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ORIGINAL ARTICLE

An inverse association between magnesium in 24-h urine and cardiovascular risk factors in middle-aged subjects in 50 CARDIAC Study populations

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Serum, plasma and dietary magnesium (Mg) have been reported to be inversely associated with cardiovascular disease risk factors. We examined the associations between the 24-h urinary Mg/creatinine (Cre) ratio and cardiovascular disease risk factors, such as body mass index (BMI), blood pressure (BP), serum total cholesterol (TC) and prevalence of obesity, hypertension and hypercholesterolemia. A cross-sectional analysis was conducted among 4211 participants (49.7% women) aged 48–56 years in 50 population samples from 22 countries in the World Health Organization-coordinated Cardiovascular Diseases and Alimentary Comparison (CARDIAC) Study (1985–1994). In linear regression analyses, Mg/Cre ratio was inversely associated with BMI, systolic BP (SBP), diastolic BP (DBP) and TC (*P* for linear trend <0.001 for each). These associations were not markedly altered by adjustment for traditional risk factors, urinary markers or cohort effects. Multivariate-adjusted mean values for the subjects in the highest Mg/Cre ratio quintile were 6.3, 3.4, 5.3 and 4.6% lower than those for the subjects in the lowest quintile for BMI, SBP, DBP and TC ($P<0.001$, respectively). The prevalence of obesity, hypertension and hypercholesterolemia was 2.10 (95% confidence interval: 1.50, 2.95), 1.55 (1.25, 1.92) and 2.06 (1.63, 2.62) times higher ($P<0.001$, respectively) among the subjects in the lowest Mg/Cre ratio quintile than in the subjects in the highest quintile. These associations were not appreciably altered by adjustment for potential confounding variables. In conclusion, higher 24-h urinary Mg/Cre ratio was associated with lower cardiovascular disease risk factors, including BMI, BP, TC, obesity, hypertension and hypercholesterolemia.
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INTRODUCTION

Magnesium (Mg), which is abundant in whole seeds, unmilled grain, green leafy vegetables, seaweeds, legumes and nuts, is a biologically active mineral that acts as a cofactor in hundreds of enzymatic reactions in the human body.[1,2] In observational studies, serum, plasma and dietary Mg have been found to be inversely associated with cardiovascular disease risk factors, such as obesity,[3] hypertension,[3,4] dyslipidemia,[5] type 2 diabetes[6] and coronary heart disease.[7–9] Twenty-four hour urinary Mg was reported to be inversely associated with blood pressure (BP).[10,11] However, few studies have reported the relationship between Mg in 24-h urine and cardiovascular disease risk factors among the global population.

We previously showed an inverse association between population averages of 24-h urinary Mg excretion and BP,[12] and we also reported that the group of individuals excreting more than the world average 24-h urinary Mg/creatinine (Cre) ratio had significantly lower cardiovascular disease risk;[13] therefore, Mg/Cre ratios were inversely associated with coronary heart disease[14] in the Cardiovascular Disease and Alimentary Comparison (CARDIAC) Study. That study confirmed that there was a close relationship between the population averages of cardiovascular disease risk and dietary customs by analyzing various dietary biomarkers in 24-h urine, such as sodium (Na), potassium (K), calcium (Ca), Mg and taurine (Tau; a biological marker of seafood intake[14,15]).[16] However, the population averages are greatly influenced by the genetic background of the individuals and the environmental factors in the regions examined. Therefore, in the present study, we investigated associations with and without adjustment for confounding variables between 24-h urinary Mg/Cre ratio and cardiovascular disease risk factors in individuals such as obesity, hypertension and hypercholesterolemia among the CARDIAC Study

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populations disregarding genetic background, living conditions and gender.

METHODS

Study population

The WHO-coordinated CARDIAC Study was initiated in 1985 as a multi-center cross-sectional study with a standard research protocol, and a total of 12 335 men and women participated in the study. Details of the study design and methods of the CARDIAC Study have been reported elsewhere.[16–19] Briefly, in each center, 100 men and 100 women aged 48–56 years were selected randomly from the general population. In the present study, 50 population samples from 22 countries were included. These 22 countries include various ethnic groups and diverse populations: Australia (97 participants), Brazil (244), Belgium (165), Bulgaria (209), Canada (160), China (686), Ecuador (254), France (158), Georgia (65), Greece (35), Israel (50), Italy (82), Japan (920), New Zealand (140), Portugal (115), Russia (31), Spain (274), Sweden (28), Tanzania (51), Nigeria (40), UK (224), and USA (183) (Figure 1). The study was approved by the CARDIAC Study's institutional review board committee.

