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Association between Serum Magnesium Concentration with Metabolic Syndrome, Diabetes, Hypertension and Hyperuricemia in Knee Osteoarthritis

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Abstract

Objectives: This cross-sectional study aimed to examine associations between serum magnesium (Mg) concentration with the prevalence of metabolic syndrome (MetS), diabetes (DM), hypertension (HP) and hyperuricemia (HU) in radiographic knee osteoarthritis (OA) patients. It was hypothesized that serum Mg concentration was inversely associated with these diseases.

Methods: The present study was conducted at the Health Management Center of Xiangya Hospital. Radiographic OA was evaluated in patients aged over than 40 years with basic characteristics and blood biochemical assessment.

Results: A total of 962 radiographic knee OA patients were included. The multivariable-adjusted OR (95% CI) showed a significant lower prevalence of MetS in the second (OR=0.58, 0.36-0.94, P=0.026) and highest quintile (OR=0.56, 95CI% 0.34-0.93, P=0.024) compared with the reference quintile of serum Mg. Meanwhile, a significant lower prevalence of DM was observed in the second (OR=0.38, 0.22-0.67, P=0.001), third (OR=0.35, 0.19-0.64, P=0.001), fourth (OR=0.27, 0.14-0.53, P<0.001) and highest quintile (OR=0.21, 95CI% 0.10-0.41, P<0.001). A significant lower prevalence of HU was observed in the third (OR=0.36, 0.20-0.63, P<0.001), fourth (OR=0.54, 0.31-0.93, P=0.026) and highest quintile (OR=0.39, 95CI% 0.22-0.68, P=0.001). However, there was no significant association between serum Mg and HP in OA patients.

Conclusions: The present study indicated that the serum Mg concentration was inversely associated with the prevalence of MetS, DM and HU in radiographic knee OA patients. Thus, elevating serum Mg level is more likely to be associated with the decreasing prevalence of MetS, DM and HU among subjects with knee OA.

Level of Evidence: Level Ⅲ, cross-sectional study.

Key words: osteoarthritis, magnesium, metabolic syndrome, diabetes, hypertension, hyperuricemia

Strengths and limitations of this study

1. This is the first study examining the associations between serum magnesium (Mg) and the prevalence of metabolic syndrome, diabetes mellitus, hypertension and hyperuricemia in radiographic knee osteoarthritis patients.
2. The multivariable logistical regression models in this study were adjusted by a considerable number of potential confounding factors, which greatly improved the reliability of the results.
3. Kidney is the key organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by adding estimated glomerular filtration rate into multivariable logistic regression models, and the reverse associations remained significant.
4. This study adopted cross-sectional design which precluded causal correlations.
5. Serum Mg concentration was adopted as the indicator of body Mg content in this study which was not the best indicator of body status.

Introduction

The association between metabolic diseases, especially metabolic syndrome (MetS)[1 2] and diabetes mellitus (DM),[3-5] with osteoarthritis (OA) has drawn increasing attention in the past few years, and OA has also been classified into three specific phenotypes including metabolic OA, age-related OA and injure-related OA.[6] A large number of researches have indicated that the prevalence of MetS,[7-9] DM[10-18] and hypertension (HP)[7 9-13 19 20] are either higher in OA patients or associated with OA. In addition, some other studies reported that MetS,[21 22] DM[23 24] and HP[21 22] are the risk factors of OA progression. Thus, it appears necessary to pay more attention to the high prevalence of metabolic diseases in OA patients and even take measures to reduce their prevalence, which also seems to be beneficial in delaying OA progression.

Serum magnesium (Mg), one of the most important micronutrients for human health, has been reported to be negatively associated with MetS,[25-29] DM[30-38] and HP[30 39-41] by lots of studies. Furthermore, our previous study showed an inverse association between serum Mg with hyperuricemia (HU).[42] However, to our best knowledge, there is not yet a study examined the association between the serum Mg concentration with the aforementioned metabolic diseases (MetS, DM, HP and HU) in OA patients. In addition, another study of ours indicated that the serum Mg concentration may be inversely associated with radiographic knee OA.[43] Therefore, it is reasonably speculated that the prevalence of MetS, DM, HP and HU in OA patients may be reduced by elevating the level of serum Mg, which can in turn delay OA progression. Thus, the objective of the present study was to examine the associations between the serum Mg concentration with the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. It was hypothesized that serum Mg concentration was inversely associated with these diseases.

Methods

Study population

The present study was conducted at the Health Management Center of Xiangya Hospital between October 2013 and November 2014. The study design has been published previously.[42-46] The protocol of this study was reviewed and approved by the local Ethics and Research Committee, and the methods were carried out in "accordance" with the approved guidelines. Also the study population gave informed consent. Registered nurses interviewed all participants during the examination using a standard questionnaire, with the purpose to collect information on demographic characteristics and health-related habits. Participants were selected according to the following inclusion criteria: 1) 40 years old or above; 2) undergoing weight-bearing bilateral anteroposterior radiography of the knee, and diagnosed with knee OA according to Kellgren-Lawrence (K-L) radiographic atlas (knee joint was graded K-L 2 or above); 3) availability of all basic characteristics, including age, gender, body mass index (BMI) and blood pressure; 4) availability of biochemical test results, including serum Mg concentration; 5) availability of information related to the living habits, including education background, activity level, smoking, drinking and medication status. Initially, this cross-sectional study included 1820 radiographic knee OA patients aged over than 40 years with sound basic characteristics and needed blood biochemical assessment (including serum Mg concentration). Among them, 962 patients offered demographic characteristics and health-related habits and they were finally included in this study.

Blood biochemistry

All blood samples were drawn after a 12-hour overnight fast and were kept at 4°C until analysis. All blood test were undertaken using a Beckman Coulter AU 5800 (Beckman Coulter Inc., Brea, CA, USA). The inter- and intra-assay coefficients of variation were tested by low concentrations (2.5 mmol/L for glucose, 118 μmol/L for

uric acid and 0.60 mmol/L for serum Mg) and high concentrations (6.7 mmol/L for glucose, 472 μmol/L for uric acid and 1.00 mmol/L for serum Mg) of standard human samples. The intra-assay coefficients of variation were 0.98% (2.5 mmol/L) and 1.72% (6.7 mmol/L) for glucose, 1.39% (118 μmol/L) and 0.41% (472 μmol/L) for uric acid, and 1.86% (0.60 mmol/L) and 1.65% (1.00 mmol/L) for serum Mg. The inter-assay coefficients of variation were 2.45% (2.5 mmol/L) and 1.46% (6.7 mmol/L) for glucose, 1.40% (118 μmol/L) and 1.23% (472 μmol/L) for uric acid, and 1.87% (0.60 mmol/L) and 1.70% (1.00 mmol/L) for serum Mg.

