Dietary Micronutrient Intakes Are Associated with Markers of Inflammation but Not with Markers of Subclinical Atherosclerosis\textsuperscript{1,2}

Marcia C. C. de Oliveira Otto,\textsuperscript{3} Alvaro Alonso,\textsuperscript{4} Duk-Hee Lee,\textsuperscript{5} George L. Delclos,\textsuperscript{3} Nancy S. Jenny,\textsuperscript{6} Rui Jiang,\textsuperscript{7} Joao A. Lima,\textsuperscript{8} Elaine Symanski,\textsuperscript{9} David R. Jacobs Jr,\textsuperscript{4} and Jennifer A. Nettleton\textsuperscript{3*}

\textsuperscript{3}Division of Epidemiology, Human Genetics and Environmental Sciences, University of Texas School of Public Health, Houston, TX 77030; \textsuperscript{4}Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN 55454; \textsuperscript{5}Kyungpook National University, Seoul 700-422, South Korea; \textsuperscript{6}Department of Pathology, University of Vermont College of Medicine, Burlington, VT 05446; \textsuperscript{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 \textsuperscript{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), \(\beta\)-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 \(\mu\)mol/L, and 9.20, 9.00, 8.65, 8.76, and 8.33 \(\mu\)mol/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/mL across increasing quintiles of Zn and 1.72, 1.76, 1.83, 1.86, and 1.94 mg/mL 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.


Introduction

Several studies (1–4), although not all (5,6), have reported significant associations between dietary micronutrients and the development of cardiovascular disease (CVD).\textsuperscript{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), \(\beta\)-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,
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 years 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 immunosassay (IMx Homocysteine Assay, Axis Bioscience) 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 Bio-medical 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/m²) 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 intake 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 >
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).

**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 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...
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 (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).

**Discussion**

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.

**Interactions**

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 > 2.5 mm) 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 Minéraux 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 processes.

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

<table>
<thead>
<tr>
<th></th>
<th>Heme iron</th>
<th>Nonheme iron</th>
<th>Mg</th>
<th>Zn</th>
<th>Vitamin C</th>
<th>Vitamin E</th>
<th>β-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heme iron</td>
<td>1</td>
<td>-0.03</td>
<td>0.02*</td>
<td>0.39</td>
<td>0.07</td>
<td>-0.09</td>
<td>0.25</td>
</tr>
<tr>
<td>Nonheme iron</td>
<td>1</td>
<td>0.57</td>
<td>0.37</td>
<td>0.39</td>
<td>0.08</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>1</td>
<td>0.61</td>
<td>0.44</td>
<td>-0.15</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>1</td>
<td>0.20</td>
<td>-0.18</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1</td>
<td>0.11</td>
<td>0.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.08</td>
</tr>
</tbody>
</table>

1 All coefficients are significant, P < 0.05, except *P = 0.14.
2 Correlations adjusted for age, gender, and race-ethnicity.
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, 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 (13.6). 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
saturated fat and processed foods. After further adjustment for correlated with red meat consumption, such as higher intakes of possible that this relationship may reflect other dietary factors intake and CRP was independent of red meat intake, it is possible that antioxidant intake may influence CVD risk (4,51) Artery Risk Development in Young Adults Study (50). It is also assessment of nutrient intake, as reported by the Coronary 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 temporal 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 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 trials 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.

Acknowledgments
M.C.C.O. and J.A.N. designed research project, performed statistical analysis, and wrote this manuscript; G.L.D., E.S., D.H.L., A.A., D.R.J., R.J., N.S.J., and J.A.L. assisted in study design and writing the manuscript. All authors read and approved the final manuscript.

Literature Cited