Data collection

All participants were invited to a local hospital or health center for a physical examination, and a 15-ml overnight fasting blood sample was taken. Twenty-four-hour urine samples were collected using a standard aliquot cup that allowed participants to collect an exact portion of voided urine repeatedly.[18–20] BP was measured using a standard automated sphygmomanometer (Khi machine, VINE, Tokyo, Japan), and these measurements were repeated three times.[18,21] A structured questionnaire was used for face-to-face interviews during the field survey, and it included items on demographic data, lifestyle factors and medical history.[18] The urine and blood samples were frozen at −20 °C and analyzed centrally in the laboratory of the WHO-collaborating Center for Research on Primary Prevention of Cardiovascular Disease, Izumo, Japan (in 1993, this center was transferred to the Graduate School of Human and Environmental Studies, Kyoto University, Japan). Standardized laboratory methods were used.[18] Quality controls were carefully maintained by internal and external quality surveillance procedures. Measurements included in the present report are body mass index (BMI), BP, serum total cholesterol (TC) and urinary Na, K, Mg, Ca, Cre and Tau excretion levels.

Statistical analysis

Obese subjects were defined as those with BMI $\geqslant 30\,kg\,m^{-2}$.[22] Patients with hypertension were defined as those with systolic BP (SBP) $\geqslant 140$ mm Hg or diastolic BP (DBP) $\geqslant 90$ mm Hg or those who were receiving anti-hypertensive drug therapy. Hypercholesterolemic subjects were defined as those with serum TC $\geqslant 220\,mg\,dl^{-1}$. The markers in 24-h urine are expressed as the ratio of each parameter to Cre. We categorized the 24-h urinary Mg/Cre ratio in quintiles. The distributions of 24-h urinary Na/Cre, K/Cre, Ca/Cre and Tau/Cre ratio were highly skewed, and thus log transformations were performed to achieve a normal distribution. In all analyses, the log-transformed values were then used. For easy interpretation, nontransformed values are reported in the tables.

Differences between baseline characteristics of participants within each quintile were analyzed using the Cochrane–Armitage test for trends for

