Magnesium and Zinc Intake Ratio Mediates the Increase of Coronary Artery Calcification through Upregulating Interleukin 6

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ORIGINAL ARTICLE

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ABSTRACT

The relation between dietary minerals and coronary artery calcification (CAC) has been emphasized. However, the effects of multiple dietary minerals on CAC progression remain unclear. This study Investiagetes the effect of combined dietary mineral intake on the progression of CAC. We analyzed a population-based cohort with 6814 participants from the Multi-Ethnic Study of Atherosclerosis (MESA). CAC scores were measured at baseline and subsequent follow-up examinations by Multi-detector computed tomography (MDCT) scans with Agatston scores. Then, the progression of CAC was defined through increased CAC scores in the follow-up from the baseline exam. The results revealed that the dietary intake of individual minerals did not show significant differences across CAC progression vs non progression groups. However, participants with CAC progression had an increased Magnesium (Mg):Zinc (Zn) ratio ($P < 0.05$). This effect was significant in logistic regression after adjusting for multiple established risk factors of CAC progression (OR 1.050; 95% CI 1.003, 1.099; $P = 0.038$). The increased risk of CAC associated with Mg/Zn was mediated through an increase level of IL-6, which increased with association to the Mg: Zn ratio. In conclusion, the dietary of Mg: Zn ratio, rather than individual mineral intake is associated with increased risk of CAC progression, which is mediated by pro-calcific IL-6. Therefore, the consideration of dietary intake of Zn and Mg together would play a cardio protective role among CAC patients.

1. Introduction

Cardiovascular diseases (CVD) are a group of serious diseases that lead to death. As known, traditional risk factors of CVD include age, obesity, hypertension, dyslipidemia, chronic kidney disease, and diabetes mellitus (DM) [1]. The non traditional risk factors of CVD such as vascular calcification (VC) have been recently emphasized [2]. Further, it is thought that VC is a result of the interaction among multiple complex cellular signaling pathways [3] that are enhanced by the up-regulation of key proinflammatory cytokines [4]. Ultimately, it is an outcome of the osteogenic differentiation of vascular smooth muscle cells (VSMC). Furthermore, several inflammatory mediators such as C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), and Interleukins (IL) have been identified to directly promote VC [5]. The progression of coronary artery calcium (CAC) score, which is used in clinical practice as a radiological diagnostic tool of VC is associated with major cardiac adverse events (MACE) [6–8]. Moreover, CAC is a strong predictor of increased vascular morbidity and mortality [9,10]. It's involved in the assessment of atherosclerosis risks and progression [11]. Recently, CAC has an increased clinical significance in the early detection of atherosclerosis and the risk of progression among cardiovascular patients [12,13].

As yet no specific pharmacological therapies are available for treating CAC. Dietary intervention seems to be a potential method to reduce CAC progression [14] and improve CVD outcomes [15,16]. In addition, over recent years, an increasing number of studies investigated the effect of different dietary patterns and nutritional constituents on CAC [14,17–19]. Though no population-specific beneficial dietary advice could have been formulated because of limited studies, advanced analysis of the database could provide new information that could rise specific beneficial dietary advice. Notably, a particular food item is a complex of different nutrients and their individual effects often differ from their collective effect [20]. On the other hand, minerals consist an important part of the diet and are essential for cardiovascular nutrition. Since micronutrient supplements are not consumed independently in the diet,

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it is often inappropriate to assess the association between single micronutrient intake and CAC [20]. Therefore, the effect of combinations of multiple minerals from diet in a large population-based cohort analysis seems to enlighten our understanding on vascular nutrition.

A range of nutritional studies reported both beneficial, as well as harmful effects of particular dietary mineral intakes on CAC, warranting further research [21–23]. Zinc (Zn) is one of the most important micro minerals that was lately focused to have a relationship with CAC. Several observational and animal studies have reported that increased dietary Zn intake attenuated VC [21,24]. But, the relationship between dietary magnesium (Mg) intake and VC is doubtful. Some studies reported an inverse association between Mg intake and CAC [25]. Conversely, a cross-sectional study reported no association between dietary Mg and CAC [26]. Moreover, another population-based study has shown that high serum Mg is associated with increased thoracic aortic calcification among diabetic patients [27].

