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Dietary Micronutrient Intakes Are Associated with Markers of Inflammation but Not with Markers of Subclinical Atherosclerosis[1,2]

Marcia C. C. de Oliveira Otto,[3] Alvaro Alonso,[4] Duk-Hee Lee,[5] George L. Delclos,[3] Nancy S. Jenny,[6] Rui Jiang,[7] Joao A. Lima,[8] Elaine Symanski,[3] David R. Jacobs Jr,[4] and Jennifer A. Nettleton[3]*

[3]Division of Epidemiology, Human Genetics and Environmental Sciences, University of Texas School of Public Health, Houston, TX 77030; [4]Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN 55454; [5]Kyungpook National University, Seoul 0 700-422, South Korea; [6]Department of Pathology, University of Vermont College of Medicine, Burlington, VT 05446; [7]Department of Medicine, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032; and [8]Department of Cardiology, John Hopkins University, Baltimore, MD 21205

Abstract

Few studies have examined associations of dietary micronutrients with markers of inflammation and subclinical atherosclerosis. The present study investigated associations of heme iron, nonheme iron, zinc (Zn), magnesium (Mg), β-carotene, vitamin C, and vitamin E with C-reactive protein (CRP), IL-6, total homocysteine (tHcy), fibrinogen, coronary artery calcium, and common and internal carotid artery intima media thickness. Micronutrient intakes and markers of inflammation and subclinical atherosclerosis were studied in 5181 participants from the Multi-Ethnic Study of Atherosclerosis who were aged 45–84 y and free of diabetes and cardiovascular disease. Models were adjusted for energy intake, demographics, lifestyle characteristics, and BMI. Dietary nonheme iron and Mg intakes were inversely associated with tHcy concentrations (mean tHcy: 9.11, 8.86, 8.74, 8.71, and 8.50 μmol/L, and 9.20, 9.00, 8.65, 8.76, and 8.33 μmol/L across increasing quintiles of nonheme iron and Mg, respectively; P-trend < 0.001 for both). However, dietary Zn and heme iron were positively associated with CRP [mean: 1.73, 1.75, 1.78, 1.88, and 1.96 mg/L across increasing quintiles of Zn and 1.72, 1.76, 1.83, 1.86, and 1.94 mg/L across increasing quintiles of heme iron (P-trend = 0.002 and 0.01, respectively). Other tested micronutrient-marker associations were not significant. In conclusion, of the 49 tested associations, only 7 were significant. Although this study does not provide strong support for associations between the micronutrients and markers of inflammation and subclinical atherosclerosis, the results are consistent with dietary guidelines that advocate for a balanced diet that includes a variety of plant foods containing Mg, Zn, and nonheme iron. J. Nutr. 141: 1508–1515, 2011.

Introduction

Several studies (1–4), although not all (5,6), have reported significant associations between dietary micronutrients and the development of cardiovascular disease (CVD).[9] Inflammation and oxidative stress are key mechanisms underlying the development and progression of such conditions (7–9). In the Multi-Ethnic Study of Atherosclerosis (MESA), cross-sectional studies have shown that greater consumption of fruits, whole grains, nuts, and seeds and dietary patterns characterized by high intake of these foods are associated with lower concentrations of inflammatory markers (10–12). Micronutrients found in these foods are known for their antioxidant properties [e.g. zinc (Zn), β-carotene, and vitamins C and E] and essential roles in enzymatic function [e.g. magnesium (Mg) and Zn] (13,14); therefore, these micronutrients may partly explain associations of foods with inflammation. On the other hand, several prospective studies have shown a positive relationship between heme iron intake and the risk of CVD (1,5,15), consistent with observations of a positive association between red meat (a primary source of heme iron) and CVD risk (16,17). When present in quantities that exceed the physiologic iron-binding capacity, free iron can catalyze the generation of many reactive species (18) and consequently induce LDL oxidation (19,20) or amplify the inflammatory response (20,21). Yet little is known about the relationships of Mg, Zn, iron, and other antioxidants,

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[9] Abbreviations used: CAC, coronary artery calcification; CC, common carotid; CRP, C-reactive protein; CT, computed tomography; CVD, cardiovascular disease; IC, internal carotid; IMT, intima media thickness; MESA, Multi-Ethnic Study of Atherosclerosis; tHcy, total homocysteine.
* To whom correspondence should be addressed. E-mail: Jennifer.A.Nettleton@uth.tmc.edu.

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such as β-carotene and vitamins C and E, with inflammation and early stages of atherosclerosis.

Despite the suspected beneficial properties of these nutrients with respect to chronic diseases such as CVD, many U.S. adults do not meet the daily requirements for Zn, Mg, and foods rich in antioxidants in their diet (22–24). Thus, studying the role that these nutrients may play in inflammation and the development of atherosclerosis may provide additional incentive for intervention strategies that could reduce the public health burden of CVD.

This study describes the cross-sectional associations of dietary intakes of heme iron and nonheme iron, Zn, Mg, β-carotene, vitamin C, and vitamin E with markers of inflammation and measures of subclinical atherosclerosis in a multi-ethnic, population-based cohort. We hypothesized that intake of heme iron would be positively associated with markers of inflammation and subclinical atherosclerosis, whereas intakes of Zn, Mg, and vitamin antioxidants would be inversely associated with the same markers.

Methods

Study population. The MESA is a population-based, longitudinal study initiated in July 2000 designed to investigate the prevalence and progression of subclinical CVD as well as the potential factors involved in different stages of CVD development (25). The MESA study included 6814 participants aged 45–84 y and free of clinical CVD at baseline, recruited from 6 U.S. communities: Baltimore City and County, MD; Chicago, IL; Forsyth County, NC; New York, NY; Los Angeles County, CA; and St Paul, MN. All participants provided written informed consent. The study was approved by the institutional review board at the university affiliated with each participating field center. The cross-sectional analysis presented here excluded participants taking antiinflammatory medications at baseline (n = 150) and those who provided inadequate dietary information (n = 801). In addition, due to the potential for recent dietary change that would alter the meaning of the cross-sectional diet measure, participants with suspected diabetes at baseline [self-reported, receiving pharmacologic treatment for diabetes, or with single high fasting glucose concentration (>7 mmol/L)] were also excluded (n = 859). In total, 5181 participants were included in our analyses. The study population is similar to the original sample with respect to the distribution of risk factors included in the statistical models.