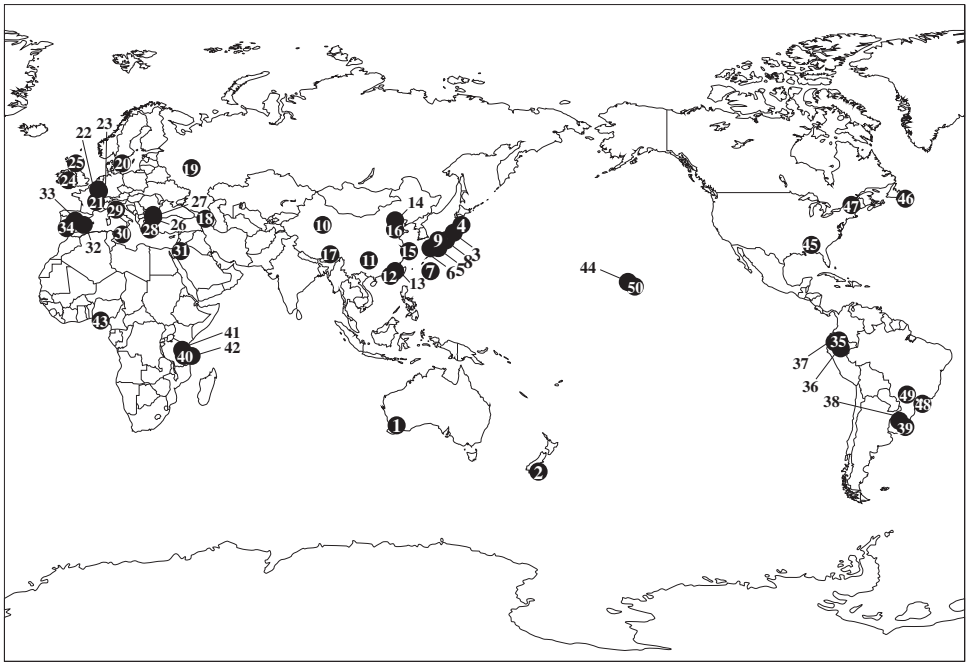

Figure 1 Geographical distribution of the population samples of the Cardiovascular Disease and Alimentary Comparison (CARDIAC) Study (1985–1994). The following are the 50 study sites in 22 countries, the number of participants and their principal investigators: (1) Perth (97): LJ Beilin, MST Hobbs and K Jamrozik; (2) Dunedin (140): FO Simpson; (3) Toyama (179): S Kagamimori; (4) Hirosaki (126): T Kanazawa; (5) Beppu (116): S Kodama; (6) Kurume (175): H Toshima; (7) Okinawa (120): G Mimura and K Taira; (8) Hiroshima (60): M Yamakido; (9) Ohda (144): Y Yamori; (10) Urumqi (56): BX He; (11) Guiyang (96): MX Zhang and XL Wu; (12) Guangzhou (116): ZD Huang; (13) Meixian (63): I Lee; (14) Beijing (19): LS Liu; (15) Shanghai (133): GS Zhao; (16) Shijiazhuang (153): HX Zhang; (17) Lhasa (50): SF Sun; (18) Georgia (65): SM Dalakishivili; (19) Moscow (31): RG Oganov; (20) Gothenburg (28): L Wilhelmsen; (21) Orleans (158): A Marie; (22) Leuven (82): A Amery; (23) Ghent (83): G De Backer; (24) Belfast (91): AE Evans; (25) Stornoway (133): CA Birt; (26) Sofia (urban) (109): N Nicolov and I Tomov; (27) Sofia (rural) (100): N Nicolov and I Tomov; (28) Athens (35): A Ioanidis; (29) Milan (39): G Cerasola; (30) Palermo (43): GC Cesana; (31) Tel Aviv (50): T Rosenthal; (32) Navas (135): A Fernandez-Cruz; (33) Madrid (139): A Fernandez-Cruz; (34) Lisbon (115): MO Carrageta; (35) Quito (146): PD Dillon; (36) Vilcabamba (74): V Del Pozo; (37) Manta (34): V Del Pozo; (38) Uruguaiana (12): Y Moriguchi and E Moriguchi; (39) Bagé (117): Y Moriguchi and E Moriguchi; (40) Handeni (34): J Mtabaji and M Njelekela; (41) Shinya (7): J Mtabaji and M Njelekela; (42) Dar es Salaam (10): J Mtabaji and M Njelekela; (43) Ibadan (40): OO Akinkungbe; (44) Honolulu (16): G Mimura; (45) Jackson (95): HG Langford; (46) Newfoundland (64): G Fodor and A Chockalingam; (47) Montreal (96): P Hamet; (48) Sao Paulo (30): Y Moriguchi and E Moriguchi; (49) Campo Grande (85): Y Moriguchi and E Moriguchi; and (50) Hilo (72): M Kanahele.

Table 1 Baseline characteristics of participants by quintiles of 24-h urinary Mg/Cre ratio in the CARDIAC Study, 1985–1994

