Nutrient intakes				
Carbohydrate (% of energy)	52 ± 9.4	52 ± 9.9	53 ± 10.1	56 ± 8.5
Protein (% of energy)	17 ± 3.3	16 ± 3.5	16 ± 3.6	16 ± 3.1
Fat (% of energy)	31 ± 8.5	33 ± 8.5	31 ± 9.6	29 ± 7.6
Dietary fiber (g/day)	17 ± 7.0	14 ± 7.1	16 ± 7.8	15 ± 6.5
Total magnesium intake (mg/day)*	335 ± 198	261 ± 170	279 ± 191	277 ± 152
Dietary magnesium intake (mg/day)†	263 ± 100	222 ± 140	229 ± 127	219 ± 96
Markers of inflammation				
hs-CRP (mg/l)	5.01 ± 7.2	5.91 ± 15.4	4.66 ± 5.5	2.11 ± 3.95
IL-6 (pg/ml)	3.26 ± 5.0	3.68 ± 4.58	3.14 ± 3.67	2.87 ± 6.24
TNF-α-R2 (pg/ml)	2,785 ± 871	2,423 ± 819	2,517 ± 767	2,292 ± 688
Markers of endothelial dysfunction				
sICAM-1 (ng/ml)	328 ± 94	270 ± 106	319 ± 103	255 ± 79
sVCAM-1 (ng/ml)	819 ± 278	639 ± 224	738 ± 301	690 ± 243
E-selectin (ng/ml)	47.0 ± 28.1	45.4 ± 24.5	48.7 ± 28.1	40.2 ± 23.5

Data are means ± SD unless otherwise indicated. *n* = 3,713. *Total magnesium intake includes intake from dietary and supplemental sources. †Dietary magnesium includes intake from diet alone. MET, metabolic equivalent.

α-R2 (*P* for linear trend = 0.10) in relation to magnesium intake. After additionally controlling for fruit and vegetable, folate, and saturated and *trans* fat intake, dietary magnesium intake remained significantly associated with hs-CRP and IL-6. Multivariable adjusted geometric means across increasing quintiles of dietary magnesium were 3.08, 2.63, 2.31, 2.53, and 2.16 mg/l for hs-CRP (*P* for linear trend = 0.005) and 2.91, 2.63, 2.45, 2.27, and 2.26 pg/ml for IL-6 (*P* for linear trend = 0.0005).

Similarly, we observed significant trends of an inverse association of dietary magnesium intake with concentrations of sVCAM-1 (*P* for linear trend = 0.06) and E-selectin (*P* for linear trend = 0.0007) (Table 2). After further adjustment for dietary fiber intake, the inverse association with sVCAM-1 remained strong (*P* for linear trend = 0.05) and the association with E-selectin was diminished. After additional adjustment for dietary factors, only sVCAM-1 remained significantly associated with dietary magnesium intake; multivariable-adjusted geometric means of sVCAM-1 across increasing quintiles of dietary magnesium were 707, 681, 673,

671, and 656 ng/ml (*P* for linear trend = 0.04). Tests of variance inflation indicated no serious multicollinearity issues in the models (variance inflation factor <5 for all models).

Assuming a linear relation, an increment of 100 mg/day in dietary magnesium intake was inversely associated with hs-CRP ($\beta = -0.23 \pm 0.07$; *P* = 0.002) and IL-6 ($\beta = -0.14 \pm 0.05$; *P* = 0.004) (model 3) (Table 3). After correction for measurement error, the association was strengthened for both hs-CRP ($\beta = -0.31 \pm 0.10$) and IL-6 ($\beta = -0.19 \pm 0.07$) (data not shown). There was a suggestion that the association of dietary magnesium with hs-CRP concentration varied by ethnicity, with strong associations among white and African American women but no associations among Hispanic and Asian-Pacific Islander women ($P_{\text{interaction}} = 0.05$). Inverse associations of dietary magnesium with IL-6 concentration were observed in each ethnicity, without statistical evidence of heterogeneity ($P_{\text{interaction}} = 0.77$). Compared with those in normal-weight women (BMI <25 kg/m²), concentrations of hs-CRP and IL-6 were significantly higher among

overweight and obese women (BMI ≥25 kg/m²), and inverse correlations with dietary magnesium were more pronounced (Fig. 1A and B). Similarly, in subgroup analyses stratified by smoking status (Fig. 1C and D), concentrations of hs-CRP and IL-6 were higher and demonstrated a stronger inverse association among ever smokers than never smokers (*P* for linear trend among ever smokers: hs-CRP *P* = 0.01 and IL-6 *P* = 0.0008). Tests of interaction were also significant for alcohol intake (IL-6 *P* = 0.04 and E-selectin *P* = 0.008) and dietary fiber intake (TNF-α-R2 *P* = 0.05).