Dietary assessment. At the baseline examination, a 120-item, self-administered FFQ was used to assess usual food intake over the previous year. Participants reported frequency of intake (categories of times per day) and portion sizes (small, medium, and large) for selected foods and beverages. A section of the questionnaire included information on frequency of dietary supplement use, allowing quantification of intakes of nutrients from foods alone, as well as nutrients from foods plus supplements. The FFQ was modified from that used in the Insulin Resistance and Atherosclerosis Study to include items from Chinese cuisine (26). Validity of the parent FFQ was evaluated against eight 24-h recalls in Hispanics, African Americans, and non-Hispanic whites (26) and criterion validity of the modified MESA-specific FFQ was evaluated against plasma lipid concentrations within the MESA cohort (27). Energy-adjusted Pearson correlation coefficients between log-transformed nutrients quantified from FFQ vs. eight 24-h recalls were 0.40 for dietary vitamin C, 0.20 for dietary vitamin E, and 0.36 for total energy intake (unadjusted). Correlations were substantially higher among participants reporting no material change in their diet over 2–4 y between the administration of FFQ. For example, among urban non-Hispanic white women, correlation coefficients were 0.43 for vitamin E, 0.64 for vitamin C, and 0.81 for total energy intake (26).

Nutrient intakes from foods included in the MESA FFQ were estimated by multiplying the reported amount (frequency × serving size) of food consumed by the nutrient content of that food (Nutrition Data Systems for Research, University of Minnesota). Heme iron intake was estimated as 40% of the total iron content of all food items containing red meat, poultry, and fish (28). In most cases the results of the analyses described were not materially different when expressed as total nutrient intake (foods + supplemental sources) vs. nutrients from food sources only. Thus, for simplicity, only data using food-derived nutrients are shown.

Assessment of biomarkers of inflammation and subclinical atherosclerosis. Participants were required to fast, avoid heavy exercise 12 h prior to the exam, and avoid smoking on the morning of the examination (12). Blood samples were stored at −80°C and analyzed in batches in a single analytic laboratory. Details on analytical methods for each biomarker are provided below.

Inflammatory markers. Plasma IL-6 was measured by ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems); the laboratory analytical CV was 6.3%. Plasma C-reactive protein (CRP) was measured with a particle-enhanced immunonephelometric assay by a BNII nephelometer (High Sensitivity CRP; Dade Behring) and the analytical CV was 3.6%. Plasma total homocysteine (tHcy) was measured with a fluorescence polarization immunoassay (IMx Homocysteine Assay, Axis Biochemicals) using the IMx Analyzer (Abbott Diagnostics); the analytical CV was 4.5%. The detection range for this assay is 0.5–50 μmol/L. Fibrinogen antigen was measured using the BNII nephelometer (Antiserum to Human Fibrinogen, Dade Behring) and the analytical CV was 2.7%. The detection range for this assay is 0.58–183 g/L. The intra-assay and inter-assay CV are 2.7 and 2.6%, respectively.

CRP, IL-6, and fibrinogen were measured at the Laboratory for Clinical Biochemistry Research (University of Vermont) and tHcy was measured at the Biochemical Genetics Clinical Laboratory at Fairview-University Medical Center.

Subclinical atherosclerosis. Coronary artery calcification (CAC) was measured using chest computed tomography (CT) with either an electron beam CT scanner (Imatron C-150; Imatron; at the Chicago, Los Angeles, and New York centers) or a multi-detector CT (Baltimore, Forsyth County, and St. Paul field centers) (29,30). All participants were scanned twice by certified technologists and CT scans were read by a radiologist or a cardiologist at the central reading center of the Los Angeles Biomedical Research Institute at Harbor (University of California Medical Center) (30). CAC scores (Agatston scores) were determined by CT analysts unaware of participants' status regarding subclinical atherosclerosis. CAC presence was defined as an Agatston score > 0 (29). Agatston score > 100 was evaluated as an additional measure represented more advanced CAC burden.

Intima-media thickness of the common carotid artery (CC-IMT) and of the internal carotid artery (IC-IMT) were measured using high resolution B-mode ultrasonography (Logiq 700 ultrasound machine; GE Medical Systems) and calculated at the MESA ultrasound reading center (Tufts-New England Medical Center). The presence of atherosclerotic plaque was defined as any stenosis in either the right or left carotid artery (dichotomous variable) (29). IMT values were dichotomized with high IMT defined as ≥ the 75th percentile of the population distribution for each IC-IMT and CC-IMT separately (1.21 and 0.96 mm, respectively).

Assessment of additional covariates. Questionnaires were used to obtain information on demographics, education, medication use, smoking, and drinking status at the baseline examination. A detailed questionnaire was used to quantify physical activity for each participant. BMI was calculated using body weight and height (kg/m^2) measured at the baseline examination.

Statistical analysis. In this analysis, micronutrient intakes were adjusted for total energy intake using the nutrient residual methods (31). Participants were categorized into quintiles of energy-adjusted nutrient intakes for each of the 7 micronutrients of interest. To meet the criterion of normality for the linear models, concentrations of inflammatory markers were transformed and analyzed on the natural log scale. Values were subsequently back transformed to geometric means and 95% CI for presentation. Logistic regression was used to estimate OR and 95% CI for CAC score > 0 or IMT values ≥75th percentile across categories of each nutrient intake using the lowest intake category as the reference category. Results from analysis using different cutpoints for CAC (Agatston score >

0 and > 100) did not differ and therefore we present only results from CAC > 0. Likewise, because the patterns of association from logistic regression analyses were similar to those from linear regression analyses using continuous measures of the markers of subclinical atherosclerosis, only results from the former analyses are presented here.

Multivariable linear regression was used to evaluate cross-sectional associations between nutrients and inflammatory markers and risk of subclinical atherosclerosis across categories of each nutrient intake using the lowest intake category as the reference category. Model 1 adjusted for sex, age (continuous), race-ethnicity, total energy intake (continuous), and field center. Model 2 adjusted for variables in model 1 plus highest attained education level (less than high school, high school, more than high school), active and inactive leisure (active leisure activities included walking, sport, and conditioning activities, and inactive leisure activities included television viewing, reading, and light sitting activities; in metabolic equivalent-min/wk, both variables continuous), alcohol intake (10 g ethanol/d, continuous), smoking status (never, former, current smoker), BMI (continuous), daily total fiber intake (grams, continuous), and dietary supplement use (at least weekly, yes/no). Further adjustment for other micronutrients did not materially affect the results and thus are not presented, with the few exceptions noted in the text. To evaluate potential influence by other components of the food matrix, relevant associations were further adjusted for the main dietary sources of the nutrients of interest. Tests of linear trend were performed by modeling intake of the energy-adjusted micronutrient of interest as a continuous variable in the regression models.

Additional assessment of interactions with alcohol consumption (categorized as 0–9.99, 10.00–29.99, and ≥30.00 g ethanol/d), gender, and ethnicity is provided, because these factors were shown to alter nutrient metabolism and could potentially modify such relationships as well (15,32–34). In instances where relationships between strata appeared to materially differ, statistical tests of interaction were then performed by including an interaction term in model 2.

All *P*-values were 2 sided, with $P < 0.05$ indicating significance. All analyses were conducted with SAS version 9.2 (SAS Institute).

