Dietary Magnesium, C-Reactive Protein and Interleukin-6:

The Strong Heart Family Study

Nandana D Rao

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Amanda Fretts

Rozenn Lemaitre

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Abstract

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Nandana D Rao

Chair of the Supervisory Committee:

Amanda Fretts, Assistant Professor

Department of Epidemiology

Background: Recent studies suggest that dietary factors, particularly magnesium (Mg) intake, influence systemic inflammation. Whether these associations are modified by genes associated with Mg metabolism and transport is unknown.

Objective: To examine the associations of reported Mg intake, and the interaction of reported Mg intake with single nucleotide polymorphisms (SNPs) related to Mg metabolism and transport, on markers of inflammation (i.e., C-reactive protein (CRP) and interleukin 6 (IL-6)) among American Indians (AIs).

Methods: This cross-sectional study included AI participants (n=1,924) from the Strong Heart Family Study. Intake of Mg from foods and dietary supplements was ascertained using a 119-item Block food frequency questionnaire. CRP and IL-6 were measured from blood collected

after a 12-hour fast, and candidate SNP (rs3740393) was genotyped using MetaboChip. Generalized estimating equations were used to examine associations of Mg intake, and the interaction of rs3740393 x dietary Mg, on CRP and IL-6.

Results: Reported Mg intake was not associated with CRP or IL-6. We observed no interaction of reported Mg intake with rs3740393 on CRP. However, we observed a significant interaction (p-interaction=0.018) of reported Mg intake with rs3740393 on IL-6. Among participants with the G/G genotype, for every 1 SD higher in log-Mg, log-CRP was 0.04 (95% CI: -0.10 to 0.17) mg/l higher. Among participants with the C/G genotype, for every 1 SD higher in log-Mg, log-CRP was 0.08 (95% CI: -0.21 to 0.05) mg/l lower, and among participants with the C/C genotype, for every 1 SD higher in log-Mg, log-CRP was 0.19 (95% CI: -0.38 to -0.01) mg/l lower.

Conclusion: Among SHFS participants, dietary intake of Mg is not associated with CRP (irrespective of genotype). However, Mg intake is associated with lower IL-6 among carriers of the C allele at rs3740393. Future research is necessary to replicate this finding, and to examine other Mg-related genes that may influence associations of Mg intake with inflammation.

Introduction

Magnesium (Mg) is a critical element in human nutrition, involved in over 600 enzymatic reactions and multiple metabolic pathways that influence a myriad of physiologic systems.[1] Major sources of dietary Mg include leafy green vegetables, nuts, seeds, and whole grains.[2] Recent studies have linked dietary Mg intake with markers of inflammation and subsequent metabolic diseases such as hypertension, diabetes, and cardiovascular disease (CVD), and colorectal cancer,[3,4] but findings remain inconsistent.

Published studies typically use serum levels of C-reactive protein (CRP) and interleukin 6 (IL-6) as markers of inflammation. Observational studies suggest that higher levels of reported intake of dietary Mg are associated with lower levels of inflammation (CRP) compared to lower levels of intake, even after adjustment for other foods and nutrients.[3,4,5] In addition, individual randomized, controlled trials show no impact of oral Mg supplementation on reducing CRP,[6,7,8,9,10] yet a meta-analysis[11] including some of these trials indicates that supplementation reduces CRP levels, with the effect being greater in those with higher baseline CRP (e.g., CRP ≥ 20 mg/l) compared to those with lower baseline CRP. Furthermore, the relationship of dietary Mg and IL-6 remains unclear.[3,12,13]

Several biological mechanisms may in part explain the relationship of Mg and markers of inflammation. Both animal and in vitro studies suggest that lower levels of Mg increase intracellular Ca^{2+}, a signal thought to initiate inflammatory responses by cells.[1] Genetics may also play a role in the body's ability to properly utilize dietary Mg. Loci that are associated with abnormal Mg metabolism[14] and Mg transport[15] have been identified, but to date, no studies have assessed the influence of these loci on the relationship of dietary Mg with inflammation.

Better understanding potential interactions of genetic loci associated with Mg metabolism and dietary Mg intake on inflammatory markers may provide further insight about potentially relevant biological mechanisms.