CONCLUSIONS— In this large, ethnically diverse cohort of postmenopausal women, dietary magnesium intake was inversely associated with plasma concentrations of hs-CRP, IL-6, TNF-α-R2, sVCAM-1, and E-selectin independent of known risk factors for metabolic outcomes. Adjustment for dietary fiber intake attenuated but did not significantly alter these associations except in the case of E-selectin. After further adjustment for fruit and vegetable intake, folate intake, and saturated and *trans* fat intake, these

Table 2—Mean plasma concentrations of biomarkers of inflammation by quintile of dietary magnesium intake among postmenopausal women in the WHI-OS

	Dietary magnesium					P for linear trend
	Q1	Q2	Q3	Q4	Q5	
Median (mg/day)	168.5	204.4	233.2	263.1	310.2	
hs-CRP (mg/l)						
Model 1*	2.89 (2.59–3.23)	2.58 (2.31–2.87)	2.31 (2.07–2.58)	2.60 (2.31–2.92)	2.29 (2.03–2.58)	0.003
Model 2†	2.99 (2.61–3.41)	2.61 (2.33–2.92)	2.32 (2.07–2.59)	2.57 (2.28–2.90)	2.23 (1.96–2.55)	0.009
Model 3‡	3.08 (2.67–3.55)	2.63 (2.34–2.94)	2.31 (2.07–2.59)	2.53 (2.24–2.86)	2.16 (1.87–2.50)	0.005
IL-6 (pg/ml)						
Model 1*	2.91 (2.70–3.13)	2.63 (2.45–2.83)	2.46 (2.28–2.64)	2.28 (2.11–2.46)	2.27 (2.10–2.46)	<0.0001
Model 2†	2.86 (2.62–3.13)	2.62 (2.43–2.82)	2.45 (2.28–2.64)	2.29 (2.11–2.48)	2.30 (2.11–2.51)	0.0005
Model 3‡	2.91 (2.64–3.19)	2.63 (2.44–2.84)	2.45 (2.27–2.64)	2.27 (2.09–2.46)	2.26 (2.05–2.49)	0.0005
TNF- α -R2 (pg/ml)						
Model 1*	2,461 (2,392–2,532)	2,506 (2,437–2,577)	2,426 (2,357–2,497)	2,424 (2,352–2,498)	2,338 (2,267–2,411)	0.0006
Model 2†	2,429 (2,347–2,514)	2,493 (2,422–2,566)	2,423 (2,354–2,494)	2,434 (2,360–2,510)	2,361 (2,282–2,443)	0.10
Model 3‡	2,422 (2,335–2,513)	2,493 (2,421–2,567)	2,422 (2,353–2,493)	2,433 (2,358–2,511)	2,358 (2,270–2,448)	0.15
sICAM-1 (ng/ml)						
Model 1*	300 (291–310)	303 (293–313)	300 (290–311)	303 (292–314)	293 (282–304)	0.31
Model 2†	303 (291–316)	304 (294–315)	300 (290–311)	302 (291–314)	291 (279–303)	0.13
Model 3‡	301 (288–315)	304 (293–315)	300 (290–311)	302 (291–314)	292 (278–305)	0.29
sVCAM-1 (ng/ml)						
Model 1*	695 (673–718)	677 (655–699)	673 (651–695)	675 (652–699)	665 (642–689)	0.06
Model 2†	703 (676–731)	680 (657–703)	673 (651–696)	673 (650–697)	660 (634–686)	0.05
Model 3‡	707 (678–738)	681 (658–704)	673 (651–696)	671 (648–696)	656 (628–685)	0.04
E-selectin (ng/ml)						
Model 1*	45.5 (43.2–47.8)	42.5 (40.4–44.7)	40.8 (38.8–43.0)	40.8 (38.7–43.1)	40.7 (38.5–43.0)	0.0007
Model 2†	43.9 (41.2–46.7)	41.9 (39.8–44.1)	40.7 (38.6–42.9)	41.3 (39.1–43.6)	41.8 (39.4–44.5)	0.43
Model 3‡	43.8 (41.0–46.8)	41.9 (39.7–44.2)	40.7 (38.7–42.9)	41.4 (39.1–43.8)	42.1 (39.4–45.1)	0.62