Assessment of other exposures

Blood pressure was measured by an electronic sphygmomanometer. The weight and height of each subjects was measured respectively to calculate the BMI. Participants were asked about their average frequency of physical activity (never, one to two times per week, three to four times per week, five times and above per week) and average duration of physical activity (within half an hour, half an hour to one hour, one to two hours, more than two hours). The smoking, alcohol drinking and medication status were asked face to face.

Assessment of MetS, DM, HP and HU

MetS was diagnosed according to the Chinese Diabetes Society (CDS) criteria.[47-49] CDS criteria for metabolic syndrome requires 3 items or all the four items: (1) BMI ≥25 kg/m2; (2) Fasting plasma glucose (FPG) ≥6.1 mmol/L, or diagnosed DM; (3) Systolic blood pressure (BP)≥140 mmHg or diastolic BP≥90 mmHg, or treatment of previously diagnosed HP; (4) Triglycerides ≥1.7 mmol/L and/or HDL-cholesterol <0.9 mmol/L in male or <1.0 mmol/L in female, or treatment for this lipid abnormality. Subjects with the fasting glucose ≥7.0 mmol/L or currently undergoing

drug treatment for blood glucose control were regarded as DM patients, and subjects with the systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or currently using antihypertensive medication were regarded as HP patients. HU was defined as uric acid ≥416 μmol/L for male and ≥360 μmol/L for female or currently undergoing drug treatment for uric acid control.

Statistical analysis

The continuous data are expressed as mean (standard deviation), and the category data are expressed in percentage. Differences in continuous data were evaluated by one-way classification ANOVA (normally distributed data) or Kruskal-Wallis H test (non-normally distributed data), while differences in category data were assessed by the $\chi 2$ test. The serum Mg was classified into five categories based on the quintile distribution: ≤0.85, 0.86-0.89, 0.90-0.92, 0.93-0.96 and ≥0.97 mmol/L. Logistic regression was conducted in two models in order to calculate the adjusted ORs with 95% CIs for the associations of serum Mg with MetS, DM, HP and HU. Three models were adjusted for the association. Model 1 were adjusted for age and sex. Then, model 2, a multivariable model was adopted. Covariates were chosen based on previous similar studies.[27 33 50 51] Model 2 for the association between serum Mg and MetS was adjusted by age (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data) and alcohol drinking status (yes, no). Model 2 for the association between serum Mg and diabetes was adjusted by age (continuous data), BMI (≥25 kg/m2, <25 kg/m2), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), HP (yes, no), and dyslipidemia (yes, no). Dyslipidemia was defined by triglycerides ≥1.7 mmol/L and/or HDL-cholesterol <0.9 mmol/L in male or <1.0 mmol/L in female, or treatment for this lipid abnormality.

Model 2 for the association between serum Mg and hypertension was adjusted by age (continuous data), BMI (≥25 kg/m2, <25 kg/m2), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), diabetes (yes, no), and dyslipidemia (yes, no). Model 2 for the association between serum Mg and HU was adjusted by age (continuous data), BMI (≥25 kg/m2, <25 kg/m2), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), HP (yes, no), DM (yes, no) and dyslipidemia (yes, no). Model 3 for all associations were adjusted based on model 2, with additional factor of estimated glomerular filtration rate (eGFR). eGFR was calculated by serum creatinine (Scr), sex, and patients' age. The calculation formula was: 186 × SCr−1.154× age−0.203× 1.210 (if black) × 0.742 (if female).[52] Tests for linear trends were conducted based on logistic regression using a median variable of Mg concentration in each category. All data analyses were performed using SPSS 17.0; P≤0.05 was considered to be statistically significant. All test were two tailed.

Results

A total of 962 subjects were included in the present cross-sectional study. The characteristics of the study population according to quintiles of serum Mg were illustrated in Table 1. The mean age of the subjects was 54.9±7.6 years old, and there were 377 females (39.2%). The overall prevalence of MetS, DM, HP and HU in OA patients were 21.4%, 12.0%, 38.5% and 18.3% respectively. Significant differences were observed across quintiles of serum Mg for fasting glucose, the prevalence of DM and HU.

Outcomes of multivariable adjusted associations between MetS and serum Mg concentration were shown in Table 2. The age-sex adjusted OR values (Model 1)

suggested a significant lower prevalence of MetS in the second (OR=0.61, 95CI% 0.38-0.97, P=0.038) and highest quintile (OR=0.59, 95CI% 0.36-0.96, P=0.035) compared with the reference quintile of serum Mg in OA patients, and the P for trend was 0.090. The multivariable adjusted OR values (Model 2) showed similar outcomes (OR=0.60, 95CI% 0.37-0.96, P=0.035 in the second quintile; OR=0.61, 95CI% 0.37-0.99, P=0.047 in the fifth quintile), and the P for trend was 0.120. The sensitivity analysis, by adding eGFR into model 2, also reached similar outcomes - a significant lower prevalence of MetS in the second (OR=0.58, 0.36-0.94, P=0.026) and highest quintile (OR=0.56, 95CI% 0.34-0.93, P=0.024) compared with the reference quintile of serum Mg, and the P for trend was 0.066.

Table 3 indicated the multivariable adjusted relations of serum Mg and DM in OA patients. Both age-sex adjusted OR values (Model 1) and multivariable adjusted OR values (Model 2) suggested a strong inverse association between serum Mg and diabetes. The age-sex adjusted ORs for the prevalence of diabetes were 0.38 (95CI% 0.22-0.66, P=0.001), 0.34 (95CI% 0.19-0.61, P<0.001), 0.29 (95CI% 0.15-0.55, P<0.001), and 0.20 (95CI% 0.10-0.40, P<0.001) in the second, third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was smaller than 0.0001. The multivariable adjusted ORs for the prevalence of diabetes were 0.38 (95CI% 0.22-0.66, P=0.001), 0.34 (95CI% 0.19-0.62, P<0.001), 0.27 (95CI% 0.14-0.52, P<0.001), and 0.20 (95CI% 0.10-0.40, P<0.001) in the second, third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was smaller than 0.0001. The sensitivity analysis, by adding eGFR into model 2, showed similar outcomes - a significant lower prevalence of DM in the second (OR=0.38, 0.22-0.67, P=0.001), third (OR=0.35, 0.19-0.64, P=0.001), fourth (OR=0.27, 0.14-0.53, P<0.001), and highest quintile (OR=0.21, 95CI% 0.10-0.41, P<0.001) compared with the reference quintile of serum Mg, and the P for trend was <0.001.