Results

Among the 5181 MESA participants included in this analysis, 42.8% were non-Hispanic whites, 24.4% were African Americans, 20.6% were Hispanics, and were 12.2% Chinese Americans. The mean age of the cohort was 61.8 y. Approximately two-thirds of the participants had >12 y of formal education. About 50% of the cohort never smoked, >80% reported drinking <10 g/d of alcohol (**Table 1**).

After adjustment for age, gender, and race-ethnicity, correlations between energy-adjusted nutrients ranged between −0.18 and 0.61 (**Table 2**).

TABLE 1 Baseline characteristics of MESA participants (n = 5181)[1]

Characteristics and plasma markers	
Demographic and lifestyle characteristics	
Sex, %	
Female	52.4
Male	47.6
Age, *y*	61.8 ± 10.3
Race/ethnicity, %	
White	42.8
African American	24.4
Hispanic	20.6
Asian	12.2
Education, %	
<High school	16.1
High school	17.6
>High school	66.3
Physical activity,[2] *MET- min/wk*	
Inactive leisure	1680 ± 1120
Active leisure	2500 ± 3050
Cigarette smoking status, %	
Never	50.7
Former	36.6
Current	12.7
BMI, kg/m^2	27.9 ± 5.2
Alcohol intake, *g/d*	5.6 ± 12.3
Dietary intakes	
Total energy,[3] *kcal/d*	1680 ± 797
Dietary nonheme iron, *mg/d*	11.2 ± 3.5
Dietary heme-iron, *mg/d*	0.78 ± 0.64
Dietary Zn, *mg/d*	8.3 ± 4.4
Dietary Mg, *mg/d*	255 ± 119
Dietary vitamin C, *mg/d*	100 ± 60
Dietary vitamin E, *mg/d*	10.4 ± 6.7
Dietary β-carotene, *mg/d*	3.26 ± 2.41
Dietary fiber intake, *g/d*	16.8 ± 8.4
Plasma markers of inflammation and subclinical atherosclerosis	
IL-6, *ng/L*	1.15 (1.16)
CRP, *mg/L*	1.79 (5.74)
tHcy, *μmol/L*	8.60 (3.81)
Fibrinogen, *g/L*	3.33 (7.13)

[1] Values are percent, mean ± SE, or median (SE).
[2] Metabolic equivalents in minutes per week.
[3] 1 kcal/d = 4.18 kJ/d.

Markers of inflammation

Iron. Dietary intake of nonheme iron was inversely associated with plasma concentrations of tHcy after adjustment for demographics, lifestyle characteristics, and BMI. The mean tHcy concentration was reduced by ~7% (95% CI: 3.8, 10.2) for participants in the highest quintile compared with those in the lowest quintile (*P*-trend < 0.0001; **Table 3**). The association remained significant after further adjustment for dietary intakes of Mg, Zn, folate, vitamin B-6, and B-12 (data not shown).

The food groups with the greatest contribution to nonheme iron intake in the MESA cohort were: white bread (22%), whole grains (17%), red meat (6%), and legumes (5%). The inverse association between nonheme iron and tHcy was independent of intakes of white bread, red meat, and legumes (data not shown). However, when whole grain intake was included in model 2, the association was attenuated and no longer significant (mean tHcy concentrations for increasing quintiles of nonheme- iron intake: 8.91, 8.75, 8.73, 8.81, and 8.71 μmol/L; *P*-trend = 0.18).

Heme iron was positively associated with plasma concentrations of CRP (Table 3). Those in the highest quintile of heme iron intake had 12% (95% CI: 3.0, 21.8) higher plasma CRP concentrations compared with those in the lowest quintile. After further adjustment for dietary intakes of Mg and Zn, mean CRP concentrations for increasing quintiles of heme iron intake were 1.75, 1.77, 1.83, 1.85, and 1.89 mg/L (*P*-trend = 0.11). The association with CRP was independent of consumption of red meat, poultry, and fish (the primary sources of heme iron in MESA; data not shown). Iron (heme or nonheme) was not associated with IL-6 or fibrinogen (Table 3).

Mg. Dietary Mg intake was significantly inversely associated with plasma concentrations of tHcy but positively associated with fibrinogen. Participants in the highest quintile of Mg intake had 10% (95% CI: 7.0, 12.9) lower concentrations of plasma tHcy. In contrast, participants in the highest quintile of Mg

TABLE 2 Adjusted Spearman correlation coefficients between energy-adjusted nutrient intakes (MESA, $n = 5181$)[1,2]

	Heme iron	Nonheme iron	Mg	Zn	Vitamin C	Vitamin E	β-Carotene
Heme iron	1	−0.03	0.02*	0.39	0.07	−0.09	0.25
Nonheme iron		1	0.57	0.37	0.39	0.08	0.35
Mg			1	0.61	0.44	−0.15	0.39
Zn				1	0.20	−0.18	0.25
Vitamin C					1	0.11	0.55
Vitamin E						1	0.08

[1] All coefficients are significant, $P < 0.05$, except *$P = 0.14$.
[2] Correlations adjusted for age, gender, and race-ethnicity.

intake had ~3% (95% CI: 0.01, 4.7) higher plasma fibrinogen concentrations compared with those in the lowest quintile (Table 3). The association between Mg and fibrinogen was not significant after adjustment for intakes of Zn and heme iron intake (geometric means for fibrinogen by quintile of Mg intake: 3.29, 3.33, 3.34, 3.35, and 3.41 g/L; *P*-trend = 0.07).

The main sources of Mg intake in the MESA population were milk (10%), fruits (8.5%), whole grains (7%), white bread (7%), and legumes (6.5%). Associations with plasma tHcy and fibrinogen remained significant after further adjustment for each of those food items (data not shown). Dietary Mg intake was not associated with other markers of inflammation (Table 3).

Zn. Dietary Zn was positively associated with plasma CRP concentrations [difference between extreme quintiles: 13% (95% CI: 7.0, 12.9)]. The association remained significant after further adjustment for the intakes of heme iron and Mg intake (data not shown). In contrast, dietary Zn was inversely associated with tHcy concentrations [difference between extreme quintiles: 7.7% (95% CI: 5.2, 10.1)], but the association with tHcy was attenuated and not significant when adjusted for other nutrients (geometric means for ascending quintiles: 9.00, 8.89, 8.77, 8.66, and 8.58 μmol/L; *P*-trend = 0.25; model 3, data not tabulated).

Important dietary sources of Zn in the MESA population were red meat (14%), milk (9%), whole grains (9%), fish (7.5%), and poultry (7%). The association between Zn and plasma CRP was independent of further adjustment for all selected food sources (data not shown). On the other hand, the inverse association between Zn and tHcy was attenuated and no longer significant after further adjustment for whole grain intake (mean tHcy concentrations for increasing quintiles of Zn intake were 9.10, 8.92, 8.77, 8.62, and 8.50 μmol/L; *P*-trend = 0.18; data not tabulated). Dietary Zn intake was not associated with other markers of inflammation (Table 3).