Although the increased studies considering the dietary intakes of different minerals with CAC, the direct effect of mineral intakes remains controversial. Recent studies argued the absence of clinical experimental evidences of the effect of individual mineral intakes on CAC. Thus, most of these studies concluded that mineral intake could be a significant risk factor increasing the occurrence of CAC [28]. However, in spite of the rising numbers of nutritional research discussing the effect of individual mineral dietary intake, clear mechanistic details on the effect of combination of dietary mineral intakes on CAC are still unavailable. Therefore, within the Multi-Ethnic Study of Atherosclerosis (MESA) population, we investigated the relationship between multiple mineral intakes and CAC progression. We further investigated the possible role of inflammatory cytokines mediating the relationship. This study has a clinical significance because it highlights a new area of atherosclerosis risks regarding dietary mineral intakes in cardiovascular diseases.

2. Materials and methods

2.1. Study population

The MESA is an observational cohort study which aimed to determine characteristics related to progression from subclinical to clinical CVD. A total of 6814 participants consisting of Caucasian, African-American, Chinese and Hispanic, aged between 45 and 84 years from 6 different regions of the USA who were free from any CVD, were enrolled in 2000–2002 for exam 1 (baseline). Participants were followed up in exam 2 (2002–2004), exam 3 (2004–2005), exam 4 (2005–2007), and exam 5 (2010–2012). Procedural details of MESA study have been published [29].

2.2. Assessment of dietary intake

Data were collected from 120 item food frequency questionnaire that included participant's usual dietary pattern during the previous year. Data regarding mineral consumption were obtained based on the participants' usual dietary pattern. In addition to individual intakes, we also used ratios of different minerals to investigate the combined effect of minerals on CAC.

2.3. Measurement of CAC score

CAC was measured as mean Agatston scores (Phantom adjusted) for every participant at baseline (exam 1). Follow-up CT scans with measurements of CAC scores were obtained at subsequent examinations (exams 2 and 3) from 2002–2005. Methodology of CT scans and CAC measurements has been published previously. CAC severity was divided into 3 levels of increasing baseline CAC score: mild CAC (Agatston1-100), moderate CAC (Agatston100-400), and severe CAC (Agatston>400). Change in CAC scores (Δ CAC) was calculated as arithmetical difference between follow-up and baseline scores. CAC progression is a categorical variable which is defined to have had occurred when Δ CAC>1. Remaining participants (Δ CAC≤1) were grouped as CAC non progression. In this study, we only included the participants with baseline CAC measurements and at least one follow-up CAC measurement, and eliminated all missing values, thereby enrolling a total of 6814 participants for final analyses.

2.4. Additional measurements

Demographic data and lifestyle risk factors of every participant were obtained at baseline by interviewer and self-administered questionnaire. Anthropometry and laboratory data were derived from standardized physical examination and venipuncture. Body mass index (BMI) was calculated using WHO category as weight (kg) divided by square of height (m). History of hypertension was defined as systolic BP (SBP) ≥140 mmHg and diastolic BP (DBP) ≥90 mmHg. Seated/ resting SBP and DBP have been measured three times for each participant and the average of 2nd and 3rd measurement has been considered for analyses. History of DM has been obtained by 2003 ADA criteria. A fasting plasma glucose ≥126 mg/dl or use of antidiabetic medication was considered as DM. Estimated glomerular filtration rate (eGFR) was measured by CKD-EPI equation.

2.5. Statistical analyses

Baseline characteristics of the participants were analyzed using mean ± standard deviation (SD) or median (25th, 75th percentiles) for continuous variables and percentages for categorical variables. We used ANOVA and Chi square test for analyzing the differences among categories of CAC score (progression vs non progression). Multivariable logistic regression was performed to examine the independent variable across CAC progression categories (CAC score progression vs non progression). Initially, we performed an unadjusted model (model 1) and multivariable logistic regression analysis for significant and potential confounding variables (models 2, 3, and 4) to investigate the association between dietary mineral intake and CAC progression. Further, we performed sub-group analyses by age, sex, BMI, eGFR, presence of DM and hypertension, smoking, and alcohol drinking status. Variables were adjusted with age, sex, race, BMI, eGFR, triglycerides, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total cholesterol, and smoking.