Data are adjusted geometric means (95% CI). *Model 1 adjusted for matching factors (age, race/ethnicity, clinical center, and time of blood draw), smoking, alcohol, total energy expenditure from recreational physical activity/week, total energy intake, BMI, and case-control status. †Model 2 adjusted for variables in model 1 plus dietary fiber intake. ‡Model 3 adjusted for variables in model 2 plus fruit and vegetable intake, folate intake, and total saturated and *trans* fat intake. Q, quintile.

inverse trends remained significant for hs-CRP, IL-6, and sVCAM-1, suggesting that dietary magnesium may influence concentrations of these biomarkers independently of other dietary factors associated with inflammation.

Our findings support the notion that magnesium intake improves systemic inflammation and endothelial dysfunction and may play a role in the prevention of type 2 diabetes and metabolic syndrome, a longstanding relation with causality yet to be confirmed. These data are consistent with a considerable body of experimental evidence in animals suggesting that acute magnesium deficiency leads to an inflammatory response (5). Observational data in humans provide additional support for this relation. To date, several cross-sectional studies have reported a link between both low dietary magnesium intake

(1,6) and serum magnesium concentrations (19) and elevated hs-CRP in white populations. Fiber intake and dietary patterns high in magnesium were also inversely associated with hs-CRP concentrations in the Nurses' Health Study (8) and in NHANES 1999–2000 (20). However, the relation of magnesium intake to IL-6 and TNF- α is less clear. No association was reported between dietary magnesium intake and TNF- α -R2 or IL-6 in the Nurses' Health Study (6). However, a "western" dietary pattern, lower in magnesium-containing foods, was found to be associated with elevated IL-6 and other markers of inflammation and endothelial dysfunction (8). Our findings provide evidence that hs-CRP and IL-6 may serve as sensitive markers of inflammation that may directly benefit from increased magnesium intake through dietary sources.

CRP is an acute-phase reactant secreted by the liver in response to inflammatory cytokines including IL-6 and TNF- α and is an independent predictor of cardiovascular disease (21) and type 2 diabetes (11). IL-6 and TNF- α are proinflammatory cytokines secreted by macrophages and T-cells to stimulate an immune response to trauma. Low plasma magnesium concentrations and the subsequent disruption in intracellular magnesium homeostasis may play a role in activating the inflammatory response (22). Because hs-CRP is a more sensitive and robust marker of systemic inflammation than other inflammatory markers (23), it may be more readily detected. Our findings also suggest that IL-6 may be sensitive to fluctuations in dietary intake of magnesium. Although TNF- α -R2, a cell surface receptor believed to modulate the

Table 3—Linear regression coefficients for the relation between each increase of 100 mg/day in dietary magnesium intake and log-transformed biomarkers of inflammation and endothelial dysfunction among postmenopausal women in the WHI-OS