Antioxidants. No association was found between dietary intakes of β-carotene or vitamin E and markers of inflammation. However, vitamin C was positively associated with tHcy concentrations. After further adjustment for intakes of dietary Mg, Zn, and heme iron, mean tHcy concentrations for increasing quintiles of vitamin C intake were 8.67, 8.67, 8.76, 8.89, and 8.92 μmol/L; *P*-trend = 0.01).

Markers of subclinical atherosclerosis

The studied micronutrients showed no significant associations with any of the markers of subclinical atherosclerosis, with the exception of an inverse association between Mg and CC-IMT. Participants in the highest quintile of Mg intake had a 24% lower odds of high CC-IMT compared with those in the lowest quintile (**Table 4**).

Interactions

There was no evidence of interaction between the studied nutrients and sex, race-ethnicity, or drinking status. Also, no significant interactions were found between smoking status and β-carotene, vitamin C, and vitamin E.

Discussion

In this cross-sectional analysis of 5181 non-Hispanic white, African American, Hispanic, and Chinese American adults, plasma tHcy was inversely associated with intakes of nonheme iron and Mg after adjustment for demographic, lifestyle, and dietary confounders, including related micronutrients (vitamins B-6 and B-12 and folate). In contrast to these favorable micronutrient associations, greater intakes of heme iron and Zn were each associated with higher plasma CRP concentrations. Associations between micronutrient intakes and markers of atherosclerosis (CAC and IMT) were weak and inconsistent. Results were similar across the 4 race-ethnicities and similar between men and women.

Very few epidemiological studies have evaluated associations of micronutrients with markers of inflammation and atherosclerosis. A nested case-control study using data from the Atherosclerosis Risk in Communities study evaluated associations between the intakes of several micronutrients and tHcy concentrations in people with (IMT > 2.5mm) and without (IMT ≤ 2.5 mm) subclinical atherosclerosis (35). In this study, greater total dietary iron intake was associated with lower tHcy concentrations among those who did not use supplements (35). However, the association was no longer significant after further adjustment for intake of cold cereal, which is in agreement with our observations. The attenuation of measures of association after adjustment for cold cereal or whole grains in general suggests that results were confounded by the generally favorable association of these foods on CVD and its risk factors (36,37). Overall, our findings are consistent with previous epidemiological studies evaluating the main food sources of Mg, nonheme iron, and other suspected antioxidant micronutrients. For example, intakes of whole grains, fruits, and vegetables, which are important sources of Mg and nonheme iron, have each been inversely associated with tHcy (11,12). In addition, a higher red meat intake (predominant source of heme iron) was associated with higher CRP concentrations in another study (38). Also concordant with our findings are the results from the Supplementation en Vitamines et Mineraux Antioxydants, a randomized trial conducted in France that showed no association between total dietary iron and IMT in men and women (6). On the other hand, we did not observe previously reported inverse associations between Mg and IL-6 (39), Mg and CRP (40), and vitamins C and E and CRP (41).

The inverse association between Mg and tHcy observed in our data is biologically plausible and consistent with the hypothesis that greater intake of nutrients with antioxidative/antiinflammatory properties would be associated with lower levels of analytes reflecting inflammatory processes. However, the mechanism underlying this relationship is uncertain. Mg is an essential cofactor for several enzymes (42,43). As observed with vitamins B-6 and B-12 and folate (44), low intakes of Mg could inhibit tHcy metabolism, resulting in increased plasma tHcy concentrations. We also observed an inverse association between nonheme iron and tHcy, which may be a consequence of the food source more than the nutrient per se. Higher intakes of vegetables and whole grains, sources of nonheme iron, may be part of a dietary pattern that influences the inflammatory

TABLE 3 Association of dietary micronutrient intakes with plasma biomarkers of inflammation in 5181 MESA participants[1–3]