The multivariable-adjusted relations between serum Mg and HP in OA patients were listed in Table 4. According to the age-sex adjusted ORs (Model 1) and multivariable

adjusted ORs (Model 2), there was no significant association between serum Mg and hypertension, and the P for trend was 0.929 and 0.423, respectively. The sensitivity analysis, by adding eGFR into model 2, showed the same results.

The multivariable-adjusted relations of serum Mg and HU in OA patients were illustrated in Table 5. Both the age-sex adjusted OR values (Model 1) and the multivariable adjusted OR values (Model 2) suggested significant decreased prevalence of HU in the third quintile (age-sex adjusted OR=0.44, 95CI% 0.26-0.75, P=0.002; multivariable adjusted OR=0.42, 95CI% 0.24-0.73, P=0.002) and fifth quintile (age-sex adjusted OR=0.51, 95CI% 0.30-0.85, P=0.010; multivariable adjusted OR=0.50, 95CI% 0.29-0.86, P=0.012) compared with the lowest quintile of serum Mg, and the P for trend was 0.008 and 0.007, respectively. The sensitivity analysis, by adding eGFR into model 2, showed similar outcomes - a significant lower prevalence of HU in the third (OR=0.36, 0.20-0.63, P<0.001), fourth (OR=0.54, 0.31-0.93, P=0.026), and highest quintile (OR=0.39, 95CI% 0.22-0.68, P=0.001) compared with the reference quintile of serum Mg, and the P for trend was <0.001.

Discussion

The results of this study suggested that the serum Mg concentration was negatively associated with the prevalence of MetS, DM and HU in subjects with radiographic knee OA. In order to control potential confounders, several covariates such as characteristics, living habits and underlying diseases were selected, and even the eGFR was added into the multivariable logistic regression models to eliminate the influence of renal function on Mg excretion. The reverse associations mentioned above remained significant after adjustments of confounders. However, such negative association between serum Mg and the prevalence of HP was not observed in radiographic knee OA patients.

Mg, the fourth most abundant cation in human body and the second most profuse intracellular cation, is a metallic cofactor for over 300 enzymatic reactions. It appears to play an important role in glucose metabolism and insulin homeostasis, which are highly correlated with metabolic diseases, especially MetS and DM. The mechanisms involved in the Mg deficiency with MetS, DM and HU are probably multifactorial. The most important one may be insulin resistance, as Mg is essential for insulin action and is a critical cofactor for several enzymes in carbohydrate metabolism, which is important for phosphorylation reactions of tyrosine-kinase in the insulin receptor.[31 53-57] Incidentally, our previous prospective study involving 62897 person-years of follow-up showed that hematocrit was independently associated with the incidence of HU through, with a high possibility, the insulin resistance mechanism.[58] Other potential mechanisms included cellular calcium homeostasis,[54] glucose transportation,[56] oxidative stress[56] and inflammatory cytokines.[59-61] Of course, it is necessary to highlight the fact that insulin can also induce Mg excretion[62] and produce a significant decline of plasma Mg through ion exchange.[63] Thus, there seems to be a vicious circle between Mg deficiency and insulin resistance.

MetS[21 22] and DM[4 23 24] were reported to be the risk factors of OA progression. It seems that OA progression may be delayed by elevating the serum Mg level through decreasing the prevalence of MetS and DM. Some other studies proved that the serum Mg level was significantly associated with the high-sensitive C-reactive protein (CRP) concentration,[27 64-66] and higher CRP might serve as a prediction factor for OA progression.[67 68] Thus, OA progression may also be delayed by elevating the serum Mg level through decreasing the level of CRP. Above all, the present study indicated that elevating serum Mg level has the potential to reduce the prevalence of MetS, DM and HU in knee OA patients and may delay the progression of knee OA (Figure 1). However, the specific mechanism needs to be further explored.

The present study has several strengths. Firstly, this is the first study examining the associations between serum Mg and the prevalence of MetS, DM, HP and HU in

radiographic knee OA patients. The results of this study will provide a new insight into the treatment of knee OA. Secondly, the multivariable logistical regression models were adjusted by a considerable number of potential confounding factors, which greatly improved the reliability of the results. Thirdly, kidney is the key organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by adding eGFR into multivariable logistic regression models, and the reverse associations remained significant.

Limitations of the present study should also be admitted. The cross-sectional design precludes causal correlations, so further prospective studies and intervention trials should be undertaken to establish a causal association between serum Mg with the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. Since no previous research investigated such associations in knee OA patients, the value of this study should not be blotted out by the cross-sectional nature. Another limitation of this study lies in the relatively small sample size, and thus, extensive high-quality researches based on a larger sample are needed. Last but not the least, it is important to highlight that Mg is an intracellular ion; therefore, the serum Mg concentration must be considered as a poor indicator of body magnesium content,[69] even though this parameter has been used in many studies. However, blood magnesium level is the second best indicator of body status.[70]

Conclusions

The present study indicated that the serum Mg concentration was inversely associated with the prevalence of MetS, DM and HU in radiographic knee OA patients. Thus, elevating serum Mg level is more likely to be associated with the decreasing prevalence of MetS, DM and HU among subjects with knee OA.

Contributors

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. GHL, YLW and JW conceived the study. GHL, YLW and JW were responsible for conception and design of the study and drafted the manuscript. CZ, TY, HL, YC and DXX contributed to data collection. WJ contributed to preparation and data analysis. BX, ZCL, JTL, and SDJ contributed to study retrieval. GHL contributed to revision of the manuscript. All the authors contributed to the interpretation of the data and critically reviewed the manuscript for publication.

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Competing interests

The authors declare that they have no conflict of interest.

Ethics approval

The protocol of this study was reviewed and approved by the Ethics Committee at Xiangya Hospital.

Data sharing statement

The datasets during the current study available from the corresponding author on reasonable request.