Ethnicity and biomarkers	Dietary magnesium		
	Model 1*	Model 2†	Model 3‡
All (n = 3,713)			
hs-CRP (mg/l)	-0.12 ± 0.04 (0.004)§	-0.17 ± 0.06 (0.006)	-0.23 ± 0.07 (0.002)
IL-6 (pg/ml)	-0.15 ± 0.03 (<0.0001)	-0.12 ± 0.04 (0.005)	-0.14 ± 0.05 (0.004)
TNF-α-R2 (pg/ml)	-0.04 ± 0.01 (0.0004)	-0.04 ± 0.02 (0.02)	-0.04 ± 0.02 (0.06)
sICAM-1 (ng/ml)	-0.01 ± 0.01 (0.30)	-0.04 ± 0.02 (0.05)	-0.03 ± 0.02 (0.15)
sVCAM-1 (ng/ml)	-0.02 ± 0.01 (0.13)‡	-0.04 ± 0.02 (0.05)	-0.04 ± 0.02 (0.07)
E-selectin (ng/ml)	-0.07 ± 0.02 (0.0005)	-0.02 ± 0.03 (0.47)	-0.01 ± 0.03 (0.71)
White (n = 1,922)			
hs-CRP (mg/l)	-0.07 ± 0.05 (0.13)	-0.16 ± 0.07 (0.03)	-0.27 ± 0.09 (0.004)
IL-6 (pg/ml)	-0.14 ± 0.04 (0.0001)	-0.09 ± 0.05 (0.08)	-0.19 ± 0.07 (0.006)
TNF-α-R2 (pg/ml)	-0.06 ± 0.01 (<0.0001)	-0.05 ± 0.02 (0.03)	-0.04 ± 0.03 (0.13)
sICAM-1 (ng/ml)	-0.03 ± 0.01 (0.01)	-0.02 ± 0.02 (0.37)	-0.02 ± 0.02 (0.41)
sVCAM-1 (ng/ml)	-0.03 ± 0.02 (0.07)	-0.02 ± 0.02 (0.38)	-0.03 ± 0.03 (0.30)
E-selectin (ng/ml)	-0.09 ± 0.03 (0.0003)	-0.04 ± 0.04 (0.25)	-0.03 ± 0.05 (0.48)
African American (n = 1,123)			
hs-CRP (mg/l)	-0.17 ± 0.07 (0.02)	-0.21 ± 0.11 (0.06)	-0.34 ± 0.14 (0.01)
IL-6 (pg/ml)	-0.18 ± 0.05 (0.0006)	-0.11 ± 0.08 (0.16)	-0.17 ± 0.10 (0.08)
TNF-α-R2 (pg/ml)	-0.02 ± 0.02 (0.37)	-0.007 ± 0.03 (0.83)	-0.008 ± 0.04 (0.83)
sICAM-1 (ng/ml)	0.009 ± 0.04 (0.79)	-0.08 ± 0.05 (0.12)	-0.08 ± 0.07 (0.21)
sVCAM-1 (ng/ml)	-0.02 ± 0.02 (0.50)	-0.04 ± 0.04 (0.24)	-0.03 ± 0.04 (0.42)
E-selectin (ng/ml)	-0.05 ± 0.03 (0.13)	-0.01 ± 0.05 (0.80)	-0.02 ± 0.06 (0.79)
Hispanic (n = 423)			
hs-CRP (mg/l)	-0.12 ± 0.10 (0.25)	-0.16 ± 0.16 (0.34)	0.07 ± 0.20 (0.73)
IL-6 (pg/ml)	-0.07 ± 0.08 (0.43)	-0.16 ± 0.13 (0.21)	-0.03 ± 0.16 (0.87)
TNF-α-R2 (pg/ml)	-0.04 ± 0.03 (0.19)	-0.09 ± 0.05 (0.05)	-0.09 ± 0.06 (0.12)
sICAM-1 (ng/ml)	-0.04 ± 0.03 (0.20)	-0.06 ± 0.05 (0.22)	0.01 ± 0.06 (0.85)
sVCAM-1 (ng/ml)	-0.01 ± 0.04 (0.71)	-0.10 ± 0.06 (0.12)	-0.14 ± 0.08 (0.07)
E-selectin (ng/ml)	-0.07 ± 0.06 (0.22)	-0.05 ± 0.09 (0.55)	-0.07 ± 0.11 (0.51)
Asian/Pacific Islander (n = 245)			
hs-CRP (mg/l)	-0.14 ± 0.34 (0.68)	-0.04 ± 0.52 (0.94)	0.23 ± 0.65 (0.72)
IL-6 (pg/ml)	-0.36 ± 0.14 (0.01)	-0.33 ± 0.21 (0.12)	-0.48 ± 0.27 (0.07)
TNF-α-R2 (pg/ml)	0.04 ± 0.05 (0.39)	0.001 ± 0.07 (0.98)	0.02 ± 0.09 (0.81)
sICAM-1 (ng/ml)	0.08 ± 0.05 (0.11)	0.03 ± 0.08 (0.73)	0.12 ± 0.09 (0.19)
sVCAM-1 (ng/ml)	0.02 ± 0.06 (0.71)	-0.06 ± 0.09 (0.48)	-0.07 ± 0.11 (0.52)
E-selectin (ng/ml)	0.09 ± 0.09 (0.30)	0.22 ± 0.13 (0.09)	0.44 ± 0.16 (0.008)

Data are means ± SEM (P). n = 3,713. *Model 1 adjusted for matching factors (age, race/ethnicity, clinical center, and time of blood draw), smoking, alcohol, total energy expenditure/week, total energy intake, BMI, and case-control status. †Model 2 adjusted for variables in model 1 plus dietary fiber. ‡Model 3 adjusted for variables in model 2 plus fruit, vegetable, folate intake, and total saturated and trans fat intake. §P values are from multiple linear regression models for the relation between dietary magnesium intake (per 100 mg/day increase) and log-transformed biomarkers.

action of TNF-α, was inversely associated with dietary magnesium intake before adjustment for dietary fiber intake, this relation was attenuated after adjustment, suggesting that the inverse association with TNF-α-R2 is most likely partially explained by the association with dietary fiber.