	Quintiles of nutrient intake					
	Q1	Q2	Q3	Q4	Q5	*P*-trend
n	1037	1036	1035	1037	1036	
Heme iron, *mg/d*	≤0.44	0.45–0.62	0.63–0.79	0.80–1.06	≥1.07	
IL-6, *ng/L*	1.24 (1.19, 1.28)	1.16 (1.11, 1.20)	1.17 (1.12, 1.21)	1.18 (1.14, 1.23)	1.16 (1.12, 1.21)	0.09
CRP, *mg/L*	1.72 (1.61, 1.83)	1.76 (1.65, 1.87)	1.83 (1.72, 1.95)	1.86 (1.75, 1.98)	1.94 (1.82, 2.08)	0.01
tHcy, *μmol/L*	8.86 (8.71, 9.02)	8.85 (8.71, 9.00)	8.73 (8.59, 8.88)	8.73 (8.59, 8.88)	8.72 (8.57, 8.88)	0.15
Fibrinogen, *g/L*	3.33 (3.29, 3.37)	3.34 (3.30, 3.37)	3.36 (3.32, 3.40)	3.36 (3.32, 3.40)	3.35 (3.31, 3.39)	0.46
Nonheme iron, *mg/d*	≤8.5	8.6–9.8	9.9–11.4	11.5–13.5	≥13.6	
IL-6, *ng/L*	1.17 (1.13, 1.23)	1.18 (1.14, 1.23)	1.20 (1.16, 1.25)	1.18 (1.13, 1.22)	1.16 (1.11, 1.22)	0.67
CRP, *mg/L*	1.89 (1.76, 2.04)	1.79 (1.68, 1.91)	1.89 (1.78, 2.01)	1.87 (1.76, 2.00)	1.67 (1.54, 1.80)	0.09
tHcy, *μmol/L*	9.11 (8.94, 9.29)	8.86 (8.71, 9.01)	8.74 (8.60, 8.89)	8.71 (8.56, 8.85)	8.50 (8.33, 8.67)	<0.0001
Fibrinogen, *g/L*	3.34 (3.29, 3.39)	3.35 (3.31, 3.39)	3.34 (3.30, 3.38)	3.36 (3.31, 3.39)	3.35 (3.30, 3.40)	0.82
Mg, *mg/d*	≤206	207–237	238–262	263–298	≥299	
IL-6, *ng/L*	1.21 (1.16, 1.26)	1.20 (1.16, 1.25)	1.15 (1.11, 1.19)	1.17 (1.13, 1.22)	1.17 (1.12,1.21)	0.22
CRP, *mg/L*	1.80 (1.67, 1.93)	1.83 (1.72, 1.95)	1.82 (1.71, 1.94)	1.81 (1.70, 1.93)	1.84 (1.71, 1.97)	0.78
tHcy, *μmol/L*	9.20 (9.02, 9.37)	9.00 (8.85, 9.15)	8.65 (8.51, 8.80)	8.76 (8.61, 8.90)	8.33 (8.17, 8.48)	<0.0001
Fibrinogen, *g/L*	3.31 (3.27, 3.36)	3.34 (3.30, 3.40)	3.34 (3.30, 3.38)	3.35 (3.31, 3.39)	3.40 (3.36, 3.44)	0.001
Zn, *mg/d*	≤6.6	6.7–7.5	7.6–8.4	8.5–9.6	≥9.7	
IL-6, *ng/L*	1.18 (1.13, 1.22)	1.19 (1.15, 1.23)	1.19 (1.15, 1.23)	1.18 (1.14, 1.23)	1.16 (1.12, 1.20)	0.46
CRP, *mg/L*	1.73 (1.62, 1.85)	1.75 (1.64, 1.86)	1.78 (1.67, 1.90)	1.88 (1.77, 2.01)	1.96 (1.84, 2.09)	0.002
tHcy, *μmol/L*	9.14 (8.98, 9.30)	8.94 (8.79, 9.09)	8.77 (8.63, 8.92)	8.61 (8.47, 8.76)	8.46 (8.32, 8.61)	<0.0001
Fibrinogen, *g/L*	3.32 (3.28, 3.36)	3.33 (3.29, 3.36)	3.37 (3.33, 3.40)	3.38 (3.34, 3.42)	3.34 (3.30, 3.38)	0.24
Vitamin C, *mg/d*	≤52	53–75	76–98	99–130	≥131	
IL-6, *ng/L*	1.23 (1.18, 1.28)	1.15 (1.11, 1.20)	1.17 (1.13, 1.21)	1.14 (1.10, 1.18)	1.21 (1.16, 1.26)	0.35
CRP, *mg/L*	1.82 (1.70, 1.96)	1.78 (1.67, 1.90)	1.89 (1.78, 2.02)	1.81 (1.70, 1.93)	1.79 (1.67, 1.92)	0.84
tHcy, *μmol/L*	8.72 (8.56, 8.88)	8.67 (8.52, 8.81)	8.75 (8.61, 8.90)	8.87 (8.73, 9.02)	8.90 (8.74, 9.06)	0.04
Fibrinogen, *g/L*	3.35 (3.31, 3.39)	3.37 (3.33, 3.41)	3.35 (3.31, 3.38)	3.34 (3.30, 3.38)	3.33 (3.29, 3.38)	0.23
Vitamin E, *mg/d*	≤6.9	7.0–8.4	8.5–9.7	9.8–11.9	≥12.0	
IL-6, *ng/L*	1.24 (1.19, 1.29)	1.15 (1.11, 1.20)	1.16 (1.12, 1.21)	1.18 (1.13, 1.22)	1.17 (1.13, 1.21)	0.38
CRP, *mg/L*	1.85 (1.73, 1.98)	1.81 (1.70, 1.92)	1.81 (1.70, 1.93)	1.82 (1.71, 1.94)	1.80 (1.69, 1.92)	0.69
tHcy, *μmol/L*	8.75 (8.60, 8.90)	8.71 (8.57, 8.86)	8.83 (8.68, 8.98)	8.82 (8.68, 8.97)	8.79 (8.64, 8.94)	0.93
Fibrinogen, *g/L*	3.37 (3.33, 3.41)	3.36 (3.32, 3.39)	3.36 (3.32, 3.40)	3.34 (3.30, 3.38)	3.29 (3.25, 3.33)	0.28
β-Carotene, *mg/d*	≤1.56	1.57–2.27	2.28–3.23	3.24–4.70	≥4.71	
IL-6, *ng/L*	1.21 (1.17, 1.26)	1.18 (1.14, 1.23)	1.18 (1.14, 1.23)	1.17 (1.13, 1.21)	1.15 (1.10, 1.20)	0.07
CRP, *mg/L*	1.78 (1.66, 1.91)	1.82 (1.71, 1.95)	1.90 (1.78, 2.02)	1.79 (1.68, 1.91)	1.80 (1.68, 1.94)	0.54
tHcy, *μmol/L*	8.76 (8.60, 8.92)	8.92 (8.77, 9.08)	8.79 (8.65, 8.94)	8.78 (8.63, 8.92)	8.66 (8.49, 8.82)	0.83
Fibrinogen, *g/L*	3.34 (3.29, 3.38)	3.34 (3.30, 3.38)	3.35 (3.31, 3.39)	3.35 (3.31, 3.39)	3.36 (3.31, 3.40)	0.70

[1] Values are geometric means and 95% CI for concentrations of the biomarkers of inflammation by quintiles of micronutrient intake.
[2] All statistical models were adjusted for energy intake (kcal/d), age (y), sex, race-ethnicity (non-Hispanic whites, African Americans, Hispanics, and Chinese Americans), education (<high school, high school, > high school), study center, alcohol intake (g/d), physical activity (active and inactive leisure in metabolic equivalents per min/wk), BMI (kg/m^2), fiber intake (g/d), cigarette smoking (never, current, or former smoker), dietary supplement use (>1/wk, yes or no).
[3] Missing values: 143, 43, 21, and 41 for IL-6, CRP, tHcy, and fibrinogen models, respectively.

process. In addition, intakes of nonheme iron, and vegetables more generally, are correlated with other indicators of healthy lifestyle that have been associated with plasma tHcy concentrations, such as physical activity and not smoking (45). Therefore, even though we did adjust for these nutrients and lifestyle, residual confounding remains as a potential explanation.

Although associations between the studied nutrients and markers of atherosclerosis were generally not significant, greater Mg intake was associated with slightly lower odds of high CC-IMT (≥75th percentile). Several possible mechanisms may explain the observed association between Mg intake and CC-IMT. Previous studies have reported that higher Mg intakes may improve insulin resistance (13,46) and lipoprotein metabolism (47), both processes associated with inflammation and atherosclerosis. Mg is also hypothesized to have antihypertensive properties (48). Although CC-IMT is a marker of subclinical atherosclerosis that is closely related to blood pressure, we did not observe a substantial alteration in the association when further adjusting for systolic and diastolic blood pressure, which suggests that Mg intake may influence CC-IMT through different mechanisms than blood pressure regulation.