REFERENCES

1. Zhuo Q, Yang W, Chen J, *et al*. Metabolic syndrome meets osteoarthritis. *Nat Rev Rheumatol* 2012;8:729-37.
2. Katz JD, Agrawal S, Velasquez M. Getting to the heart of the matter: osteoarthritis takes its place as part of the metabolic syndrome. *Curr Opin Rheumatol* 2010;22:512-9.
3. Berenbaum F. Diabetes-induced osteoarthritis: from a new paradigm to a new phenotype. *Ann Rheum Dis* 2011;70:1354-6.
4. King KB, Rosenthal AK. The adverse effects of diabetes on osteoarthritis: update on clinical evidence and molecular mechanisms. *Osteoarthritis Cartilage* 2015;23:841-50.
5. Kirkman MS. Osteoarthritis progression: is diabetes a culprit? *Osteoarthritis Cartilage* 2015;23:839-40.
6. Sellam J, Berenbaum F. Is osteoarthritis a metabolic disease? *Joint Bone Spine* 2013;80:568-73.
7. Puenpatom RA, Victor TW. Increased prevalence of metabolic syndrome in individuals with osteoarthritis: an analysis of NHANES III data. *Postgrad Med* 2009;121:9-20.
8. Shin D. Association between metabolic syndrome, radiographic knee osteoarthritis, and intensity of knee pain: results of a national survey. *J Clin Endocrinol Metab* 2014;99:3177-83.
9. Calvet J, Orellana C, Larrosa M, *et al*. High prevalence of cardiovascular co-morbidities in patients with symptomatic knee or hand osteoarthritis. *Scand J Rheumatol* 2015;1-4.

10. Rahman MM, Kopec JA, Cibere J, *et al*. The relationship between osteoarthritis and cardiovascular disease in a population health survey: a cross-sectional study. *BMJ Open* 2013;3:

11. Inoue R, Ishibashi Y, Tsuda E, *et al*. Medical problems and risk factors of metabolic syndrome among radiographic knee osteoarthritis patients in the Japanese general population. *J Orthop Sci* 2011;16:704-9.

12. Hart DJ, Doyle DV, Spector TD. Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. *J Rheumatol* 1995;22:1118-23.

13. Jungmann PM, Kraus MS, Alizai H, *et al*. Association of metabolic risk factors with cartilage degradation assessed by T2 relaxation time at the knee: data from the osteoarthritis initiative. *Arthritis Care Res (Hoboken)* 2013;65:1942-50.

14. Anagnostopoulos I, Zinzaras E, Alexiou I, *et al*. The prevalence of rheumatic diseases in central Greece: a population survey. *BMC Musculoskelet Disord* 2010;11:98.

15. Massengale M, Reichmann WM, Losina E, *et al*. The relationship between hand osteoarthritis and serum leptin concentration in participants of the Third National Health and Nutrition Examination Survey. *Arthritis Res Ther* 2012;14:R132.

16. Nieves-Plaza M, Castro-Santana LE, Font YM, *et al*. Association of hand or knee osteoarthritis with diabetes mellitus in a population of Hispanics from Puerto Rico. *J Clin Rheumatol* 2013;19:1-6.

17. Greiver M, Williamson T, Barber D, *et al*. Prevalence and epidemiology of diabetes in Canadian primary care practices: a report from the Canadian Primary Care Sentinel Surveillance Network. *Can J Diabetes* 2014;38:179-85.

18. Rahman MM, Cibere J, Anis AH, *et al*. Risk of Type 2 Diabetes among Osteoarthritis Patients in a Prospective Longitudinal Study. *Int J Rheumatol* 2014;2014:620920.

19. Reid JL, Morton DJ, Wingard DL, *et al*. Obesity and other cardiovascular disease risk factors and their association with osteoarthritis in Southern California American Indians, 2002-2006. *Ethn Dis* 2010;20:416-22.

20. Birtwhistle R, Morkem R, Peat G, *et al*. Prevalence and management of osteoarthritis in primary care: an epidemiologic cohort study from the Canadian Primary Care Sentinel Surveillance Network. *CMAJ Open* 2015;3:E270-5.

21. Yoshimura N, Muraki S, Oka H, *et al*. Accumulation of metabolic risk factors such as overweight, hypertension, dyslipidaemia, and impaired glucose tolerance raises the risk of occurrence and

progression of knee osteoarthritis: a 3-year follow-up of the ROAD study. *Osteoarthritis Cartilage* 2012;20:1217-26.

22. Monira HS, Wang Y, Cicuttini FM, *et al*. Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: a prospective cohort study. *Semin Arthritis Rheum* 2014;43:429-36.

23. Schett G, Kleyer A, Perricone C, *et al*. Diabetes is an independent predictor for severe osteoarthritis: results from a longitudinal cohort study. *Diabetes Care* 2013;36:403-9.

24. Eymard F, Parsons C, Edwards MH, *et al*. Diabetes is a risk factor for knee osteoarthritis progression. *Osteoarthritis Cartilage* 2015;23:851-9.

25. Guerrero-Romero F, Rodriguez-Moran M. Low serum magnesium levels and metabolic syndrome. *Acta Diabetol* 2002;39:209-13.

26. Guerrero-Romero F, Rodriguez-Moran M. Hypomagnesemia, oxidative stress, inflammation, and metabolic syndrome. *Diabetes Metab Res Rev* 2006;22:471-6.

27. Evangelopoulos AA, Vallianou NG, Panagiotakos DB, *et al*. An inverse relationship between cumulating components of the metabolic syndrome and serum magnesium levels. *Nutr Res* 2008;28:659-63.

28. Hjelmesaeth J, Hofso D, Aasheim ET, *et al*. Parathyroid hormone, but not vitamin D, is associated with the metabolic syndrome in morbidly obese women and men: a cross-sectional study. *Cardiovasc Diabetol* 2009;8:7.

29. Lima ML, Cruz T, Rodrigues LE, *et al*. Serum and intracellular magnesium deficiency in patients with metabolic syndrome--evidences for its relation to insulin resistance. *Diabetes Res Clin Pract* 2009;83:257-62.

30. Ma J, Folsom AR, Melnick SL, *et al*. Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin, and carotid arterial wall thickness: the ARIC study. Atherosclerosis Risk in Communities Study. *J Clin Epidemiol* 1995;48:927-40.

31. Kao WH, Folsom AR, Nieto FJ, *et al*. Serum and dietary magnesium and the risk for type 2 diabetes mellitus: the Atherosclerosis Risk in Communities Study. *Arch Intern Med* 1999;159:2151-9.

32. Wang JL, Shaw NS, Yeh HY, *et al*. Magnesium status and association with diabetes in the Taiwanese elderly. *Asia Pac J Clin Nutr* 2005;14:263-9.

33. Chambers EC, Heshka S, Gallagher D, *et al*. Serum magnesium and type-2 diabetes in African

Americans and Hispanics: a New York cohort. *J Am Coll Nutr* 2006;25:509-13.

34. Simmons D, Joshi S, Shaw J. Hypomagnesaemia is associated with diabetes: Not pre-diabetes, obesity or the metabolic syndrome. *Diabetes Res Clin Pract* 2010;87:261-6.