Furthermore, mediation analysis was conducted to explore the mediators on the relationship of mineral intake and CAC progression. Mediation existed when four conditions were met: first, the predictor (in this case mineral consumption and inflammatory markers) must have a significant relationship with the outcome variable (CAC progression) in pathway c; second, the predictor must also have a significant relationship with the potential mediators in pathway a; third, the mediator must have a significant relation with the outcome when the effect of the predictor on the outcome was controlled for pathway b; fourth, the relationship between predictor and outcome must be decreased (lower than in pathway c) when controlling for the mediators in pathway c'. If the predictor remained significant when the mediator was controlled for,

Table 1. Baseline characteristics of study populations according to CAC score progression.

Variables	No CAC progression (N = 3892)	CAC progression (N = 2922)	P value
Demographics			
Age, year	59.8 (10.10)	65.2 (9.58)	<0.001
Male, N (%)	1542 (39.6)	1671 (57.2)	<0.001
Hypertension, N (%)	1510 (38.8)	1548 (53.0)	<0.001
Diabetes, N (%)	883 (22.8)	915 (31.4)	<0.001
Smoking, N (%)	1776 (45.8)	1598 (54.8)	<0.001
Alcohol, N (%)	2057 (67.5)	1692 (69.7)	0.071
Examinations			
BMI, kg/m^2	28.3 (5.57)	28.4 (5.24)	0.233
SBP, mmHg	124.1 (21.44)	129.9 (21.10)	<0.001
DBP. mmHg	71.3 (10.35)	72.7 (10.08)	<0.001
PP, mmHg	52.8 (16.90)	57.2 (17.43)	<0.001
Biochemistry			
Fasting glucose, mg/dl	95.5 (29.41)	99.86 (31.23)	<0.001
eGFR, ml/min/1.73 m^2	80.1 (16.03)	75.3 (16.35)	<0.001
HDL, mg/dl	52.1 (15.22)	49.5 (14.17)	<0.001
LDL, mg/dl	116.3 (31.36)	118.4 (31.57)	0.009
Cholesterol, mg/dl	193.7 (35.11)	194.8 (36.54)	0.179
Triglyceride, mg/dl	128.3 (88.17)	136.0 (89.45)	<0.001
Inflammatory markers			
TNF	1316.7 (461.22)	1438.5 (445.29)	<0.001
IL 2	941.2 (443.96)	1036.2 (480.59)	<0.001
IL 6	1.5 (1.23)	1.6 (1.22)	<0.001
hsCRP	3.8 (6.07)	3.7 (5.63)	0.522
Dietary intake			
Total protein,g	60.4 (32.83)	59.9 (32.06)	0.534
Total fat, g	55.0 (35.67)	53.8 (33.84)	0.211
Total carbohydrate, g	200.3 (105.34)	196.7 (98.62)	0.178
Phosphorus, mg	1035.4 (587.60)	1037.1 (588.80)	0.909
Magnesium, mg	258.4 (130.91)	262.1 (129.83)	0.259
Zinc, mg	8.2 (4.84)	8.2 (4.63)	0.902
Calcium, mg	719.0 (521.18)	719.4 (539.10)	0.972
Mg: Zn ratio	33.3 (8.23)	33.8 (8.17)	0.028
Iron, mg	12.2 (6.39)	12.3 (6.07)	0.846
Cupper, mg	1.1 (0.62)	1.1 (0.58)	0.954
Sodium, mg	2154.7 (1276.21)	2134.7 (1222.49)	0.534
Potassium, mg	2611.0 (1308.49)	2653.3 (1319.50)	0.208
B6, mg	1.6 (0.79)	1.6 (0.76)	0.278
B12, mcg	3.6 (3.35)	3.5 (2.83)	0.625
Folate, mcg	360.0 (186.93)	356.8 (177.94)	0.489
Folate: B12	157.3 (843.07)	138.5 (117.36)	0.252
Calcium:phosphorus	0.7 (0.16)	0.7 (0.16)	0.511
Vitamin Supplements, N (%)	2138 (60.6)	1629 (61.2)	0.616

*BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, PP; pulse pressure, HDL; high-density lipoprotein cholesterol, LDL, low-density lipoprotein cholesterol, eGFR; estimated glomerular filtration rate, TNF-α; tumor necrosis factor alpha, *IL-2; Interleukin-2*, IL-6; interleukin-6, CACS; coronary artery calcium score, F-Glu; Fasting glucose, CRP; C-reactive protein, B6; Vitamin *B6*

the mediation was considered partial. When controlling for the mediator rendered the predictor non-significant, mediation was considered complete. The parameter estimates a, b, c, c' and their standard errors calculated above must be standardized according to methods MacKinnon suggested. Then the indirect effect was calculated by standardized parameters and tested for significance by Sobel test. The mediated effect size was also evaluated by a formula ab/(ab+c'), where a, b, and c' were all standardized.

We used IBM SPSS Statistics v22 for all data analyses and two-tail P value <0.05 has been considered significant.

3. Results

3.1. Characteristics of MESA participants across CAC score at baseline

6814 participants of multi ethnic populations were enrolled. As presented in Table 1; the mean age of the CAC in MESA population was 62.5 (± 9.84) years of which 57.2% were males. In addition, 1548 (53%) were hypertensive and 915 (31.4%) had a history of DM. Within the study population, 2,922 (42.8%) had detectable CAC scores by CT scan and the remaining 3892 (57.1%) were free from CAC at baseline. Participants with detectable CAC were more likely to be elderly, males, had increased systolic, diastolic, and pulse pressures. The

differences across two groups of baseline CAC have been presented in *supplementary table 1*. Participants with detectable CAC had lower intake of dietary Zn. No differences were observed in terms of other individual dietary mineral intakes except for the ratio between Mg and Zn (Mg: Zn) which was significantly higher among participants with detectable baseline CAC ($P = 0.028$) as seen in Table 1.

3.2. Characteristics of MESA participants across CAC progression groups

Baseline characteristics of MESA study population across two groups were defined by CAC progression. Similarly, participants with CAC progression were more likely to be elderly, males and more prevalence of hypertension and DM were observed, as shown in Table 1. Presumably, they exhibited increased serum fasting glucose, worse renal function (as indicated by lower eGFR), altered fasting lipid profile (indicated as combination of serum triglyceride, LDL-C, HDL-C, and total cholesterol) and upregulated inflammatory markers except high sensitivity C reactive protein (hsCRP). In terms of tested dietary mineral intakes, statistical difference was observed only for Mg:Zn which was shown to be elevated significantly within CAC progression group ($P = 0.028$).