Inflammatory responses are triggered by oxidative stress (8,49). Contrary to our hypothesis and with previous studies evaluating similar relationships, the selected antioxidants were not associated with inflammatory markers in the MESA population, except for a modest positive association between vitamin C and tHcy concentrations. In addition, we did not identify associations between antioxidant intakes and subclinical atherosclerosis. The failure to detect such associations may be due to the cross-sectional design of the study, which cannot capture

TABLE 4 Association of dietary micronutrient intakes with subclinical atherosclerosis in 5181 MESA participants[1–4]

	Quintiles of energy-adjusted nutrient intake					
	Q1	Q2	Q3	Q4	Q5	*P*-trend
n	1037	1036	1035	1037	1036	
Heme iron, *mg/d*	≤0.44	0.45–0.62	0.63–0.79	0.80–1.06	≥1.07	
CAC	1.00	1.03 (0.83, 1.26)	1.07 (0.86, 1.32)	1.02 (0.82, 1.26)	1.07 (0.87, 1.33)	0.57
IC-IMT	1.00	0.99 (0.79, 1.24)	0.83 (0.66, 1.04)	0.89 (0.70, 1.12)	0.84 (0.66, 1.06)	0.10
CC-IMT	1.00	0.94 (0.75, 1.17)	1.02 (0.81, 1.28)	1.24 (0.98, 1.57)	0.95 (0.74, 1.20)	0.82
Nonheme iron, *mg/d*	≤8.5	8.6–9.8	9.9–11.4	11.5–13.5	≥13.6	
CAC	1.00	0.90 (0.73, 1.11)	1.01 (0.81, 1.26)	1.07 (0.84, 1.36)	0.96 (0.73, 1.26)	0.85
IC-IMT	1.00	0.88 (0.69, 1.11)	1.10 (0.86, 1.41)	0.99 (0.77, 1.28)	0.85 (0.63, 1.15)	0.41
CC-IMT	1.00	0.83 (0.66, 1.06)	1.10 (0.85, 1.41)	0.85 (0.65, 1.10)	0.89 (0.65, 1.21)	0.49
Mg, *mg/d*	≤206	207–237	238–262	263–298	≥299	
CAC	1.00	0.80 (0.65, 0.99)	1.05 (0.84, 1.31)	0.87 (0.69, 1.10)	0.92 (0.71, 1.18)	0.52
IC-IMT	1.00	1.04 (0.83, 1.32)	1.14 (0.89, 1.46)	0.90 (0.70, 1.15)	1.01 (0.77, 1.34)	0.41
CC-IMT	1.00	0.93 (0.74, 1.19)	0.91 (0.71, 1.16)	0.87 (0.67, 1.12)	0.76 (0.58, 1.01)	0.001
Zn, *mg/d*	≤6.6	6.7–7.5	7.6–8.4	8.5–9.6	≥9.7	
CAC	1.00	0.81 (0.66, 1.00)	0.77 (0.63, 0.96)	0.88 (0.71, 1.08)	0.89 (0.72, 1.10)	0.64
IC-IMT	1.00	1.11 (0.88, 1.39)	0.95 (0.75, 1.19)	1.03 (0.82, 1.30)	0.94 (0.75, 1.18)	0.62
CC-IMT	1.00	0.97 (0.77, 1.21)	0.98 (0.77, 1.24)	1.01 (0.79, 1.28)	0.89 (0.70, 1.12)	0.11
Vitamin C, *mg/d*	≤52	53–75	76–98	99–130	≥131	
CAC	1.00	1.23 (1.00, 1.51)	1.24 (1.00, 1.53)	1.05 (0.84, 1.31)	1.03 (0.81, 1.32)	0.99
IC-IMT	1.00	1.23 (0.98, 1.54)	1.11 (0.88, 1.40)	1.03 (0.81, 1.31)	1.00 (0.77, 1.30)	0.71
CC-IMT	1.00	1.00 (0.79, 1.26)	1.10 (0.86, 1.40)	0.75 (0.59, 0.95)	0.95 (0.73, 1.24)	0.67
Vitamin E, *mg/d*	≤6.9	7.0–8.4	8.5–9.7	9.8–11.9	≥12.0	
CAC	1.00	0.82 (0.66, 1.01)	0.85 (0.69, 1.06)	0.94 (0.76, 1.16)	0.95 (0.77, 1.17)	0.99
IC-IMT	1.00	0.98 (0.77, 1.23)	0.90 (0.71, 1.14)	1.12 (0.89, 1.42)	1.06 (0.84, 1.33)	0.06
CC-IMT	1.00	0.97 (0.77, 1.23)	1.03 (0.81, 1.31)	1.03 (0.81, 1.31)	1.14 (0.90, 1.43)	0.98
β-Carotene, *mg/d*	≤1.56	1.57–2.27	2.28–3.23	3.24–4.70	≥4.71	
CAC	1.00	1.23 (1.00, 1.51)	1.21 (0.97, 1.49)	1.30 (1.04, 1.62)	1.24 (0.96, 1.59)	0.09
IC-IMT	1.00	0.96 (0.77, 1.20)	0.98 (0.77, 1.23)	0.83 (0.66, 1.06)	0.92 (0.70, 1.21)	0.81
CC-IMT	1.00	1.24 (0.98, 1.55)	1.12 (0.89, 1.41)	1.23 (0.97, 1.58)	1.38 (1.04, 1.82)	0.15

[1] Values are OR and 95% CI for risk of subclinical atherosclerosis according to quintiles of micronutrient intakes.
[2] All statistical models were adjusted for energy intake (kcal/d), age (y), sex, race-ethnicity (non-Hispanic whites, African Americans, Hispanics, and Chinese Americans), education (<high school, high school, > high school), study center, alcohol intake (g/d), physical activity (active and inactive leisure in metabolic equivalents per min/wk), BMI (kg/m^2), fiber intake (g/d), cigarette smoking (never, current, or former smoker), dietary supplement use (>1/wk, yes or no).
[3] Missing values: 6, 125, and 68 for CAC, IC-IMT, and CC-IMT models, respectively.
[4] CAC was defined as Agatston score > 0 vs. ≤0; IC-IMT was defined as ≥1.2 mm vs. <1.2 mm; CC-IMT was defined as ≥0.96 mm vs. < 0.96 mm.

lifetime or chronic dietary intake or to limited accuracy in the assessment of nutrient intake, as reported by the Coronary Artery Risk Development in Young Adults Study (50). It is also possible that antioxidant intake may influence CVD risk (4,51) through different pathways than reduction of CAC or IMT.

In this cohort, red meat was one of the primary sources of dietary Zn intake, accounting for ~14% of the total Zn consumption. Although the positive association between dietary Zn intake and CRP was independent of red meat intake, it is possible that this relationship may reflect other dietary factors correlated with red meat consumption, such as higher intakes of saturated fat and processed foods. After further adjustment for saturated fat intake, the association was of borderline significance (P = 0.06). Furthermore, this relationship was not significant for the sum of Zn from diet and supplements, which supports the conclusion that the association observed with dietary Zn intake may be confounded by other factors positively associated with CRP concentrations rather than Zn intake per se. This finding is consistent with a previous MESA study showing that a dietary pattern characterized by high intake of red meat was positively associated with CRP concentrations (12).