	Quintile of Mg/Cre ratio, mg g^{-1}					
	1 (low) (n = 842, median, 38.1; range, 1.1–<49.1)	*2 (n = 845, median, 57.5; range, 49.1–<65.0)*	*3 (n = 840, median, 72.7; range, 65.0–<80.9)*	*4 (n = 842, median, 90.2; range, 80.9–<103.0)*	*5 (high) (n = 842, median, 123.6; range, ⩾103.0)*	P *for trend*[a]
Male, %	67.6	61.2	52.3	38.6	32.1	<0.001
Age, years	51.9 (1.7)[b]	52.0 (1.7)	51.8 (1.6)	52.0 (1.7)	51.9 (1.6)	0.55
BMI, kg m^{-2}	25.8 (4.1)	25.4 (4.0)	24.9 (4.0)	24.7 (4.2)	23.5 (3.9)	<0.001
Systolic BP, mm Hg	128.2 (18.8)	124.8 (18.7)	123.5 (18.9)	122.9 (20.7)	123.0 (20.2)	<0.001
Diastolic BP, mm Hg	78.1 (12.2)	75.2 (11.9)	74.4 (11.9)	73.3 (13.0)	72.7 (13.0)	<0.001
Serum TC, mg dl^{-1}	193.9 (44.6)	194.3 (44.5)	189.0 (44.4)	189.8 (48.9)	178.4 (44.7)	<0.001
Anti-hypertensive treatment, %	4.5	4.3	4.2	4.8	4.5	<0.001
Obesity[c], %	12.8	11.5	9.9	10.2	6.5	<0.001
Hypertension[d], %	32.5	22.7	23.2	22.4	23.8	<0.001
Hypercholesterolemia[e], %	28.3	25.4	21.2	22.3	16.0	<0.001
Nutrient marker in 24-h urine						
Mg/Cre, mg g^{-1}	34.7 (12.1)	57.3 (4.5)	72.9 (4.6)	90.7 (6.2)	136.7 (44.9)	<0.001
Ca/Cre, mg g^{-1}	85.2 (57.9)	125.2 (55.4)	149.4 (65)	176.3 (76.9)	223.8 (108.6)	<0.001
Na/Cre, mmol mmol^{-1}	14.4 (6.5)	15.8 (6.9)	17.0 (7.1)	18.5 (7.7)	22.6 (10.1)	<0.001
K/Cre, mmol mmol^{-1}	4.66 (1.92)	4.83 (1.93)	4.95 (2.02)	5.26 (2.24)	5.72 (2.53)	<0.001
Tau/Cre, μmol mmol^{-1}	99.1 (124.8)	110.9 (122.6)	114.9 (147.9)	116.0 (130.6)	113.3 (132.1)	<0.001

Abbreviations: BMI, body mass index; BP, blood pressure; Ca/Cre, calcium/creatinine; K/Cre, potassium/creatinine; Mg/Cre, magnesium/creatinine; Na/Cre, sodium/creatinine; Tau/Cre, taurine/creatinine; TC, total cholesterol.
[a]*P*-values were determined by the Cochrane–Armitage test for trend for proportions and the Jonckheere–Terpstra trend test for continuous measures.
[b]Values are expressed as mean (s.d.).
[c]Obesity was defined as BMI ⩾ 30 kg m^{-2}.
[d]Hypertension was defined as an anti-hypertensive treatment or systolic BP ⩾ 140 and/or diastolic BP ⩾ 90 mm Hg.
[e]Hypercholesterolemia was defined as serum TC ⩾ 220 mg dl^{-1}.

proportions and the Jonckheere–Terpstra trend test for continuous measures. General linear models were used to estimate adjusted mean values of BMI, SBP, DBP and TC across quintiles of 24-h urinary Mg/Cre ratio after adjustment for potential confounding variables. Multiple linear regression models were used to estimate regression coefficients for the change in cardiovascular disease risk factors for an increase of 10 mg g^{-1} in 24-h urinary Mg/Cre ratio. The models were initially adjusted for potential confounders, such as traditional cardiac risk factors (age, sex and use of anti-hypertensive medication). The final multivariable models were additionally adjusted for natural logarithm-transformed 24-h urinary Na/Cre, K/Cre, Ca/Cre and Tau/Cre ratios, survey years (1985–1989 or 1990–1994, in which participants who had an average age of 52 years were born in 1933–1937 or 1938–1942, respectively, to adjust for potential cohort effects on the study outcomes), region (Europe, Africa, Asia, Oceania, North America or South America) and BMI. BMI was not included in the adjusted variables when the dependent variable was BMI.

To evaluate the association between Mg/Cre ratio and cardiovascular disease risk factors, we estimated adjusted odds ratios for obesity, hypertension and hypercholesterolemia in relation to quintiles of Mg/Cre using logistic regression models, including variables for age; sex; anti-hypertensive medication use; natural logarithm-transformed Na/Cre, K/Cre, Ca/Cre and Tau/Cre ratios; survey year (1985–1989 or 1990–1994); region (Europe, Africa, Asia, Oceania, North America or South America); and BMI. Adjustments were made in two stages the same as in the multiple linear regression model analyses described above. Anti-hypertensive medication use was not included in the adjusted variables in the estimation of odds ratios for hypertension.

All statistical analyses except for the Cochrane–Armitage test for trends were conducted using SPSS (Statistical Package for the Social Sciences) 15.0J for Windows (IBM Japan, Tokyo, Japan). The Cochrane–Armitage test for trends was performed using EXCEL 2003 (Microsoft, Tokyo, Japan). A two-sided *P*-value ⩽ 0.05 was considered statistically significant.