With regard to endothelial dysfunction, we observed that dietary magnesium was inversely associated with plasma concentrations of sVCAM-1 independently of other dietary factors. sVCAM-1 and sICAM-1 are cellular adhesion molecules belonging to the immunoglobulin family

and are primarily involved in the attachment and transendothelial migration of leukocytes in response to inflammatory cytokines (24). We observed no association between dietary magnesium intake and plasma concentrations of sICAM-1, a finding consistent with a cross-sectional study in the Nurses' Health Study (6). However, we observed a modest association with sVCAM-1 that remained significant even after adjustment for dietary factors associated with endothelial dysfunction and cardiovascular disease. Although the biological explanation for the variability in results across markers is not

clear, these findings further support the link between low magnesium intake and elevated concentrations of certain markers of endothelial dysfunction. E-selectin is a cellular adhesion molecule found primarily on the surface of stimulated endothelial cells and mediates the initial rolling of leukocytes along the endothelium (25). In the current study, we observed an inverse association of dietary magnesium intake with E-selectin that disappeared after we accounted for dietary factors.

In this multiethnic cohort of women, we observed notable variation in strengths of association across ethnicity. To our

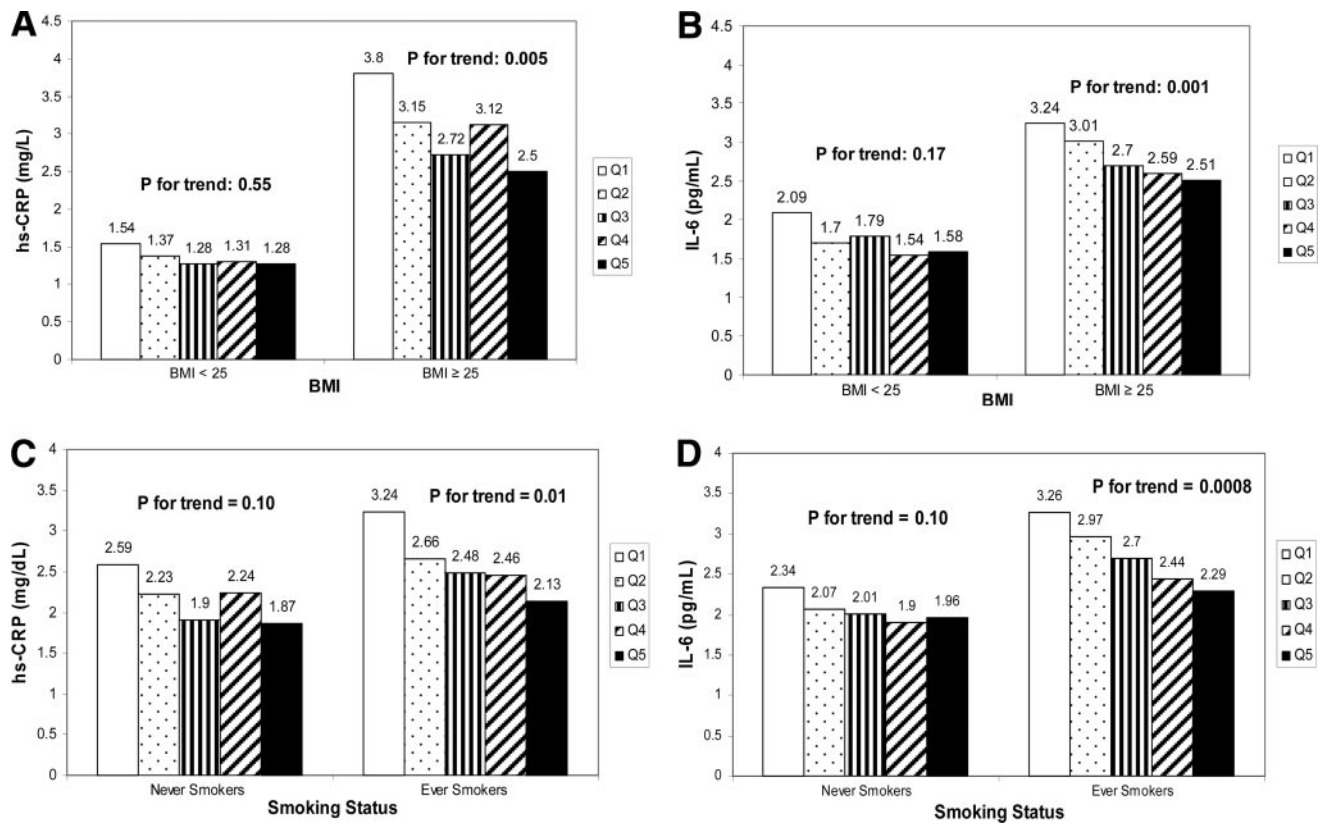


Figure 1—Multivariable adjusted means of hs-CRP (mg/l) and IL-6 (pg/ml) across increasing quintiles of dietary magnesium stratified by BMI (BMI <25 kg/m² [n = 1,047] and BMI ≥25 kg/m² [n = 2,666]) (A and B) and smoking status (never smokers [n = 1,959] and ever smokers [including past and current smokers, n = 1,707]) (C and D). Models are adjusted for matching factors (age, race/ethnicity, clinical center, and time of blood draw), smoking, alcohol, total energy expenditure/week, total energy intake, BMI, type 2 diabetes status, dietary fiber intake, fruit and vegetable intake, folate intake, and trans and saturated fat intake (model 3). Tests of linear trend were conducted by using the median value of each quintile as a continuous variable in the model.