The present investigation is not without limitations. The inconsistencies observed for some of the nutrient-marker relationships underscore the complexity of the diet and difficulty in isolating individual nutrient associations. Individual nutrients are consumed as part of food and each food is a complex mixture of ingredients designed by evolution to support the life of the organism eaten; this complex mixture is potentially highly synergistic (52). In addition, because of the cross-sectional design, no assertion can be made about the temporality of the relationships evaluated. Furthermore, limitations associated with the use of FFQ to estimate nutrient intake include the inability to comprehensively capture food intake and the restriction to a single food recipe to derive nutrients of a particular item, which may not accurately represent all the possible food recipes for that 1 item. Also, relatively low correlations between micronutrient intake from the FFQ and 24-h recall shown on validation studies may result in misclassification of dietary intake status and attenuation on the measures of association. Similarly,

even though we excluded participants with symptomatic CVD and prevalent type II diabetes, the magnitude of the associations are likely to be underestimated due to misclassification of long-term dietary intakes resulting from change in dietary behavior. Lastly, several recent trails aimed at lowering homocysteine have shown no benefit for CVD risk reduction (53). Nevertheless, research does suggest that, even if not causally related to CVD, elevated concentrations of homocysteine do denote an underlying pathologic process that increases CVD risk (54).

We are aware of the statistical implications of a large number of tests utilized in this work. A total of 7 significant associations was identified among 49 associations tested, which is 2 times the number of significant associations we would expect to observe if the null hypothesis is true (i.e. due to chance alone, using an uncorrected α where significance is <0.05). As pointed out by Rothman (5), the adjustment of test statistics to account for multiple comparison reduces type I error but also increases the chance of missing relevant associations if the alternative hypothesis is true. Willet has also indicated that such adjustment "unduly reduces power" (6), which can be particularly problematic when evaluating diet-disease associations, because they are moderate in nature. Instead of adopting *P*-value adjustment, we have interpreted our results in the light of consistency with previous observations, biological plausibility, and construct validity of the method used.

In conclusion, in this study of men and women from 4 race-ethnicity groups, greater dietary intakes of Mg and nonheme iron were associated with lower concentrations of tHcy, whereas greater intake of heme iron was associated with greater CRP concentrations. Dietary micronutrients were not consistently associated with markers of subclinical atherosclerosis. Most findings of this study showed no association with selected nutrients; however, significant findings were generally consistent with dietary recommendations. Comparing such findings to those of other studies [e.g. the study of dietary patterns within MESA (13)], a balanced diet that includes a variety of plant foods containing these nutrients, such as fruits, vegetables, and whole grains, is the best approach for maintaining health and minimizing chronic disease risk.

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Literature Cited

1. van der A DL, Peeters PH, Grobbee DE, Marx JJ, van der Schouw YT. Dietary haem iron and coronary heart disease in women. Eur Heart J. 2005;26:257–62.
2. Champagne CM. Magnesium in hypertension, cardiovascular disease, metabolic syndrome, and other conditions: a review. Nutr Clin Pract. 2008;23:142–51.
3. Singh RB, Niaz MA, Rastogi SS, Bajaj S, Gaoli Z, Shoumin Z. Current zinc intake and risk of diabetes and coronary artery disease and factors associated with insulin resistance in rural and urban populations of north India. J Am Coll Nutr. 1998;17:564–70.
4. Asplund K. Antioxidant vitamins in the prevention of cardiovascular disease: a systematic review. J Intern Med. 2002;251:372–92.
5. Klipstein-Grobusch K, Grobbee DE, den Breeijen JH, Boeing H, Hofman A, Witteman JC. Dietary iron and risk of myocardial infarction in the Rotterdam study. Am J Epidemiol. 1999;149:421–8.
6. Vergnaud AC, Bertrais S, Zureik M, Galan P, Blacher J, Hercberg S, Czernichow S. Dietary iron intake and serum ferritin in relation to 7.5 years structure and function of large arteries in the SUVIMAX cohort. Diabetes Metab. 2007;33:366–71.
7. Weinberg MD, Hooper WC, Dangas G. Cardiac biomarkers for the prediction and diagnosis of atherosclerotic disease and its complications. Curr Mol Med. 2006;6:557–69.
8. Ceriello A, Motz E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? the common soil hypothesis revisited. Arterioscler Thromb Vasc Biol. 2004;24:816–23.
9. Ross R. Mechanisms of disease: atherosclerosis-an inflammatory disease. N Engl J Med. 1999;340:115–26.
10. Jiang R, Jacobs DR Jr, Mayer-Davis E, Szklo M, Herrington D, Jenny NS, Kronmal R, Barr RG. Nut and seed consumption and inflammatory markers in the Multi-Ethnic Study of Atherosclerosis. Am J Epidemiol. 2006;163:222–31.
11. Lutsey PL, Jacobs DR Jr, Kori S, Mayer-Davis E, Shea S, Steffen LM, Szklo M, Tracy R. Whole grain intake and its cross-sectional association with obesity, insulin resistance, inflammation, diabetes and subclinical CVD: The MESA study. Br J Nutr. 2007;98:397–405.
12. Nettleton JA, Steffen LM, Mayer-Davis EJ, Jenny NS, Jiang R, Herrington DM, Jacobs DR Jr. Dietary patterns are associated with biochemical markers of inflammation and endothelial activation in the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Clin Nutr. 2006;83:1369–79.
13. Guerrero-Romero F, Rodriguez-Moran M. Hypomagnesemia, oxidative stress, inflammation, and metabolic syndrome. Diabetes Metab Res Rev. 2006;22:471–6.
14. Helmersson J, Arnlov J, Larsson A, Basu S. Low dietary intake of b-carotene, a-tocopherol and ascorbic acid is associated with increased inflammatory and oxidative stress status in a Swedish cohort. Br J Nutr. 2009;101:1775–82.
15. Lee DH, Folsom AR, Jacobs DR Jr. Iron, zinc, and alcohol consumption and mortality from cardiovascular diseases: The Iowa Women's Health Study. Am J Clin Nutr. 2005;81:787–91.
16. Hu FB, Rimm EB, Stampfer MJ, Ascherio A, Spiegelman D, Willett WC. Prospective study of major dietary patterns and risk of coronary heart disease in men. Am J Clin Nutr. 2000;72:912–21.
17. Bernstein AM, Sun Q, Hu FB, Stampfer MJ, Manson JE, Willet WC. Major dietary protein sources and risk of coronary heart disease in women. *Circulation*. 2010;122:876–83.
18. Lee DH, Zacharski LR, Jacobs DR Jr. Comparison of the serum ferritin and percentage of transferrin saturation as exposure markers of iron-driven oxidative stress-related disease outcomes. Am Heart J. 2006; 151:1247.e1–7.
19. Yuan XM, Anders WL, Olsson AG, Brunk UT. Iron in human atheroma and LDL oxidation by macrophages following erythrophagocytosis. Atherosclerosis. 1996;124:61–73.
20. You SA, Wang Q. Ferritin in atherosclerosis. Clin Chim Acta. 2005; 357:1–16.
21. Fernandez-Real JM, Lopez-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes. 2002;51:2348–54.
22. Ford ES, Li C, McGuire LC, Mokdad AH, Liu S. Intake of dietary magnesium and the prevalence of the metabolic syndrome among U.S. adults. Obesity (Silver Spring). 2007;15:1139–46.
23. Casagrande SS, Wang Y, Anderson C, Gary TL. Have Americans increased their fruit and vegetable intake? The trends between 1988 and 2002. Am J Prev Med. 2007;32:257–63.
24. Briefel RR, Bialostosky K, Kennedy-Stephenson J, McDowell MA, Ervin RB, Wright JD. Zinc intake of the U.S. population: findings from the Third National Health and Nutrition Examination Survey, 1988–1994. J Nutr. 2000;130 Suppl 5:S1367–73.
25. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR Jr, Kronmal R, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. Am J Epidemiol. 2002;156: 871–81.
26. Mayer-Davis EJ, Vitolins MZ, Carmichael SL, Hemphill S, Tsaroucha G, Rushing J, Levin S. Validity and reproducibility of a food frequency interview in a multi-cultural epidemiology study. Ann Epidemiol. 1999;9:314–24.
27. Nettleton JA, Rock CL, Wang Y, Jenny NS, Jacobs DR Jr. Associations between dietary macronutrient intake and plasma lipids demonstrate criterion performance of the Multi-Ethnic Study of Atherosclerosis (MESA) food frequency questionnaire. Br J Nutr. 2009;102:1220–7.