RESULTS

After excluding the participants who had missing data or who failed to complete the 24-h urine collection, the remaining 4211 participants (2120 men and 2091 women) were included in the data analyses.

The mean values and proportions of each characteristic by quintile of 24-h urinary Mg/Cre ratio are shown in Table 1. There was an ≈3.2-fold difference in the Mg/Cre ratio between the highest and lowest quintiles of the study population (medians: 123.6 mg g^{-1} day^{-1} in the highest quintile, 38.1 mg g^{-1} day^{-1} in the lowest). There was no association between Mg/Cre ratio and mean age, but higher Mg/Cre ratios were associated with lower BMI, SBP, DBP and TC and with higher Na/Cre, K/Cre, Ca/Cre and Tau/Cre ratios. Participants with lower Mg/Cre ratios were more likely to be male, obese, hypertensive or hypercholesterolemic than participants with higher Mg/Cre ratios.

Table 2 shows adjusted mean values of cardiovascular disease risk factors by 24-h urinary Mg/Cre ratio quintile. Mg/Cre ratios were inversely associated with BMI, SBP, DBP and TC. Adjustment of the analysis for age, sex and anti-hypertensive drug use in Model 1 did not markedly attenuate these associations between Mg/Cre ratio and BMI, SBP, DBP and TC. Upon further adjustment for Na/Cre, K/Cre, Ca/Cre and Tau/Cre ratios, survey year and region as for BMI, and additional adjustment for BMI as for SBP, DBP and TC in Model 2, the inverse trends remained significant for all the parameters (*P* for linear trend <0.001 for all comparisons across quintiles).

Linear regression coefficients for the cardiovascular disease risk factors in relation to an increase of 10 mg g^{-1} in the 24-h urinary Mg/Cre ratio are shown in Table 3. Inverse associations were consistently observed between Mg/Cre ratio and BMI, SBP, DBP

Table 2 Adjusted means (95% CIs) of BMI, BP and serum TC according to the levels of 24-h urinary Mg/Cre ratio in the CARDIAC Study, 1985–1994

	Quintiles of Mg/Cre ratio, mg g^{-1}					
	1 (Low) (n = *842, median, 38.1; range, 1.1–<49.1)*	*2* (n = *845, median, 57.5; range, 49.1–<65.0)*	*3* (n = *840, median, 72.7; range, 65.0–<80.9)*	*4* (n = *842, median, 90.2; range, 80.9–<103.0)*	*5 (High)* (n = *842, median, 123.6; range, ⩾103.0)*	P *for linear trend*[a]
BMI, kg m^{-2}						
Crude	25.8 (25.1, 26.4)	25.4 (24.7, 26.1)	24.9 (24.2, 25.6)	24.7 (24.1, 25.4)	23.5 (23.2, 23.7)	<0.001
Model 1[b]	25.9 (25.2, 26.5)	25.5 (24.8, 26.1)	24.9 (24.3, 25.6)	24.7 (24.0, 25.3)	23.4 (23.1, 23.7)	<0.001
Model 2[c]	25.5 (24.8, 26.2)	25.2 (24.6, 25.9)	24.9 (24.3, 25.5)	24.8 (24.2, 25.4)	23.9 (23.7, 24.2)	<0.001
Systolic BP, mm Hg						
Crude	128.2 (125.0, 131.3)	124.8 (121.6, 128.0)	123.5 (120.4, 126.7)	122.9 (119.8, 126.1)	123.0 (121.7, 124.4)	<0.001
Model 1[b]	127.9 (124.7, 131.1)	124.6 (121.4, 127.8)	123.6 (120.5, 126.8)	123.0 (119.9, 126.2)	123.3 (122.0, 124.6)	<0.001
Model 2[c]	128.1 (124.4, 131.7)	124.3 (121.0, 127.7)	123.5 (120.3, 126.8)	122.7 (119.5, 125.9)	123.8 (122.4, 125.3)	<0.001
Diastolic BP, mm Hg						
Crude	78.1 (76.1, 80.1)	75.2 (73.2, 77.2)	74.4 (72.4, 76.4)	73.3 (71.3, 75.3)	72.7 (71.8, 73.5)	<0.001
Model 1[b]	77.6 (75.6, 79.6)	74.9 (72.9, 76.9)	74.4 (72.4, 76.4)	73.6 (71.6, 75.6)	73.2 (72.3, 74.0)	<0.001
Model 2[c]	77.8 (75.5, 80.0)	74.7 (72.5, 76.8)	74.2 (72.2, 76.3)	73.4 (71.3, 75.4)	73.7 (72.8, 74.5)	<0.001
Serum TC, mg dl^{-1}						
Crude	193.9 (186.5, 201.3)	194.3 (186.9, 201.7)	189.0 (181.6, 196.4)	189.8 (182.4, 197.3)	178.4 (175.4, 181.5)	<0.001
Model 1[b]	195.2 (187.7, 202.8)	195.1 (187.6, 202.6)	189.2 (181.7, 196.7)	188.9 (181.5, 196.3)	177.1 (174.0, 180.2)	<0.001
Model 2[c]	193.1 (185.4, 200.9)	191.1 (183.9, 198.3)	187.8 (180.7, 194.8)	189.2 (182.3, 196.1)	184.3 (181.3, 187.3)	<0.001