knowledge, no previous work has examined potential ethnic differences in the relation of magnesium intake to systemic inflammation and endothelial dysfunction. Our findings of possible interactions in the current study should be interpreted with caution (i.e., hypothesis-generating) because ethnicity-specific sample sizes were small and the differences could partially be due to residual confounding from demographic and lifestyle factors. More importantly, the generally consistent patterns across the four ethnic groups provide additional evidence to support the notion that increased magnesium intake may have beneficial effects on alleviation of systemic inflammation and endothelial dysfunction. We also observed stronger associations of dietary magnesium intake with hs-CRP and IL-6 concentrations among subgroups with the highest concentrations of these inflammatory markers, i.e., overweight women and smokers. Similar findings among these high-risk subgroups were reported in the Women's Health Study (1), suggesting that higher magnesium intake may be most ben-

eficial among women who are predisposed to systemic inflammation.

There are several limitations that merit consideration. First, the measurement of dietary magnesium intake may be inaccurate because of self-report inconsistencies; however, magnesium intake assessed by our FFQ has a correlation of 0.7 when validated against dietary records (17). Furthermore, in analyses that corrected for measurement error, the relation between magnesium intake and biomarkers was strengthened. Second, dietary magnesium intake is highly correlated with several nutrients including dietary fiber, potassium, and folate, and magnesium is found in high concentrations in foods such as whole grains, nuts, and fruits and vegetables. Therefore, parsing out the independent effects of dietary magnesium is a challenge. In the current study, we sought to examine the independent effects of dietary magnesium through adjustment for dietary fiber as well as fruit and vegetable intake, folate intake, and saturated and *trans* fat intake in multiva-

riable-adjusted models to control for potential confounding. Our findings suggest that dietary magnesium is associated with several markers of inflammation and endothelial dysfunction independent of these dietary factors, although we could not completely exclude the possibility of residual confounding. This, coupled with the cross-sectional design, does limit our ability to make causal inferences regarding the effect of magnesium intake on markers of inflammation and endothelial dysfunction.

In summary, we found that dietary intake of magnesium was independently and inversely associated with plasma concentrations of hs-CRP, IL-6, and sVCAM-1 in postmenopausal women. These findings are consistent with those of previous studies mostly in whites and support the notion that diets high in magnesium-rich foods including whole grains, nuts, and leafy green vegetables should be encouraged for metabolic disease prevention.