The purpose of this study was to examine the associations of reported intake of dietary Mg with inflammatory biomarkers (CRP and IL-6) among American Indians (AI) who participated in the Strong Heart Family Study (SHFS). Additionally, we examined potential interactions of Mg intake with single nucleotide polymorphism (SNP) rs3740393, previously identified as related to Mg transport in relation to markers of inflammation (CRP and IL-6). Given the high prevalence of diabetes and CVD among AIs, it is important to understand the interplay of dietary Mg, genes, and inflammatory markers in order to help inform nutritional guidelines and CVD prevention strategies in this underserved population.

Methods

Study Setting

The Strong Heart Family Study is a study of cardiovascular disease and its risk factors among 12 AI communities located in Arizona, North Dakota, South Dakota, and Oklahoma. The cohort completed two study examinations (2001-2003 and 2007-2009) over eight years. Both study exams included a personal interview, physical examination, medication review, laboratory work-up, and dietary assessment (completion of a 119-item Block food frequency questionnaire (FFQ) and ethnic foods supplemental questionnaire). Details on the study methods and data collection instruments have been reported previously.[16] In total, 1,122 men and 1,658 women from 92 families took part in the study.

Study Subjects

The analytic sample included SHFS participants with available data on demographics, diet, and markers of inflammation (i.e., CRP and IL-6) at the baseline study exam (2001-2003). Subjects with unreliable dietary data were excluded from analysis. This included subjects who did not complete the FFQ (n=117), skipped more than 15% of the questions on the FFQ (n=31), or who reported extreme caloric intake [intakes of <600 kcal/day or >6000 kcal/day for women (n=73) and <600 or >8000 kcal/day for men (n=41), as in previous SHFS analyses[17]]. The resulting 1,924 subjects comprised the study sample for analyses that assessed the associations of dietary Mg intake with CRP and IL-6. For analyses that examined the interaction of Mg-related SNPs with dietary intake of Mg on CRP and IL-6, we additionally excluded participants missing genotype information (n=338); in total, 1,586 subjects comprised the study sample for genetic analyses.

Dietary Assessment and Exposure Measures

Average food intake over the past year was measured using an interviewer-administered Block FFQ[18], a widely used food questionnaire with well-established reliability and validity.[17] Participants were asked how often, on average, they consumed a particular food (i.e. seasonally, never, a few times per year, once per month, 2-3 times per month, once per week, twice per week, 2-3 times per week, 5-6 times per week, daily) and portion size. Portion size was described by natural portions (e.g. 1 banana, 2 eggs, etc.) or volume/weight, with photographs of different portions used as visual aids. In addition to the 119 items included on the standard Block FFQ, participants were also asked about the intake of common American Indian foods including menudo, pozole, guysava, red or green chili, Indian taco, frybread, corn

tortilla, flour tortilla, and spam. Average daily energy and nutrient intakes, including dietary Mg, were then calculated for each participant for all foods (and then summed) using the Block database (Block Dietary Systems, Berkeley, CA).[17]

The use of multivitamins (regular once-a-day, B-complex type, or antioxidant combination) and single vitamins (supplements containing only one vitamin or mineral) were also ascertained using the Block FFQ. For our analyses, total Mg intake is described as the summation of Mg from diet and vitamins.

Genotype Assessment

Participants were genotyped using the Illumina Cardio-Metabo DNA Analysis BeadChip (MetaboChip). Details of the SNP genotyping have been described previously.[19] Of the SNPs genotyped, rs3740393 was selected for gene x diet analysis and allele G was coded as the risk allele. Located in CNNM2, a gene thought to play an important role in Mg homeostasis and transport, this SNP showed a significant association (p=8.58E-07) with serum Mg concentrations in previous research regarding common genetic variants in genes coding for Mg transport proteins.[20] Prior genetic studies across various ethnic groups have also found associations between SNPs rs1205 and rs3091244 with serum CRP.[21,22,23,24] These SNPs were selected as additional covariates for the gene x diet analysis for the outcome CRP in order to reduce the variance of CRP and improve sensitivity. For SNP rs1205, allele G was coded as the risk allele, while allele A was coded as the risk allele for SNP rs3091244.

Outcome Measures

Blood samples were collected at each exam after a 12-hour fast and circulating levels of CRP were assessed using the BN II Systems. The sensitivity of the assay was 0.175 mg/l and the

CV was less than 4.1%. The IL-6 assay was performed on the Bioplex 100 (Luminex, X-MAP) instrument and had an intra-assay CV of 3.51% and inter-assay CV of 4.48%.