Abbreviations: BMI, body mass index; BP, blood pressure; CI, confidence interval; Mg/Cre; magnesium/creatinine; TC, total cholesterol.
[a]From the multiple linear regression models for the relationship between 24-h urinary Mg/Cre ratio and each biomarker associated with cardiovascular diseases.
[b]Model 1 was adjusted for age, sex and use of anti-hypertensive drugs.
[c]Model 2 was adjusted for model 1 plus log-transformed 24-h urinary sodium/creatinine, potassium/creatinine, calcium/creatinine, taurine/creatinine, survey year, region as for BMI and additionally adjusted for BMI as for BPs and TC.

and TC after adjustment for age, sex, anti-hypertensive drug use, 24-h urinary Na/Cre, K/Cre, Ca/Cre and Tau/Cre ratios, survey year and region as for BMI and additional adjustment for BMI as for SBP, DBP and TC.

Odds ratios for obesity, hypertension and hypercholesterolemia are presented in Table 4 by the 24-h urinary Mg/Cre ratio quintile. The Mg/Cre ratio was significantly inversely associated with obesity, hypertension and hypercholesterolemia. The prevalence of obesity and hypercholesterolemia increased in a dose-dependent manner from the highest Mg/Cre ratio quintile to the lowest. Participants with the lowest Mg/Cre ratios were 2.1 and 2.1 times more likely to have obesity and hypercholesterolemia, respectively, than those in the highest Mg/Cre ratio quintile. Further adjustment for confounding variables did not markedly change these inverse associations. In the univariate analyses of the prevalence of hypertension, participants with the lowest Mg/Cre ratios were 1.6 times more likely to have hypertension than those in the highest Mg/Cre ratio quintile. However, the risk of hypertension among the subjects in the second to fourth quintiles was not significantly higher than that among the subjects in the highest quintile. Age- and sex-adjusted analyses and further adjustment for other urinary markers, survey year, region and BMI did not markedly change these associations between hypertension and the Mg/Cre ratio.

DISCUSSION

In this multi-center cross-sectional study, a high 24-h urinary Mg/Cre ratio was associated with low BMI, SBP and DBP, TC, obesity, hypertension and hypercholesterolemia. Adjustment for potential confounders did not markedly change these associations. Although dietary and serum Mg have been previously reported to be inversely associated with cardiovascular disease risk factors in large-scale observational studies,[3–9] 24-h urinary Mg has only been reported to be inversely associated with BP. Our study is the first to report an inverse association between the 24-h urinary Mg/Cre ratio and

Table 3 Linear regression coefficients for the relationship between each increase of 10 mg g^{-1} in the 24-h urinary Mg/Cre ratio and BMI, BP and serum TC in the CARDIAC Study, 1985–1994

	24-h Urinary Mg/Cre ratio, mg g^{-1} [a]								
	Crude			*Model 1*[b]			*Model 2*[c]		
	Mean	*s.e.*	P-*value*	*Mean*	*s.e.*	P-*value*	*Mean*	*s.e.*	P-*value*
BMI, kg m^{-2}	−0.19	0.02	<0.001	−0.20	0.02	<0.001	−0.12	0.02	<0.001
Systolic BP, mm Hg	−0.36	0.07	<0.001	−0.32	0.07	<0.001	−0.24	0.09	<0.01
Diastolic BP, mm Hg	−0.38	0.05	<0.001	−0.30	0.05	<0.001	−0.23	0.06	<0.001
Serum TC, mg dl^{-1}	−1.49	0.17	<0.001	−1.68	0.18	<0.001	−0.86	0.19	<0.001