35. Sales CH, Pedrosa LF, Lima JG, *et al*. Influence of magnesium status and magnesium intake on the blood glucose control in patients with type 2 diabetes. *Clin Nutr* 2011;30:359-64.

36. Lecube A, Baena-Fustegueras JA, Fort JM, *et al*. Diabetes is the main factor accounting for hypomagnesemia in obese subjects. *PLoS One* 2012;7:e30599.

37. Xu J, Xu W, Yao H, *et al*. Associations of serum and urinary magnesium with the pre-diabetes, diabetes and diabetic complications in the Chinese Northeast population. *PLoS One* 2013;8:e56750.

38. Yang SJ, Hwang SY, Baik SH, *et al*. Serum magnesium level is associated with type 2 diabetes in women with a history of gestational diabetes mellitus: the Korea National Diabetes Program study. *J Korean Med Sci* 2014;29:84-9.

39. Singh RB, Rastogi V, Niaz MA, *et al*. Epidemiological study of magnesium status and risk of hypertension in a rural population of north India. *Magnes Res* 1996;9:173-81.

40. Peacock JM, Folsom AR, Arnett DK, *et al*. Relationship of serum and dietary magnesium to incident hypertension: the Atherosclerosis Risk in Communities (ARIC) Study. *Ann Epidemiol* 1999;9:159-65.

41. Guerrero-Romero F, Rodriguez-Moran M, Hernandez-Ronquillo G, *et al*. Low Serum Magnesium Levels and Its Association with High Blood Pressure in Children. *J Pediatr* 2016;168:93-8.

42. Zeng C, Wang YL, Wei J, *et al*. Association between low serum magnesium concentration and hyperuricemia. *Magnes Res* 2015;28:56-63.

43. Zeng C, Wei J, Li H, *et al*. Relationship between Serum Magnesium Concentration and Radiographic Knee Osteoarthritis. *J Rheumatol* 2015;42:1231-6.

44. Wei J, Zeng C, Gong QY, *et al*. Associations between Dietary Antioxidant Intake and Metabolic Syndrome. *PLoS One* 2015;10:e130876.

45. Xie DX, Xiong YL, Zeng C, *et al*. Association between low dietary zinc and hyperuricaemia in middle-aged and older males in China: a cross-sectional study. *BMJ Open* 2015;5:e8637.

46. Wei J, Zeng C, Gong QY, *et al*. The association between dietary selenium intake and diabetes: a cross-sectional study among middle-aged and older adults. *Nutr J* 2015;14:18.

47. Expert Panel on Metabolic Syndrome of Chinese Diabetes Society: Recommendations on metabolic syndrome of Chinese Diabetes Society (Chinese). *Chin J Diabetes* 2004;14:156-61.

48. Pang C, Jia L, Hou X, *et al*. The significance of screening for microvascular diseases in Chinese community-based subjects with various metabolic abnormalities. *PLoS One* 2014;9:e97928.

49. Zhou H, Guo ZR, Yu LG, *et al*. Evidence on the applicability of the ATPIII, IDF and CDS metabolic syndrome diagnostic criteria to identify CVD and T2DM in the Chinese population from a 6.3-year cohort study in mid-eastern China. *Diabetes Res Clin Pract* 2010;90:319-25.

50. Joosten MM, Gansevoort RT, Mukamal KJ, *et al*. Urinary magnesium excretion and risk of hypertension: the prevention of renal and vascular end-stage disease study. *Hypertension* 2013;61:1161-7.

51. Choi MK, Bae YJ. Association of Magnesium Intake with High Blood Pressure in Korean Adults: Korea National Health and Nutrition Examination Survey 2007-2009. *PLoS One* 2015;10:e130405.

52. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002;39:S1-266.

53. Grundy SM, Cleeman JI, Daniels SR, *et al*. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112:2735-52.

54. Barbagallo M, Dominguez LJ, Galioto A, *et al*. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. *Mol Aspects Med* 2003;24:39-52.

55. Song Y, Ridker PM, Manson JE, *et al*. Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* 2005;28:1438-44.

56. Guerrero-Romero F, Rodriguez-Moran M. Complementary therapies for diabetes: the case for chromium, magnesium, and antioxidants. *Arch Med Res* 2005;36:250-7.

57. Huerta MG, Roemmich JN, Kington ML, *et al*. Magnesium deficiency is associated with insulin resistance in obese children. *Diabetes Care* 2005;28:1175-81.

58. Zeng C, Wei J, Yang T, *et al*. Higher blood hematocrit predicts hyperuricemia: a prospective study of 62,897 person-years of follow-up. *Sci Rep* 2015;5:13765.

59. Bonora E, Targher G, Zenere MB, *et al*. Relationship of uric acid concentration to cardiovascular risk factors in young men. Role of obesity and central fat distribution. The Verona Young Men Atherosclerosis Risk Factors Study. *Int J Obes Relat Metab Disord* 1996;20:975-80.

60. Lyngdoh T, Marques-Vidal P, Paccaud F, *et al*. Elevated serum uric acid is associated with high circulating inflammatory cytokines in the population-based Colaus study. *PLoS One* 2011;6:e19901.

61. Kirilmaz B, Asgun F, Alioglu E, *et al*. High inflammatory activity related to the number of metabolic syndrome components. *J Clin Hypertens (Greenwich)* 2010;12:136-44.

62. Djurhuus MS, Skott P, Hother-Nielson O, *et al*. Insulin increases renal magnesium excretion: a possible cause of magnesium depletion in hyperinsulinaemic states. *Diabet Med* 1995;12:664-9.

63. Paolisso G, Sgambato S, Passariello N, *et al*. Insulin induces opposite changes in plasma and erythrocyte magnesium concentrations in normal man. *Diabetologia* 1986;29:644-7.

64. Chacko SA SYNL. Relations of dietary magnesium intake to biomarkers of inflammation and endothelial dysfunction in an ethnically diverse cohort of postmenopausal women. *Diabetes Care* 2010;33:304-10.

65. Bo S DMGS. Dietary magnesium and fiber intakes and inflammatory and metabolic indicators in middle-aged subjects from a population-based cohort. *Am J Clin Nutr* 2006;84:1062-9.

66. Kim DJ XPLK. Magnesium intake in relation to systemic inflammation, insulin resistance, and the incidence of diabetes. *Diabetes Care* 2010;33:2604-10.

67. Spector TD HDND. Low-level increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. *Arthritis Rheum* 1997;40:723-7.

68. Smith JW MTGE. Significance of C-reactive protein in osteoarthritis and total knee arthroplasty outcomes. *Ther Adv Musculoskelet Dis* 2012;4:315-25.