Other Covariates

The baseline exam included a standardized personal interview, physical examination, and laboratory work-up. Information regarding previous/current medical conditions, education, smoking, and alcohol consumption was collected at the personal interview. Anthropometric measures were obtained with the participant wearing lightweight clothing and no shoes. Body mass index (BMI) was calculated as body weight divided by height-squared (kg/m^2). Accusplit AE120 pedometers were used to assess "usual" levels of ambulatory physical activity (i.e., steps per day over a 7-day period). Blood pressure was measured three times on the right arm using standard mercury sphygmomanometers after five minutes rest; data collected as part of the personal interview and medication review, and measures of systolic and diastolic blood pressure were used to determine hypertension status. Based on the American Diabetes Association criteria, any participant taking insulin or oral anti-diabetic medications at the baseline exam, or with fasting plasma glucose ≥ 126 mg/dl was considered to have diabetes. Participants with prevalent cardiovascular diseases included those who had ever experienced a myocardial infarction or stroke, and/or had previously been diagnosed with coronary heart disease or congestive heart failure based off of data collected at the personal interviews and medical record review.

Statistical Analyses

Generalized estimating equations (GEE) with an independence working correlation structure and robust standard errors were used to examine the cross-sectional relationship

between dietary Mg and CRP, dietary Mg and IL-6, and to examine the role of gene x diet interactions in these relationships. Given the family-based sampling of the SHFS, GEE was selected to address potential familial correlation within the data. All statistical analyses were conducted using R version 3.5.1.

Log-Mg intake was examined both continuously and categorically using quintiles. Quintiles were defined according to total Mg intake relative to total calorie intake, and the lowest quintile was used as the reference group. All analyses were adjusted for total calorie intake in order to avoid false associations of nutrients with inflammation due to over or under reporting of dietary intake, differences in energy intake due to body size, metabolic activity, or physical activity, and confounding. All covariates were selected a priori based on previous studies, in order to make our analyses more comparable to published work, and on their potential associations with dietary Mg or the outcome biomarkers. Mg, CRP and IL-6 were all highly skewed, and were log transformed in order obtain normality.

Two models were fit to examine the association of Mg with CRP and IL-6. A crude model (Model A) adjusted for age, sex, site, and total calorie intake. Our primary model (Model B) additionally adjusted for education (yrs), smoking (packs per yr), alcohol use (never, ever, current), pedometer-determined ambulatory activity levels (steps/day), BMI (kg/m^2), diabetes status (yes/no), hypertension status (yes/no), CVD status (yes/no), and other dietary factors including fiber (g/day), folate (mcg/day), total fat (% of calories), and fruit and vegetable servings per day.

Given the high prevalence of diabetes and CVD in this population and the high levels of inflammation present among participants with these diseases, we conducted sensitivity

analyses that excluded participants with diabetes and CVD. In addition, we also assessed potential interaction of reported Mg intake with age, sex, and BMI on CRP and IL-6 in exploratory analyses to investigate whether these factors modify the association of Mg intake with CRP or IL-6.

We examined the associations of SNPs rs3740393, rs1205, and rs3091244 with CRP and IL-6, controlling for age and sex. Additionally, we assessed the interaction of reported Mg intake with rs3740393 on levels of CRP and IL-6 by including an interaction term (Mg x rs3740393) in a model also adjusted for Mg, rs3740393, and crude model (Model A) or primary model (Model B) covariates. For the outcome of CRP, Model B further adjusted for SNPs associated with CRP levels (i.e., rs1205, rs3091244) in order to reduce the variance of CRP.

Multiple imputation was used to account for occasional missing values of covariates in order to conserve maximum sample size [missing values: education (n=7), BMI (n=6), physical activity (n=123), smoking-packs per year (n=43), diabetes status (n=3), and hypertension status (n=4)]. This imputation was implemented using the predictive mean matching method with the MICE package in R. Imputed values were predicted across 10 datasets using age, sex, site, total calorie intake, alcohol use, and other dietary variables and final analysis results were obtained by aggregating the results of our models from these 10 datasets.