28. Monsen ER, Hallberg L, Layrisse M, Hegsted DM, Cook JD, Mertz W, Finch CA. Estimation of available dietary iron. Am J Clin Nutr. 1978;31:134–41.
29. Nettleton JA, Schulze MB, Jiang R, Jenny NS, Burke GL, Jacobs DR Jr. A priori-defined dietary patterns and markers of cardiovascular disease risk in the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Clin Nutr. 2008;88:185–94.
30. Budoff MJ, Nasir K, McClelland RL, Detrano R, Wong N, Blumenthal RS, Kondos G, Kronmal RA. Coronary calcium predicts events better with absolute calcium scores than age-sex-race/ethnicity percentiles: MESA (Multi-Ethnic Study of Atherosclerosis). J Am Coll Cardiol. 2009;53:345–52.
31. Willett WC, Stampfer M. Implications of total energy intake for epidemiologic analyses. In: Willet W, editor. Nutritional epidemiology. 2nd ed. New York: Oxford University Press; 1998. p. 273–301.
32. Sullivan JL. Iron and the sex difference in heart disease risk. Lancet. 1981;1:1293–4.
33. Bhattacharya SK, Ahokas RA, Carbone LD, Newman KP, Gerling IC, Sun Y, Weber KT. Macro- and micronutrients in African-Americans with heart failure. Heart Fail Rev. 2006;11:45–55.
34. Lindquist CH, Gower BA, Goran MI. Role of dietary factors in ethnic differences in early risk of cardiovascular disease and type 2 diabetes. Am J Clin Nutr. 2000;71:725–32.
35. Shimakawa T, Nieto FJ, Malinow MR, Chambless LE, Schreiner PJ, Szklo M. Vitamin intake: a possible determinant of plasma homocyst(e)ine among middle-aged adults. Ann Epidemiol. 1997;7:285–93.
36. Mozaffarian D, Kumanyika SK, Lemaitre RN, Olson JL, Burke GL, Siscovick DS. Cereal, fruit, and vegetable fiber intake and the risk of cardiovascular disease in elderly individuals. JAMA. 2003;289:1659–66.
37. Jacobs DR Jr, Gallaher DD. Whole grain intake and cardiovascular disease: a review. Curr Atheroscler Rep. 2004;6:415–23.
38. Azadbakht L, Esmaillzadeh A. Red meat intake is associated with metabolic syndrome and the plasma C-reactive protein concentration in women. J Nutr. 2009;139:335–9.
39. Chacko SA, Song Y, Nathan L, Tinker L, de Boer IH, Tylavsky F, Wallace R, Liu S. Relations of dietary magnesium intake to biomarkers of inflammation and endothelial dysfunction in an ethnically diverse cohort of postmenopausal women. Diabetes Care. 2010;33:304–10.
40. 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;85:1068–74.
41. Valtuena S, Pellegrini N, Franzini L, Bianchi MA, Ardigo D, Del Rio D, Piatti P, Scazzina F, Zavaroni I, et al. Food selection based on total antioxidant capacity can modify antioxidant intake, systemic inflammation, and liver function without altering markers of oxidative stress. Am J Clin Nutr. 2008;87:1290–7.
42. Rubin H. Central roles of Mg2+ and MgATP2- in the regulation of protein synthesis and cell proliferation: Significance for neoplastic transformation. Adv Cancer Res. 2005;93:1–58.
43. Barbagallo M, Dominguez LJ, Galioto A, Ferlisi A, Cani C, Malfa L, Pineo A, Busardo' A, Paolisso G. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. Mol Aspects Med. 2003; 24:39–52.
44. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA. 1993;270:2693–8.
45. Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, Ueland M, Kvale G. Total plasma homocysteine and cardiovascular risk profile. The hordaland homocysteine study. JAMA. 1995;274:1526–33.
46. Guerrero-Romero F, Tamez-Perez HE, Gonzalez-Gonzalez G, Salinas-Martinez AM, Montes-Villarreal J, Trevino-Ortiz JH, Rodriguez-Moran M. Oral magnesium supplementation improves insulin sensitivity in non-diabetic subjects with insulin resistance. A double-blind placebo-controlled randomized trial. Diabetes Metab. 2004;30:253–8.
47. Altura BT, Brust M, Bloom S, Barbour RL, Stempak JG, Altura BM. Magnesium dietary intake modulates blood lipid levels and atherogenesis. Proc Natl Acad Sci USA. 1990;87:1840–4.
48. Touyz RM. Role of magnesium in the pathogenesis of hypertension. Mol Aspects Med. 2003;24:107–36.
49. Federico A, Morgillo F, Tuccillo C, Ciardiello F, Loguercio C. Chronic inflammation and oxidative stress in human carcinogenesis. Int J Cancer. 2007;121:2381–6.
50. Simon JA, Murtaugh MA, Gross MD, Loria CM, Hulley SB, Jacobs DR Jr. Relation of ascorbic acid to coronary artery calcium: The Coronary Artery Risk Development in Young Adults Study. Am J Epidemiol. 2004;159:581–8.
51. Willcox BJ, Curb JD, Rodriguez BL. Antioxidants in cardiovascular health and disease: key lessons from epidemiologic studies. Am J Cardiol. 2008;101:D75–86.
52. Jacobs DR Jr, Gross MD, Tapsell LC. Food synergy: an operational concept for understanding nutrition. Am J Clin Nutr. 2009;89:S1543–8.
53. Clarke R, Halsey J, Lewington S, Lonn E, Armitage J, Manson JE, Bonaa KH, Spence JD, Nygard O, et al. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: Meta-analysis of 8 randomized trials involving 37 485 individuals. Arch Intern Med. 2010;170:1622–31.
54. Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA. 2002;288:2015–22.