69. Topf JM, Murray PT. Hypomagnesemia and hypermagnesemia. *Rev Endocr Metab Disord* 2003;4:195-206.

70. Jr. Sabatier M PFAM. A compartmental model of magnesium metabolism in healthy men based on two stable isotope tracers. *Am J Physiol Regul Integr Comp Physiol* 2003;285:R656-63.

364 Table 1 Basic characteristics of included subjects according to quintiles of serum Mg (n=962)

	Quintiles of serum Mg					P
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
Age (years)	53.8 (7.3)	54.6 (7.6)	55.2 (7.9)	55.3 (7.1)	56.1 (8.0)	0.062
BMI (kg/m^2)	25.2 (3.2)	24.9 (3.2)	25.0 (3.7)	25.2 (3.4)	24.6 (3.2)	0.464
Female (%)	37.5	42.3	36.8	42.3	37.0	0.627
Smoking (%)	27.5	27.4	21.6	24.4	21.7	0.457
Alcohol drinking (%)	34.5	36.3	40.5	41.1	38.1	0.645
High school diploma (%)	45.0	47.4	45.3	56.5	48.1	0.184
Activity level (h/w)	2.0 (3.5)	2.0 (3.3)	2.3 (3.5)	2.1 (3.1)	2.4 (3.5)	0.457

Fasting glucose (mmol/l)	6.6 (3.0)	5.7 (1.7)	5.7 (1.4)	5.5 (0.9)	5.5 (1.6)	0.009
Systolic pressure (mm Hg)	129.2 (16.9)	128.3 (17.9)	130.4 (16.2)	128.8 (16.3)	129.6 (17.7)	0.837
Diastolic pressure (mm Hg)	81.2 (11.8)	79.8 (12.1)	80.7 (11.0)	80.7 (10.7)	80.3 (10.5)	0.654
HDL-cholesterol (mmol/l)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	1.5 (0.3)	1.5 (0.4)	0.374
Triglyceride (mmol/l)	2.1 (1.9)	1.8 (1.5)	2.0 (2.1)	1.8 (1.0)	2.3 (2.9)	0.620
Uric acid (µmol/l)	337.3 (101.7)	329.0 (80.7)	321.3 (86.3)	331.5 (78.0)	329.4 (81.7)	0.590
eGFR (ml/min/1.73m^2)	80.2 (14.4)	77.7 (10.7)	76.0 (10.6)	75.8 (10.7)	74.3 (12.0)	<0.001
MetS (%)	26.5	17.7	25.8	19.6	17.5	0.059
DM (%)	23.5	10.7	10.0	8.3	6.3	<0.001
HP (%)	40.0	33.5	37.4	42.3	40.2	0.432
HU (%)	25.5	19.1	13.2	18.5	14.8	0.018

Data are mean (Standard Deviation), unless otherwise indicated; Mg, magnesium; OA, osteoarthritis; BMI, body mass index; HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate; MetS, metabolic syndrome; DM, diabetes mellitus; HP, hypertension; HU, hyperuricemia.

P values are for test of difference across all quintiles of serum Mg.

379 Table 2 Multivariable-adjusted relations of serum Mg and MetS in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
MS (%)	26.5	17.7	25.8	19.6	17.5	-
Model 1*	1.00 (reference)	0.61 (0.38, 0.97)	0.97 (0.61, 1.52)	0.69 (0.42, 1.14)	0.59 (0.36, 0.96)	0.090
P value	-	0.038	0.881	0.150	0.035	-
Model 2*	1.00 (reference)	0.60 (0.37, 0.96)	1.00 (0.63, 1.57)	0.70 (0.42, 1.15)	0.61 (0.37, 0.99)	0.120
P value	-	0.035	0.99	0.160	0.047	-
Model 3*	1.00 (reference)	0.58 (0.36, 0.94)	0.95 (0.60, 1.50)	0.66 (0.40, 1.10)	0.56 (0.34, 0.93)	0.066
P value	-	0.026	0.818	0.109	0.024	

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; MetS, metabolic syndrome.

*Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

393 Table 3 Multivariable-adjusted relations of serum Mg and diabetes in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
Diabetes (%)	23.5	10.7	10.0	8.3	6.3	-
Model 1*	1.00 (reference)	0.38 (0.22, 0.66)	0.34 (0.19, 0.61)	0.29 (0.15, 0.55)	0.20 (0.10, 0.40)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-
Model 2*	1.00 (reference)	0.38 (0.22, 0.66)	0.34 (0.19, 0.62)	0.27 (0.14, 0.52)	0.20 (0.10, 0.40)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-
Model 3*	1.00 (reference)	0.38 (0.22, 0.67)	0.35 (0.19, 0.64)	0.27 (0.14, 0.53)	0.21 (0.10, 0.41)	<0.001
P value	-	0.001	0.001	<0.001	<0.001	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis.

*Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (≥25 kg/m^2, <25 kg/m^2), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), hypertension (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

407 Table 4 Multivariable-adjusted relations of serum Mg and hypertension in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
Hypertension (%)	40.0	33.5	37.4	42.3	40.2	-
Model 1*	1.00 (reference)	0.71 (0.47, 1.06)	0.83 (0.54, 1.25)	1.00 (0.66, 1.54)	0.89 (0.59, 1.35)	0.929
P value	-	0.095	0.368	0.987	0.582	-
Model 2*	1.00 (reference)	0.78 (0.51, 1.18)	0.92 (0.60, 1.41)	1.16 (0.75, 1.80)	1.03 (0.67, 1.58)	0.423
P value	-	0.242	0.708	0.502	0.896	-
Model 3*	1.00 (reference)	0.77 (0.51, 1.17)	0.90 (0.59, 1.38)	1.13 (0.73, 1.76)	0.99 (0.64, 1.53)	0.524
P value	-	0.218	0.629	0.577	0.978	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis.

* Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (≥25 kg/m^2, <25 kg/m^2), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

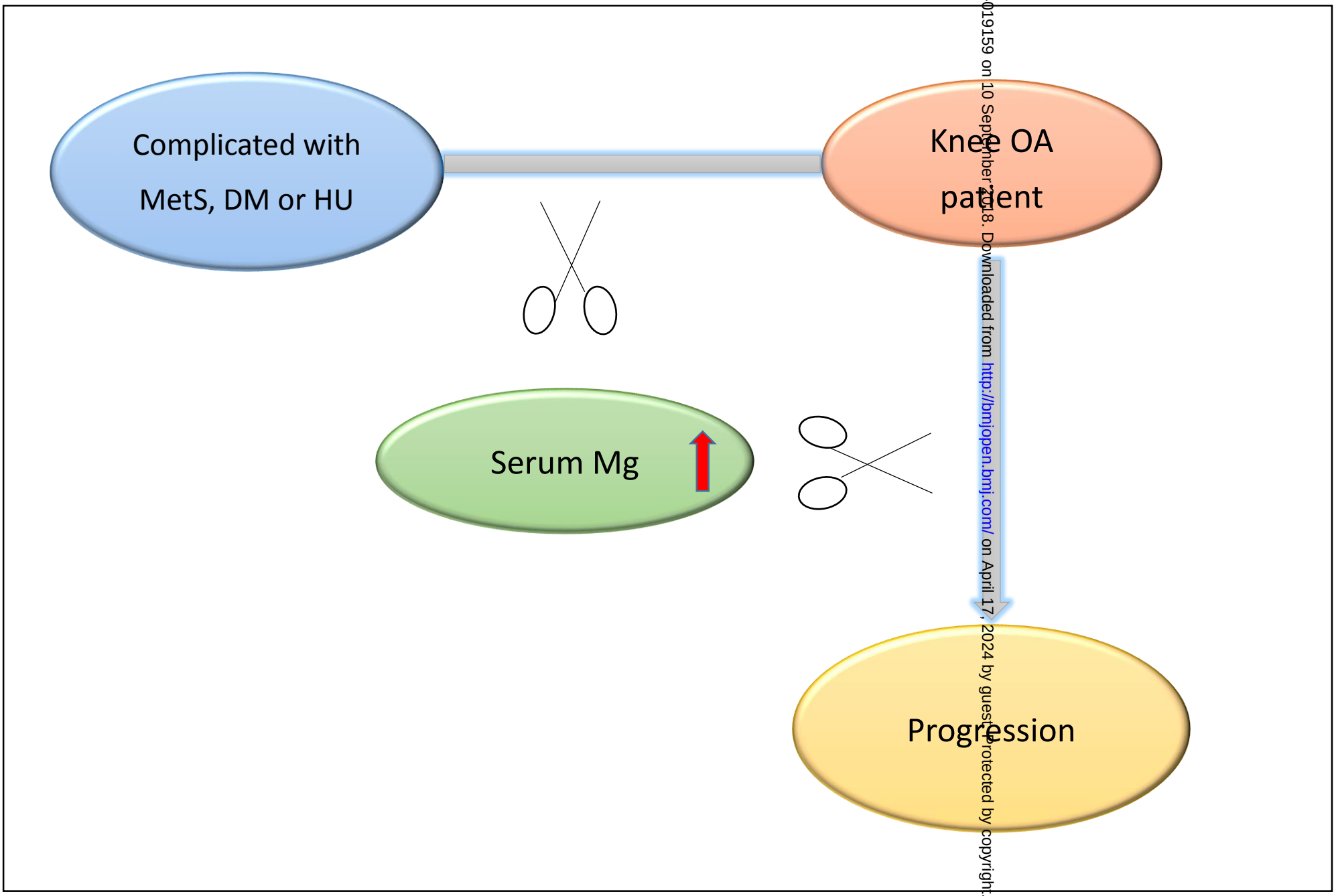
421 Table 5 Multivariable-adjusted relations of serum Mg and HU in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
HU (%)	25.5	19.1	13.2	18.5	14.8	-
Model 1*	1.00 (reference)	0.71 (0.44, 1.14)	0.44 (0.26, 0.75)	0.68 (0.41, 1.14)	0.51 (0.30, 0.85)	0.008
P value	-	0.157	0.002	0.144	0.010	-
Model 2*	1.00 (reference)	0.73 (0.45, 1.19)	0.42 (0.24, 0.73)	0.62 (0.37, 1.06)	0.50 (0.29, 0.86)	0.007
P value	-	0.205	0.002	0.082	0.012	-
Model 3*	1.00 (reference)	0.67 (0.41, 1.11)	0.36 (0.20, 0.63)	0.54 (0.31, 0.93)	0.39 (0.22, 0.68)	<0.001
P value	-	0.119	<0.001	0.026	0.001	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; HU, hyperuricemia.

* Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (≥25 kg/m^2, <25 kg/m^2), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), hypertension (yes, no), diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

Fig 1 Possible clinical significance of the present study. The present study indicates that elevating serum Mg level is more likely to be associated with decreasing prevalence of MetS, DM and HU among persons with knee OA. In addition to reduce the high-sensitive C-reactive protein level possibly, elevating serum Mg level may delay the progression of knee OA. It seems like elevating the serum Mg can cut off the connection between the prevalence of MetS, DM and HU with knee OA and delay the progression of OA. MetS, metabolic syndrome; DM, diabetes mellitus; HU, hyperuricemia; OA, osteoarthritis; Mg, magnesium.



STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Reported on Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	6-7
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	-
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	5-6

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4
		(b) Give reasons for non-participation at each stage	4
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	-
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	-
		Cross-sectional study—Report numbers of outcome events or summary measures	7-9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-9
		(*b*) Report category boundaries when continuous variables were categorized	7-9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10-11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between Serum Magnesium Concentration with Metabolic Syndrome, Diabetes, Hypertension and Hyperuricemia in Knee Osteoarthritis

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Association between Serum Magnesium Concentration with Metabolic Syndrome, Diabetes, Hypertension and Hyperuricemia in Knee Osteoarthritis

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Abstract

Objectives: To examine the associations between serum magnesium (Mg) concentration with the prevalence of metabolic syndrome (MetS), diabetes mellitus (DM), hypertension (HP) and hyperuricemia (HU) in radiographic knee osteoarthritis (OA) patients.

Methods: The present study was conducted at the Health Management Center of Xiangya Hospital. Radiographic OA was evaluated for patients aged over 40 years with basic characteristics and blood biochemical assessment. Serum Mg concentration was measured using the chemiluminescence method. MetS, DM, HP and HU were diagnosed based on standard protocols. The associations between serum Mg concentration with MetS, DM, HP and HU were evaluated by conducting multivariable adjusted logistic regression.