We further divided the study population, using the mean value (33.52) as cut off, within groups according
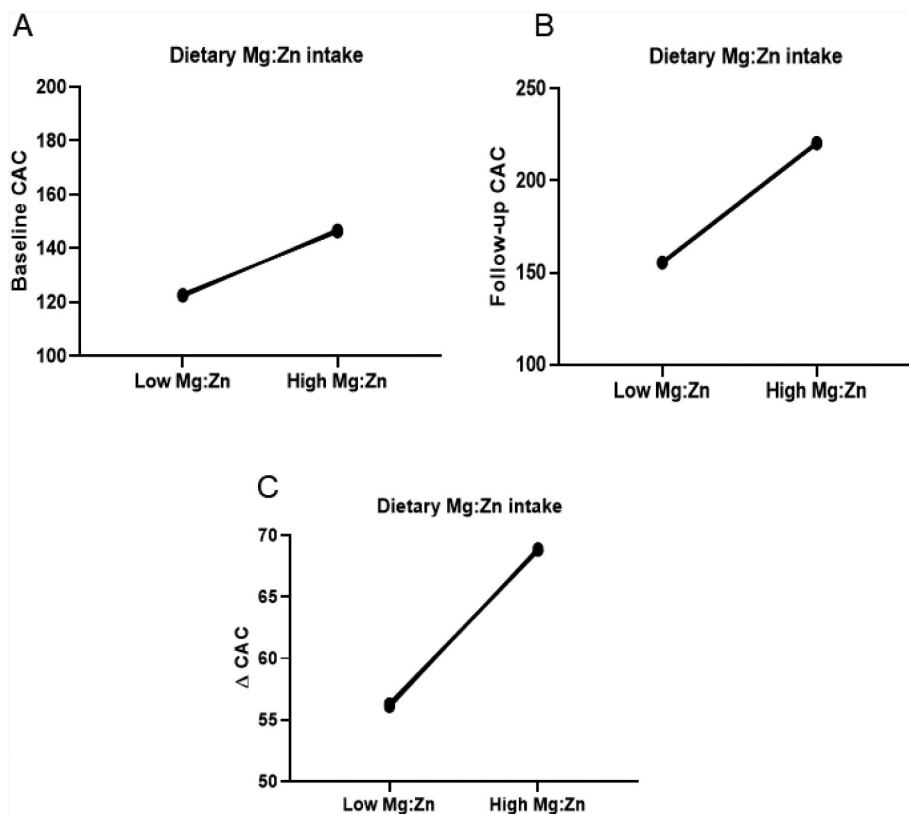

Figure 1. The association between dietary intake of Mg:Zn and coronary artery calcification. (1A) higher Mg:Zn intake was associated with increased baseline CAC, (1B) follow-up CAC, and (1 C) CAC progression.

Table 2. Multivariable logistic regression demonstrating the association between Mg:Zn ratio and CACS progression.

Dietary Mg:Zn models	OR	95% CI	P value
Model 1 (Curde)	1.007	1.001, 1.014	0.028
Model 2	1.064	1.018, 1.111	0.005
(It was adjusted with age gender, smoking, alcohol consumption, hypertension, diabetes, BMI, SBP, DBP, PP, serum fasting glucose, triglycerides, cholesterol, HDL-C, LDL-C, eGFR, IL-6)			
Model 3	1.050	1.003, 1.099	0.038
(It was adjusted with serum PO_4, serum PTH, vitamin supplements, total energy intake, dietary calcium, vitamin B6, B12, and folate)			

*BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, PP; pulse pressure, HDL-C; high density lipoprotein cholesterol, LDL-C, low density lipoprotein cholesterol, eGFR; estimated glomerular filtration rate, IL-6; interleukin 6, CACS; coronary artery calcium score, PO_4; phosphate, PTH; parathyroid hormone

to higher dietary Mg:Zn (2,834; 53.7%) and lower Mg:Zn (248; 46.3%) intake. As evident from Figure 1, higher Mg:Zn intake was associated with increase in baseline CAC (1A), follow-up CAC (1B) as well as CAC progression (1 C).

3.3. Association between combined Mg and Zn intake and CAC progression

The association between Mg:Zn intake and CAC progression as presented in Table 2, in crude logistic regression (model 1) there was a significant association between dietary Mg:Zn intake and CAC progression. In multivariable logistic regression (model 2)

after adjustment with demographic and biochemical risk factors for CAC progression, Mg:Zn intake remained associated with increased risk of CAC progression (OR: 1.064; 95% CI: 1.018–1.111; $P = 0.005$). Further adjustment with serum phosphate, parathyroid hormone and dietary consumption of total energy, calcium, folic acid, vitamins, and vitamin supplement-use (model 3) did not change the results (OR: 1.050; 95% CI: 1.003–1.099; $P = 0.038$).