Abbreviations: BMI, body mass index; BP, blood pressure; Mg/Cre; magnesium/creatinine; TC, total cholesterol.
[a]*P*-values are from the multiple linear regression models for the relationship between 24-h urinary Mg/Cre ratio (per 10 mg g^{-1} increase) and each biomarker associated with cardiovascular diseases.
[b]Multivariate model 1 was adjusted for age, sex and anti-hypertensive drug use.
[c]Multivariate model 2 was adjusted for model 1 plus log-transformed 24-h urinary sodium/creatinine, potassium/creatinine, calcium/creatinine, taurine/creatinine, survey year and region as for BMI and additionally adjusted for BMI as for systolic and diastolic BP and serum TC.

Table 4 ORs (and 95% CIs) for the prevalence of cardiovascular disease risk factors according to the levels of 24-h urinary Mg/Cre ratio in the CARDIAC Study, 1985–1994

	Quintiles of Mg/Cre ratio, mg g^{-1}					
	1 (Low)	*2*	*3*	*4*	*5 (High)*	P *for linear trend*[a]
Obesity[b]						
Crude	2.10 (1.50, 2.95)	1.85 (1.31, 2.62)	1.57 (1.10, 2.23)	1.63 (1.14, 2.31)	1.00 (Referent)	<0.001
Model 1[c]	2.49 (1.75, 3.53)	2.13 (1.50, 3.03)	1.72 (1.20, 2.47)	1.68 (1.18, 2.39)	1.00 (Referent)	<0.001
Model 2[d]	1.45 (0.95, 2.24)	1.38 (0.94, 2.04)	1.25 (0.85, 1.84)	1.33 (0.92, 1.93)	1.00 (Referent)	<0.001
Hypertension[b]						
Crude	1.55 (1.25, 1.92)	0.94 (0.75, 1.18)	0.97 (0.77, 1.22)	0.93 (0.74, 1.17)	1.00 (Referent)	<0.001
Model 1[c]	1.49 (1.20, 1.86)	0.91 (0.72, 1.15)	0.96 (0.76, 1.20)	0.92 (0.73, 1.15)	1.00 (Referent)	<0.001
Model 2[d]	1.54 (1.19, 2.00)	0.93 (0.73, 1.19)	0.97 (0.77, 1.23)	0.92 (0.73, 1.16)	1.00 (Referent)	<0.001
Hypercholesterolemia[b]						
Crude	2.06 (1.63, 2.62)	1.79 (1.41, 2.27)	1.41 (1.10, 1.80)	1.51 (1.18, 1.92)	1.00 (Referent)	<0.001
Model 1[c]	2.39 (1.87, 3.05)	2.01 (1.57, 2.57)	1.53 (1.19, 1.97)	1.54 (1.21, 1.98)	1.00 (Referent)	<0.001
Model 2[d]	2.73 (2.03, 3.67)	2.12 (1.62, 2.77)	1.57 (1.20, 2.05)	1.56 (1.20, 2.02)	1.00 (Referent)	<0.001

Abbreviations: CI, confidence interval; Mg/Cre, magnesium/creatinine; OR, odds ratio.
[a]Logistic regression analysis evaluating the linear relationships between 24-h urinary Mg/Cre ratio and cardiovascular diseases risk factors.
[b]Obesity, hypertension and hypercholesterolemia were defined as body mass index (BMI) ⩾ 30 kg m^{-2}, any anti-hypertensive medication use or systolic blood pressure ⩾ 140 mm Hg and/or diastolic blood pressure ⩾ 90 mm Hg and serum total cholesterol ⩾ 220 mg dl^{-1}, respectively.
[c]Model 1 was adjusted for age and sex as to hypertension and additionally adjusted for anti-hypertensive medication use as to obesity and hypercholesterolemia.
[d]Model 2 was adjusted for model 1 plus log-transformed 24-h urinary sodium/creatinine, potassium/creatinine, calcium/creatinine, taurine/creatinine, survey year and region as for BMI and additionally adjusted for BMI as for hypertension and hypercholesterolemia.

cardiovascular disease risk factors among worldwide population samples.