Results

The average age of study participants was 40.3 years and 39% were male. Median (IQR) dietary Mg intake was 283.1 (198.4 – 407.9) mg/day. There were 441 participants (22.9%) who reported taking Mg supplements or multivitamins containing Mg. Median (IQR) CRP levels were

3.7 (1.5 - 7.9) mg/l and median (IQR) IL-6 levels were 3.3 (1.6 - 6.5) pg/ml. Pearson correlation between log-transformed CRP and log-transformed IL-6 was 0.21. There were 23.9% participants with prevalent hypertension, 18.9% with prevalent diabetes, and 10.7% with prevalent CVD. Characteristics of all study participants, regardless of genotype availability, are shown by quintiles of dietary Mg in **Table 1**. Participants in the highest quintile of dietary Mg intake were more likely to be older and experienced a higher prevalence of hypertension, diabetes, and CVD compared to those in lower quintiles of dietary Mg intake.

Reported Mg intake was not associated with CRP or IL-6 (**Table 2**). We observed no interaction of reported Mg intake with rs3740393 on CRP (**Table 3**). However, we observed a significant interaction (p-interaction=0.018) of reported Mg intake with rs3740393 on IL-6 (**Table 4**). Among participants with the G/G genotype, for every 1 SD higher in log-Mg, log-CRP was 0.04 (95% CI: -0.10 to 0.17) mg/l higher. Among participants with the C/G genotype, for every 1 SD higher in log-Mg, log-CRP was 0.08 (95% CI: -0.21 to 0.05) mg/l lower, and among participants with the C/C genotype, for every 1 SD higher in log-Mg, log-CRP was 0.19 (95% CI: -0.38 to -0.01) mg/l lower.

In general, analyses examining Mg intake with CRP or IL-6 that modeled Mg in quintiles produced similar results (**Table S1**). However, in crude analyses (Model A), reported intake of Mg was inversely associated with CRP (p-trend=0.031); this finding was no longer statistically significant in the fully adjusted model (Model B) (**Table S1**). Results were not meaningfully changed in analyses that excluded participants with prevalent diabetes or CVD (**Table S2**).

There was no evidence of interaction of age or sex with reported intake of Mg on CRP or IL-6 (**Table S3**). However, in exploratory analyses, associations of reported intake of Mg with

CRP were modified by BMI (p-interaction=0.0007) (**Table S3**). Among participants with BMI of 25 kg/m^2, for every 1 SD higher in log-Mg, log-CRP was 0.10 (95% CI: -0.01 to 0.22) mg/l higher (**Table S4**). Among participants with BMI of 35 kg/m^2, for every 1 SD higher in log-Mg, log-CRP was 0.01 (95% CI: -0.13 to 0.11) mg/l lower, and among those with BMI of 45 kg/m^2, for every 1 SD higher in log-Mg, log-CRP was 0.12 (95% CI: -0.27 to 0.03) mg/l lower (**Table S4**). This suggests that higher reported Mg intake was associated with lower CRP among participants with higher BMI.

Results of analyses that assessed the associations of the selected SNPs with CRP and IL-6 are shown in **Table S5**. We observed no association of SNP rs3740393 with either CRP or IL-6. SNP rs1205 was significantly associated with CRP (p-value=0.004), suggesting that log-CRP levels were 0.12 mg/l higher with each additional copy of the G allele. SNP rs3091244 was also significantly associated with CRP (p=0.002), suggesting that log-CRP levels were 0.12 mg/l higher with each additional copy of the A allele. Neither SNP rs1205 nor rs3091244 were associated with IL-6.

Discussion

In this cross-sectional analysis of AI participants from the SHFS, rs3740393 modified the association of reported Mg intake with IL-6 (but not CRP), after adjustment for factors known to be associated with Mg intake and inflammation, including dietary factors, prevalent diabetes, and prevalent CVD. These findings suggest that genetic factors related to Mg transport and metabolism influence the association of Mg with inflammation in a population at high risk for inflammatory-related chronic diseases (i.e., CVD and diabetes).

The findings reported herein do not readily support the notion that higher dietary Mg intake is associated with lower levels of CRP, as is suggested by previous observational studies.[3,4,12,25,26] In particular, our findings are not consistent with results from the Women's Health Initiative (WHI)[3] or the National Health and Nutrition Examination Survey (NHANES) 1999-2000[25], both of which reported that Mg intake is inversely associated with CRP concentrations in samples of healthy adults. However, CRP levels among SHFS participants were much higher when compared to participants in WHI or NHANES. Mean CRP was 5.1 mg/l in WHI[3] compared to 6.6 mg/l in SFHS, and median CRP was 2.0 mg/l in NHANES[25] compared to 3.7 in SFHS. Mean age was also lower in SFHS compared to many of the other observational studies,[3,4,12] while average BMI[4,12,26] and percent of current smokers[3,4,12,25] was higher in our sample compared to others. Therefore, it is possible that differences in underlying CRP, baseline age, BMI, and smoking status in part explain these conflicting findings.