3.4. Investigating the possible mediator(s) in the association between Mg:Zn intake and CAC progression

We also investigated whether the association between Mg:Zn intake and CAC progression was mediated by any proinflammatory cytokine. In non stratified crude model, IL-1, IL-2 and tumor necrosis factor alpha (TNF-α) failed to demonstrate any mediating role (*supplementary file*, Table 2). However, as Figure 2 depicts, the effect of Mg:Zn intake on CAC progression was partially mediated by IL-6 [Sobel test score 2.24; (SE 0.0016); $P = 0.02$] in nonstratified model adjusted with gender, hypertension, DM, smoking, HDL-C, LDL-C, and eGFR. Though, TNF-α and IL-2 showed no mediating role (*supplementary file*, Table 3). We further performed mediation analysis with age-stratified adjusted model (*supplementary file, Table 4*) but none of the inflammatory markers showed any significant mediating effect in the association between dietary Mg:Zn ratio and CAC progression.
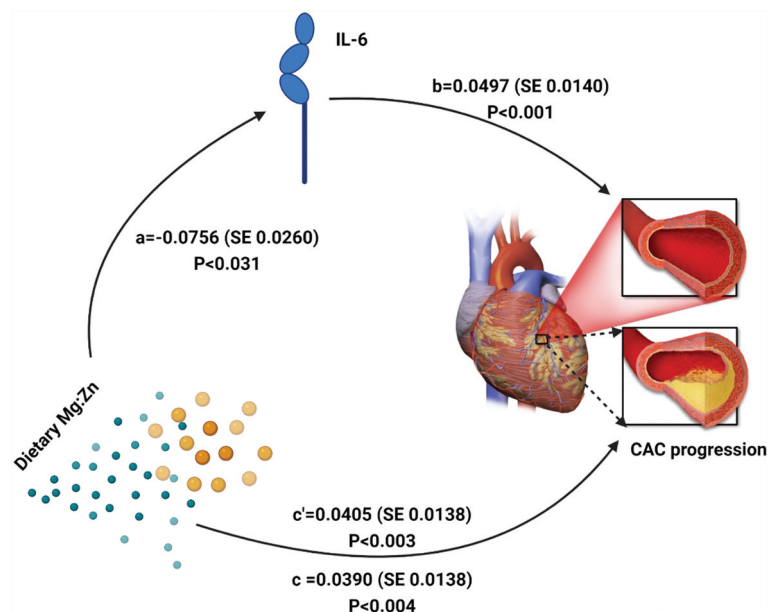

Figure 2. The effect of Mg:Zn intake on CAC progression was partially mediated by IL-6 [Sobel test score 2.24;(SE 0.0016); $P = 0.02$] in non-stratified model adjusted with gender, hypertension, DM, smoking, HDL-C, LDL-C, and eGFR. Though, TNF-α, and IL-2 showed no mediating role.

Table 3. The association between Mg:Zn intake with CAC score progression within subgroups of total study population.

Subgroups	OR	95% CI	P value	P value for interactions
Age				0.017*
≤65 years	2.571	2.139, 3.091	<0.001*	
>65 years	2.381	1.879, 3.017	<0.001*	
Sex				0.590
Female	1.009	1.000, 1.019	0.060	
Male	1.012	1.001, 1.023	0.040*	
Hypertension				0.255
present	1.006	0.996, 1.017	0.242	
absent	1.014	1.004, 1.024	0.006*	
Diabetic status				0.784
No diabetes	1.011	1.003, 1.020	0.007*	
Impaired fasting glucose	1.010	0.990, 1.030	0.327	
Diabetes present	1.005	0.982, 1.030	0.655	
Renal function				0.828
eGFR≥70 ml/min/ 1.73 m²	1.012	1.003, 1.021	0.010*	
eGFR<70 ml/min/ 1.73 m²	1.008	0.995, 1.022	0.205	
Smoking status				0.205
No smoker	1.018	1.008, 1.028	0.001*	
Former smoker	0.994	0.982, 1.006	0.342	
Current smoker	1.036	1.011, 1.061	0.004*	
Alcohol consumption				0.800
No	1.005	0.989, 1.021	0.547	
Yes	1.013	1.003, 1.002	0.011*	

*Adjusted with age, gender, smoking, hypertension, diabetes, eGFR, HDL-C, LDL-C

3.5. Sub-group analysis of dietary Mg:Zn intake and CAC progression

To explore the specific population groups where the effect of dietary Mg:Zn intake on CAC progression was more prominent, we performed sub groups analysis based on established risk factors of CAC progression. We defined old age at 65 years as recommended by WHO. As seen in Table 3, the combined effect of higher dietary Mg and lower Zn intake was associated with increased risk of CAC progression regardless of age, male sex, participants without history of chronic diseases such as hypertension, DM, renal diseases, and alcohol consumers (all $P < 0.05$). No interaction effect was noticed in the different sub groups when tested for interaction terms.