Obesity is a major risk factor for chronic diseases such as type 2 diabetes and atherosclerosis, which have been reported to be associated with low Mg status.[23] Low Mg status has been shown to occur more often in obese than in non-obese individuals.[24] Mg intake has been reported to be inversely associated with waist size among young Americans.[25] However, few studies have reported a direct relationship between Mg and obesity. Further studies are needed to explain the mechanism of interaction between Mg status and obesity.

Dietary Mg has been shown to be inversely associated with BP and hypertension in several observational studies.[3,4,26–29] In our study, the risk of hypertension was significantly higher only in the lowest Mg/Cre ratio quintile. Khan *et al.*[30] reported that there was no association between serum Mg and the risk of hypertension among the middle-aged adult participants in the Framingham Heart Study offspring cohort. The authors noted that because of the limited number of study participants with very high or low serum Mg, it had been impossible to draw conclusions regarding the risk associated with a serum Mg value far outside the normal range, and serum Mg levels might be linked to hypertension in other populations, including those with a higher prevalence of Mg deficiency. Because our study participants consisted of worldwide populations with various dietary customs, urinary Mg levels were widely distributed. As the authors noted, the risk of hypertension may not increase in a dose-dependent manner and yet still be higher in Mg-deficient conditions.

As for the mechanisms of how Mg decreases BP, numerous experimental and several clinical studies have been reported. For example, SBP and intracellular Ca in peripheral lymphocytes were found to be significantly higher in stroke-prone spontaneously hypertensive rats than in normotensive Wistar-Kyoto rats, and intracellular Mg in these rats, which is inversely correlated with intracellular Ca, was significantly lower.[31] Intracellular Ca overload acts as a trigger of cell death by inducing apoptosis, and it causes arteriosclerosis.[32] Mg activates Na–K ATPase, which controls the intracellular mineral balance and contributes to the homeostasis of electrolytes in the cell.[33] It was experimentally demonstrated that intracellular Ca and Na are reduced by oral Mg supplementation, which lowers BP in stroke-prone spontaneously hypertensive rats[34] and hypertensive patients.[35]

Although urinary Mg/Cre ratios were inversely associated with serum TC in our study, many observational studies have reported a positive association between serum or dietary Mg and serum cholesterol.[3,7,36,37] The discrepancy of these results may be partly due to differences in the subjects' characteristics. The foregoing observational studies were conducted among subjects living in particular regions, and therefore, the range of Mg levels might have been relatively narrow compared with that in our study including worldwide populations. Furthermore, dietary Mg intake has a tendency to correlate with the intake of dietary fiber,[1] which is known to lower serum TC.[38,39] This correlation may have partly influenced the inverse association between urinary Mg/Cre ratio and serum TC in our study. In streptozotocin- and fructose-fed diabetic rat models, dietary Mg administration prevents the development of dyslipidemia and preserves a normal serum lipid profile.[40] Mg has been shown to exert direct actions on lipid metabolism, interfering with several lipogenic liver enzymes and downregulating lipoprotein–lipase activity.[23]

There are some limitations to our study. First, a cause–effect association cannot be determined from the present analysis because of the cross-sectional study design. Second, we did not have some important data. TG was not determined because of the difficulty of asking the participants to fast for >12 h in a worldwide cross-country study. Low-density lipoprotein and high-density lipoprotein-cholesterol were not analyzed, because frozen serum samples had to be sent to a standardized analytical center in accordance with the CARDIAC multi-center study protocol.[18] Apolipoproteins, which are coronary heart disease risk factors,[41] were not analyzed because of the difficulty of setting up a standardized method of analysis at the time of designing the CARDIAC Study in 1985.[18] Our study also lacks data on blood glucose level and HbA1c (glycated hemoglobin), indicators of diabetes, which is one of the components of metabolic syndrome.

However, this study also has some strengths. First, the Mg/Cre ratios in 24-h urine varied widely, because the CARDIAC Study was a worldwide multi-center study including participants with various dietary customs, and thus we were able to detect significant inverse associations between the Mg/Cre ratio and cardiovascular disease risk factors. Second, we assessed the dietary intake of nutrients using 24-h urine, which enabled an objective evaluation of dietary intake.

In conclusion, higher 24-h urinary Mg/Cre ratios were associated with lower cardiovascular disease risk factor values, including BMI, SBP and DBP, TC, obesity, hypertension and hypercholesterolemia. Moreover, Mg deficiency was related to susceptibility to hypertension among the participants in the CARDIAC Study disregarding ethnic differences, living conditions and gender.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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