While individual randomized controlled trials show no effect of oral Mg supplementation on CRP, [6,7,8,9,10] a meta-analysis including some of these trials indicates that supplementation reduces CRP.[11] In general, trials varied with regards to amount and duration of supplementation. However, trials incorporated in the meta-analysis included participants with Mg deficiency,[27] hypomagnesemia,[7,28] those undergoing coronary artery bypass surgery,[29] and hemodialysis patients,[9] characteristics that are not representative of the SHFS sample.

Previous studies that have examined the association of reported Mg intake with IL-6 are inconsistent.[3,12,13,26] To our knowledge, no studies have assessed the potential effect of genes associated with Mg transport and metabolism on the association of reported Mg intake with IL-6. Our findings build upon previous work and suggest that higher intake of Mg may lower IL-6

among individuals with one or more copies of the C allele at rs3740393. On the other hand, among individuals carrying only the G allele, Mg intake is not associated with lowering IL-6. SNP rs3740393 is located in CNNM2, a gene which encodes a membrane protein necessary for Mg transport, and the G allele at this locus is associated with lower serum Mg.[20,30] Thus, the G allele may reduce the efficacy of dietary Mg because if dietary Mg is not well converted to serum Mg, no association between dietary Mg and IL-6 will be present. It is possible that we observed a significant interaction of Mg intake and rs3740393 on IL-6, but not on CRP because the role of IL-6 is further upstream in the inflammatory pathway compared to CRP and the liver secretes CRP in response to inflammatory cytokines like IL-6.[3] However, additional research is needed to better understand the interplay of SNPs, Mg, and inflammation.

This study has many strengths. This is the first study to examine the influence of genes on dietary Mg and inflammation biomarkers. The SHFS is a large, family-based sample, and use of validated and reliable instruments to collect data on a wide array of factors allowed for well-measured assessments of a variety of covariates. Additionally, this study focused on AIs, an underserved population with a high burden of diabetes and CVD.

This study also has limitations. As the SHFS only has a single measure of Mg, analyses were cross-sectional, and we are unable to infer temporality. Furthermore, genetic information for AIs from the SHFS is currently limited to those SNPs included on the MetaboChip. It is possible that other SNPs related to Mg uptake (but not currently genotyped) could provide more insight with regards to the effect of gene x diet interactions on levels of inflammatory biomarkers.

In conclusion, our results indicate that dietary intake of Mg is not associated with CRP (irrespective of genotype), but that Mg intake is associated with lower IL-6 among carriers of the C allele at rs3740393. Future research is necessary to replicate this finding, and to examine other Mg-related genes that may influence associations of Mg intake with inflammation.