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References

- Song Y, Ridker PM, Manson JE, Cook NR, Buring JE, Liu S. Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* 2005;28:1438–1444
- Song Y, Manson JE, Buring JE, Liu S. Dietary magnesium intake in relation to plasma insulin levels and risk of type 2 diabetes in women. *Diabetes Care* 2004;27:59–65
- Al-Delaimy WK, Rimm EB, Willett WC, Stampfer MJ, Hu FB. Magnesium intake and risk of coronary heart disease among men. *J Am Coll Nutr* 2004;23:63–70
- Larsson SC, Bergkvist L, Wolk A. Magnesium intake in relation to risk of colorectal cancer in women. *JAMA* 2005;293:86–89
- Malpuech-Brugère C, Nowacki W, Davéau M, Gueux E, Linaud C, Rock E, Lebreton J, Mazur A, Rayssiguier Y. Inflammatory response following acute magnesium deficiency in the rat. *Biochim Biophys Acta* 2000;1501:91–98
- Song Y, Li TY, van Dam RM, Manson JE, Hu FB. Magnesium intake and plasma concentrations of markers of systemic inflammation and endothelial dysfunction in women. *Am J Clin Nutr* 2007;85:1068–1074
- Rodríguez-Morán M, Guerrero-Romero F. Elevated concentrations of TNF- α are related to low serum magnesium levels in obese subjects. *Magn Res* 2004;17:189–196
- Lopez-Garcia E, Schulze MB, Fung TT, Meigs JB, Rifai N, Manson JE, Hu FB. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr* 2004;80:1029–1035
- Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. *Control Clin Trials* 1998;19:61–109
- Hays J, Hunt JR, Hubbell FA, Anderson GL, Limacher M, Allen C, Rossouw JE. The Women's Health Initiative recruitment methods and results. *Ann Epidemiol* 2003;13:S18–S77
- Liu S, Tinker L, Song Y, Rifai N, Bonds DE, Cook NR, Heiss G, Howard BV, Hotamisligil GS, Hu FB, Kuller LH, Manson JE. A prospective study of inflammatory cytokines and diabetes mellitus in a multi-ethnic cohort of postmenopausal women. *Arch Intern Med* 2007;167:1676–1685
- Song Y, Manson JE, Tinker L, Rifai N, Cook NR, Hu FB, Hotamisligil GS, Ridker PM, Rodriguez BL, Margolis KL, Oberman A, Liu S. Circulating levels of endothelial adhesion molecules and risk of diabetes in an ethnically diverse cohort of women. *Diabetes* 2007;56:1898–1904
- White E, Shattuck AL, Kristal AR, Urban N, Prentice RL, Henderson MM, Insull W Jr, Moskowitz M, Goldman S, Woods MN. Maintenance of a low-fat diet: follow-up of the Women's Health Trial. *Cancer Epidemiol Biomarkers Prev* 1992;1:315–323
- Kristal AR, Feng Z, Coates RJ, Oberman A, George V. Associations of race/ethnicity, education, and dietary intervention with the validity and reliability of a food frequency questionnaire: the Women's Health Trial Feasibility Study in Minority Populations. *Am J Epidemiol* 1997;146:856–869
- Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. *J Am Diet Assoc* 1988;88:1268–1271
- Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997;65:1220S–1231S
- 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
- Rosner B, Willett WC, Spiegelman D. Correction of logistic regression relative risk estimates and confidence intervals for systematic within-person measurement error. *Stat Med* 1989;8:1051–1069; discussion 1071–1073
- Rodríguez-Morán M, Guerrero-Romero F. Serum magnesium and C-reactive protein levels. *Arch Dis Child* 2008;93:676–680
- Ajani UA, Ford ES, Mokdad AH. Dietary fiber and C-reactive protein: findings from National Health and Nutrition Examination Survey data. *J Nutr* 2004;134:1181–1185
- Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000;342:836–843
- Paolisso G, Barbagallo M. Hypertension, diabetes mellitus, and insulin resistance: the role of intracellular magnesium. *Am J Hypertens* 1997;10:346–355
- Rifai N, Ridker PM. High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. *Clin Chem* 2001;47:403–411
- Albelda SM, Smith CW, Ward PA. Adhesion molecules and inflammatory injury. *FASEB J* 1994;8:504–512
- Schram MT, Stehouwer CD. Endothelial dysfunction, cellular adhesion molecules and the metabolic syndrome. *Horm Metab Res* 2005;37(Suppl. 1):49–55