4. Discussion

Our study found that (1) a diet consisting of combined Mg and Zn i.e. (Mg:Zn ratio) was associated with increased risk of CAC and CAC progression regardless of age distribution and confounding risk factors; (2) the effect of increased Mg:Zn on CAC progression was partially mediated by IL-6, an inflammatory cytokine. To the best of our knowledge, this is the first study examining the effect of dietary combined Mg and Zn intake on CAC progression, highlighting the role of inflammatory mediator.

Minerals have an important role in the regulation of metabolic and physiological pathways in the human body, while the excess or deficiencies of some minerals increase the risk of specific diseases [30,31] including CVD [32]. Therefore, a well-balanced diet has been suggested for better cardiovascular protection [33]. In addition, the protective effects of minerals among CVD patients have become an important and interesting topic for many researchers. Additionally, effects of several minerals on CAC and CAC risk factors such as hypertension, altered serum lipids, peripheral artery disease, and DM have been reported [34,35]. Furthermore, some of these studies have also shown that increased intakes of calcium (Ca), Mg, and potassium (K) have a negative correlation with blood pressure, which is also a strong risk factor for the development and progression of CAC [36,37]. Other studies have shown that Zn, iron (Fe) copper (Cu), and selenium (Se), play an important role in regulating cell metabolism [38]. Food is a matrix of different nutrients with varying degree of actions on cardio-metabolic health [39]. Since minerals are not consumed in isolation, their interactive and additive effects are crucial to be examined.

Although no prior studies have examined the direct relationship between combined dietary Mg:Zn ratio and CAC, several studies reported the relationship between individual dietary Zn and Mg intake and CVD as well as CAC [24,40–42]. The role of Zn on lowering the risk of CVD has been reported by a range of studies [43,44]. In a randomized clinical trial with 60 patients with DM, followed for 12 weeks, combined Mg and Zn supplement was shown to be beneficial for reducing fasting glucose along with inflammation and total antioxidant capacity. Though the sample size was small and no underlying mechanism was defined in this study [45]. Besides, blood pressure regulation is critically important for retarding CAC [46,47]. In another study, Zn was shown to modulate renin-angiotensin-aldosterone axis thereby regulating arterial pressure [48]. Moreover, Zn intake was associated with reduced carotid intima-media thickness, which is, in turn linked with lower risk of development of CAC [49]. Results from the clinical studies were in keeping with the experimental studies which also supported the beneficial role of Zn on VC [21,24,50].

The role of dietary Mg intake on CVD has been extensively studied. Most studies highlighted the favorable effect of Mg on CVD outcomes [51,52]. Nevertheless, these results were reported especially in patients with chronic kidney disease has been associated with worse CVD outcomes [53]. Moreover, neutral effect of Mg was also reported [54,55]. Similarly, the role of Mg intake on CAC is poorly understood [26]. A range of studies reported Mg intake was beneficial for VC [56]. In Framingham heart study, increased consumption of Mg was associated with 22% decreased risk of developing CAC, though temporal relationship could not be examined

[57]. However, in PROGREDIR study, higher dietary Mg intake was associated with the highest tertile of CAC among 373 dialysis-independent CKD patients [42]. Furthermore, in a prior study within the MESA could not demonstrate any significant effect of self-reported dietary Mg intake on CAC [58].

In either of the above scenarios, a clear underlying mechanism was lacking which called for further evaluation.