Table 1: Participant characteristics according to quintiles of total Mg intake

Variable	Total	Dietary Mg Q1	Dietary Mg Q2	Dietary Mg Q3	Dietary Mg Q4	Dietary Mg Q5
N	1924	385	385	384	385	385
Median Total Mg (mg/day)+	283.1 (198.4-407.9)	204.1 (140.9-314.7)	268.4 (187.1-399.1)	299.0 (221.0-446.1)	310.3 (223.1-454.0)	322.4 (246.3-428.8)
N using supplements	441	7	26	53	104	251
Male (%)	39.2	45.7	40.5	41.7	44.7	23.4
Age (yrs)*	40.3 (16.6)	31.1 (12.4)	35.3 (14.4)	39.1 (15.7)	45.0 (16.2)	50.9 (16.1)
Education (yrs)*	12.3 (2.3)	11.8 (1.8)	12.0 (2.3)	12.1 (2.3)	12.6 (2.3)	13.0 (2.4)
BMI (kg/m^2)*	31.5 (7.5)	31.1 (7.7)	31.8 (8.5)	32.0 (7.9)	31.5 (6.4)	31.2 (6.7)
Steps (per day)*	5907.0 (3992.7)	6431.6 (3901.4)	5894.6 (3770.2)	5781.9 (4114.1)	6110.0 (4344.3)	5322.3 (3736.3)
Smoking (%)						
Never	40.8	46.7	44.4	33.1	39.5	40.2
Ever	23.3	15.1	19.2	26.3	26.5	29.4
Current	35.9	38.2	36.4	40.6	34.0	30.4
Packs (per yr among ever/current smokers)*	11.3 (17.5)	7.1 (11.8)	9.5 (15.3)	10.3 (19.8)	14.2 (19.4)	15.1 (17.7)
Hypertension (%)	23.9	14.6	20.3	22.2	28.6	33.6
Diabetes (%)	18.9	11.2	13.0	19.1	25.8	25.7
CVD (%)	10.7	6.2	9.9	10.7	11.4	15.1
Calories (kcal/day)*	2431.8 (1334.3)	2564.2 (1321.6)	2721.2 (1404.2)	2773.3 (1502.3)	2345.5 (1183.4)	1755.7 (928.2)
Fiber (g/day)*	17.9 (11.4)	14.1 (8.5)	18.2 (10.8)	20.5 (12.5)	19.4 (11.9)	17.4 (11.7)
Folate (mcg/day)*	411.8 (244.7)	342.1 (211.6)	419.2 (230.7)	475.1 (286.2)	440.4 (248.7)	382.2 (218.1)
Total Fat (% of calories)*	38.3 (7.2)	38.2 (8.4)	38.8 (6.6)	39.2 (6.2)	38.7 (6.5)	36.8 (7.6)
Vegetable (servings/day)*	2.7 (2.1)	2.0 (1.5)	2.8 (2.0)	3.0 (2.3)	2.9 (2.2)	2.7 (2.3)

Fruit (servings/day)*	1.0 (0.9)	0.7 (0.6)	0.9 (0.7)	1.1 (0.8)	1.2 (0.9)	1.3 (1.1)
Median CRP (mg/l)[+]	3.7 (1.5-7.9)	3.1 (1.3-7.1)	3.5 (1.3-8.4)	4.3 (1.6-8.1)	3.8 (1.8-7.6)	3.9 (1.9-8.0)
Median IL-6 (pg/ml)[+]	3.3 (1.6-6.5)	3.2 (1.5-6.6)	3.3 (1.6-6.7)	3.7 (1.9-7.1)	3.1 (1.4-5.8)	3.3 (1.6-5.9)

[+] Median (IQR); *Mean (SD)

Table 2: Association of log-Mg with log-biomarkers of inflammation *(estimates corresponding to 1 SD of log-Mg)*

	log(CRP)		log(IL-6)	
	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Model A*	0.01 (-0.10, 0.11)	0.906	-0.05 (-0.16, 0.06)	0.364
Model B**	0.05 (-0.07, 0.16)	0.433	-0.02 (-0.13, 0.09)	0.689

*Adjusted for age, sex, site, total calorie intake

**Adjusted for variables in Model A plus education, alcohol consumption, smoking, BMI, steps per day, hypertension, diabetes, CVD, and dietary intake of fiber, folate, % total fat, vegetable and fruits.

Table 3: Regression coefficients for the interaction of log-Mg and rs3740393 on log-CRP

	log(CRP)	
	Estimate (95% CI)	P-value
Model A*	0.02 (-0.18, 0.23)	0.820
Model B**	0.03 (-0.14, 0.20)	0.703

*Adjusted for age, sex, site, total calorie intake

**Adjusted for variables in Model A plus education, alcohol consumption, smoking, BMI, steps per day, hypertension, diabetes, CVD, and dietary intake of fiber, folate, % total fat, vegetables and fruits, and SNPs associated with CRP (rs1205, rs3091244)

Table 4: Relationship between log-Mg and log-IL-6 for each rs3740393 genotype *(estimates corresponding to 1 SD of log-Mg)*

	log(IL-6)			
	Genotype	N (%)	Estimate (95% CI)	P-value for interaction
Model A*	C/C	5.2	-0.24 (-0.42, -0.05)	0.016
	C/G	34.0	-0.11 (-0.24, 0.02)	
	G/G	60.8	0.004 (-0.13, 0.14)	
Model B**	C/C	5.2	-0.19 (-0.38, -0.01)	0.018
	C/G	34.0	-0.08 (-0.21, 0.05)	
	G/G	60.8	0.04 (-0.10, 0.17)	

*Adjusted for age, sex, site, total calorie intake

**Adjusted for variables in Model A plus education, alcohol consumption, smoking, BMI, steps per day, hypertension, diabetes, CVD, and dietary intake of fiber, folate, % total fat, vegetable and fruits.

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Supplementary Materials

Table S1: Regression coefficients for the associations of quintiles of dietary Mg and log-biomarkers of inflammation *(Q1 used as reference)*

		log(CRP)		log(IL-6)	
	Total Mg Quartile	Estimate (95% CI)	P for trend	Estimate (95% CI)	P for trend
Model A*	Q2	0.03 (-0.13, 0.20)	0.031	0.002 (-0.19, 0.20)	0.259
	Q3	0.02 (-0.14, 0.18)		0.11 (-0.12, 0.33)	
	Q4	-0.06 (-0.22, 0.10)		-0.18 (-0.39, 0.04)	
	Q5	-0.19 (-0.36, -0.01)		-0.08 (-0.30, 0.16)	
Model B**	Q2	-0.02 (-0.18, 0.14)	0.075	-0.02 (-0.21, 0.18)	0.443
	Q3	-0.04 (-0.19, 0.10)		0.09 (-0.13, 0.31)	
	Q4	-0.10 (-0.27, 0.08)		-0.17 (-0.40, 0.06)	
	Q5	-0.18 (-0.38, 0.02)		-0.04 (-0.28, 0.20)	

*Adjusted for age, sex, site, total calorie intake

**Adjusted for variables in Model A plus education, alcohol consumption, smoking, BMI, steps per day, hypertension, diabetes, CVD, and dietary intake of fiber, folate, % total fat, vegetable and fruits.

Table S2: Association of log-Mg with log-biomarkers of inflammation among participants without diabetes or CVD (n=1,450) *(estimates corresponding to 1 SD of log-Mg)*

	log(CRP)		log(IL-6)	
	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Model A*	0.04 (-0.09, 0.17)	0.536	-0.04 (-0.17, 0.09)	0.506
Model B**	0.04 (-0.09, 0.18)	0.537	-0.001 (-0.14, 0.14)	0.986

*Adjusted for age, sex, site, total calorie intake

**Adjusted for variables in Model A plus education, alcohol consumption, smoking, BMI, physical exercise, hypertension, diabetes, CVD, fiber, folate, % total fat, vegetable and fruit intake

Table S3: Regression coefficients for the interactions of log-Mg and age, sex and BMI on markers of inflammation

	log(CRP)		log(IL-6)	
	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
Age	-0.005 (-0.01, 0.001)	0.091	-0.005 (-0.01, 0.002)	0.136
Sex	-0.002 (-0.20, 0.19)	0.981	-0.03 (-0.27, 0.21)	0.834
BMI	-0.02 (-0.03, -0.01)	0.0007	-0.01 (-0.02, 0.003)	0.124

Adjusted for age, sex, site, total calorie intake, education, alcohol consumption, smoking, BMI, steps per day, hypertension, diabetes, CVD, and dietary intake of fiber, folate, % total fat, vegetable and fruits.

Table S4: Relationship of log-Mg with log-CRP at different BMI *(estimates corresponding to 1 SD of log-Mg)*

log(CRP)		
BMI	Estimate (95% CI)	P-value for interaction
25	0.10 (-0.01, 0.22)	
30	0.05 (-0.06, 0.16)	
35	-0.01 (-0.13, 0.11)	0.0007
40	-0.06 (-0.20, 0.07)	
45	-0.12 (-0.27, 0.03)	

Adjusted for age, sex, site, total calorie intake, education, alcohol consumption, smoking, steps per day, hypertension, diabetes, CVD, and dietary intake of fiber, folate, % total fat, vegetable and fruits.

Table S5: Regression coefficients for the associations of SNPs with CRP and IL6 *(estimates corresponding to 1 copy of the risk allele)*

	log(CRP)		log(IL-6)	
	Estimate (95% CI)	P-value	Estimate (95% CI)	P-value
rs3740393	0.03 (-0.08, 0.13)	0.609	0.07 (-0.07, 0.20)	0.333
rs1205	0.12 (0.04, 0.20)	0.004	0.02 (-0.07, 0.11)	0.688
rs3091244	0.15 (0.06, 0.25)	0.002	-0.01 (-0.10, 0.09)	0.889

Adjusted for age and sex