Results from our study did not demonstrate any significant differences among individual mineral intakes across groups of CAC progression. Nevertheless, after combining two minerals only Mg: Zn ratio was shown to be associated with the increase of CAC progression risk when adjusted for established risk factors. Dietary data also suggested that even though Zn and Mg are consumed in lesser quantities (*supplementary file figure*), their combined effect on CAC surpasses those which are consumed in comparatively higher quantities. Our results are, therefore, indicating that a diet containing lower dietary Zn and higher Mg could promotes CAC.

Further, VC develops as a result of interaction between cross-linking cellular pathways. Inflammation is the key factor contributing to the development of VC [59]. In addition, a large number of researches confirmed that reactive oxygen species (ROS) [60], endothelial dysfunction [61] immune dysregulation [59] collectively contribute to osteogenic differentiation of VSMC. Besides, chronic diseases including DM, hypertension, dyslipidemia, CKD have been associated with triggering the pro calcific pathways [62]. In line with this, Zn was shown to inhibit ROS-induced oxidation of LDL-C [63], and likewise, inadequate intake of Zn was associated with increased production of ROS [64]. Moreover, another study reported that Zn down regulated expressions of ILs which is important for reduced burden of VC [59]. Furthermore, Zn deficiency is associated with immune dysfunction and induces nuclear factor kappa beta (NF-kβ) which has a strong procalcific role [65].

Studies with Mg mostly supported beneficial effect of VC biomarkers and risk factors. In a rat model, Mg was shown to alleviate oxidative stress related inflammatory insult [66]. Besides, Mg was associated with improved fasting blood sugar and lipids [67]. In contrast with the current understandings, our study shows that higher Mg intake, when combined with concomitant Zn intake, is associated with increased risk of baseline CAC and CAC progression. Thus, it seems that the effect of combined mineral intake may deviate from that of individual intake. Moreover, the sources of dietary mineral intake could be responsible for the tested outcome. It has been documented that red meat is the major source of Zn in USA [68] and rice is the major source of Zn in

Japan [69]. Similarly, chocolate, coffee, fish, and whole grains are major sources of dietary Mg [57].

This study demonstrates that among the participants of the MESA, combined effect of Zn and Mg intake, rather than individual minerals from diet leads to CAC progression through upregulation of IL-6. Alternatively, modulation of the proportion of dietary mineral intakes might have potential benefit for CAC retardation. Regarding this, future large scale clinical studies as well as experimental models should focus on the role of combination of a range of dietary minerals on various markers of calcification.

4.1. Strength and limitation

This study reports the association of dietary Mg and Zn intake and CAC progression and to further explain the association by demonstrating the mediating role of IL-6. The relationship remained statistically significant even after adjustment with established risk factors for CAC progression. MESA has a large, well-characterized, and diverse sample size which is a powerful tool to measure the relationship between dietary mineral intake and CAC. Besides, participants had repeated measurements of CAC that enabled us to monitor the change in their CAC score (Δ CAC) over time. Our study had some limitations. Firstly, dietary intake in MESA pollution was evaluated by Food Frequency Questionnaires, which may not include all foods consumed and may impair the quantification of mineral intake. Secondly, serum levels of minerals as well as the change in frequency of mineral consumption were not measured in subsequent follow-up examinations which restricted us from performing further investigations. Further, according to the analysis of multi ethnic data this study is warning the possibility of the relation between Mg:Zn dietary intake and CAC through upregulation of IL-6 which needs further experimental confirmation using animal model studies.

5. Conclusion

In conclusion, we found that dietary Mg:Zn is associated with increased risk of CAC progression. This is noticed partially mediated by IL-6. Therefore, a cardio-protective diet may be considered incorporating more amount of Zn and less amount of Mg. Moreover, pharmacological therapies are invited to test the effect of targeting the absorption of these minerals to exert more beneficial effects retarding CAC progression.

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Disclosure statement

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Author contributions

Research idea was conceived by Abdulhakim Al-qaridhi and Hui Huang. Data collection and statistical analyses were performed by Sounak Ghosh, Dongling Luo and Abdulhakim Al-qaridhi. The manuscript was written by Abdulhakim Al-qaridhi and revised by Dongling Luo and Sounak Ghosh. All authors read and agreed upon the final version of the manuscript.

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