Results: A total of 962 radiographic knee OA patients were included. Compared with the lowest quintile, the multivariable-adjusted odds ratios (ORs) and related 95% confidence intervals (95%CI) of DM were 0.38 (95%CI 0.22-0.67, P=0.001), 0.35 (95%CI 0.19-0.64, P=0.001), 0.27 (95%CI 0.14-0.53, P<0.001) and 0.21 (95%CI 0.10-0.41, P<0.001) in the second, third, fourth and highest quintiles of serum Mg, respectively (P for trend <0.001); the multivariable-adjusted ORs of HU were 0.36 (95%CI 0.20-0.63, P<0.001), 0.54 (95%CI 0.31-0.93, P=0.026) and 0.39 (95%CI 0.22-0.68, P=0.001) in the third, fourth and highest quintiles of serum Mg respectively (P for trend <0.001); and the multivariable-adjusted ORs of MetS were 0.58 (95%CI 0.36-0.94, P=0.026) in the second and 0.56 (95%CI 0.34-0.93, P=0.024) in the highest quintiles of serum Mg (P for trend =0.066). There was no significant association between serum Mg and HP in OA patients.

Conclusions: The serum Mg concentration was inversely associated with the prevalence of MetS, DM and HU in radiographic knee OA patients.

Level of Evidence: Level Ⅲ, cross-sectional study.

Key words: osteoarthritis, magnesium, metabolic syndrome, diabetes, hypertension, hyperuricemia

Strengths and limitations of this study

1. This is the first study examining the associations between serum magnesium (Mg) and the prevalence of metabolic syndrome, diabetes mellitus, hypertension and hyperuricemia in radiographic knee osteoarthritis patients.
2. The multivariable logistical regression models in this study were adjusted for a considerable number of potential confounding factors, which greatly improved the reliability of the results.
3. The kidney is the key organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by adding estimated glomerular filtration rate into the multivariable logistic regression models, and the reverse associations remained significant.
4. This study adopted cross-sectional design which precluded causal correlations.
5. Serum Mg concentration was adopted as the indicator of body Mg content in this study which may not be the best indicator of body status.

Introduction

The association between osteoarthritis (OA) and metabolic diseases, especially metabolic syndrome (MetS)[1 2] and diabetes mellitus (DM),[3-5] has drawn increasing attention in the past few years. OA includes three specific phenotypes: metabolic OA, age-related OA and injury-related OA.[6] A large number of studies have indicated that the prevalence of MetS,[7-9] DM[10-18] and hypertension (HP)[7 9-13 19 20] is either higher in OA patients or associated with OA. In addition, some other studies reported that MetS,[21 22] DM[23 24] and HP[21 22] are risk factors of OA progression. Thus, it appears necessary to pay more attention and adopt appropriate measures to reduce the high prevalence of metabolic diseases in OA patients, which also seems to be beneficial in delaying OA progression.

Serum magnesium (Mg), one of the most important micronutrients for human health, has been reported to be negatively associated with MetS,[25-29] DM[30-38] and HP[30 39-41] by lots of studies. Meanwhile, our previous study showed an inverse association between serum Mg and hyperuricemia (HU).[42] However, to the best knowledge of the authors, there is not yet a study examining the association between the serum Mg concentration and the aforementioned metabolic diseases (MetS, DM, HP and HU) in OA patients. On the other hand, we have previously shown that the serum Mg concentration may be inversely associated with radiographic knee OA.[43] Therefore, we speculate that the prevalence of MetS, DM, HP and HU in OA patients may be reduced by elevating the level of serum Mg, which can in turn delay OA progression. Thus, the objective of the present study was to examine the associations between the serum Mg concentration with the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. It was hypothesized that serum Mg concentration was inversely associated with these diseases.

Methods

Study population

The present study was conducted at the Health Management Center of Xiangya Hospital between October 2013 and November 2014. The study design has been

published previously.[42-46] The protocol has been reviewed and approved by the Ethics Committee of Xiangya Hospital, Central South University (reference numbers: 201312459), and the methods were developed in "accordance" with the approved guidelines. Informed consent has been obtained from all participants. Registered nurses were engaged to interview all participants during the examination using a standard questionnaire, with the purpose to collect information on demographic characteristics and health-related habits. Participants were selected based on the following inclusion criteria: 1) 40 years old or above; 2) undergoing weight-bearing bilateral anteroposterior radiography of the knee, and diagnosed with knee OA according to the Kellgren-Lawrence (K-L) radiographic atlas (knee joint was graded K-L 2 or above); 3) availability of all basic characteristics, including age, gender, body mass index (BMI) and blood pressure; 4) availability of biochemical test results, including serum Mg concentration; 5) availability of information related to the living habits, including education background, activity level, smoking, drinking and medication status. Initially, the present cross-sectional study retrieved 1820 radiographic knee OA patients aged over 40 years who exhibited sound basic characteristics and required blood biochemical assessment (including serum Mg concentration). Among them, 962 patients offered demographic characteristics and health-related habits and were finally included in this study.

Blood biochemistry

All blood samples were drawn after a 12-hour overnight fast and were kept at 4℃ until analysis. Blood tests were undertaken using the Beckman Coulter AU 5800 (Beckman Coulter Inc., Brea, CA, USA). The inter- and intra-assay coefficients of variation were tested at both low concentrations (2.5 mmol/L for glucose, 118 μmol/L for uric acid and 0.60 mmol/L for serum Mg) and high concentrations (6.7 mmol/L for glucose, 472 μmol/L for uric acid and 1.00 mmol/L for serum Mg) of standard human samples. The intra-assay coefficients of variation were 0.98% (2.5 mmol/L) and 1.72% (6.7 mmol/L) for glucose, 1.39% (118 μmol/L) and 0.41% (472 μmol/L) for uric acid, and 1.86% (0.60 mmol/L) and 1.65% (1.00 mmol/L) for serum Mg respectively. The

inter-assay coefficients of variation were 2.45% (2.5 mmol/L) and 1.46% (6.7 mmol/L) for glucose, 1.40% (118 μmol/L) and 1.23% (472 μmol/L) for uric acid, and 1.87% (0.60 mmol/L) and 1.70% (1.00 mmol/L) for serum Mg respectively.

Assessment of other exposures

Blood pressure was measured by an electronic sphygmomanometer. The weight and height of each subjects was measured respectively to calculate the BMI. Information on the average frequency of physical activity (never, one to two times per week, three to four times per week, five times and above per week) and average duration of physical activity (less than half an hour, half an hour to one hour, one to two hours, more than two hours) was collected through survey questionnaire. The smoking, alcohol drinking and medication status were collected during the face-to-face interview.

Assessment of MetS, DM, HP and HU

MetS was diagnosed based on the Chinese Diabetes Society (CDS) criteria,[47-49] which requires meeting at least 3 of the following 4 items: (1) BMI ≥25 kg/m^2; (2) Fasting plasma glucose (FPG) ≥6.1 mmol/L, or diagnosed DM; (3) Systolic blood pressure (BP) ≥140 mmHg or diastolic BP≥90 mmHg, or treatment of previously diagnosed HP; (4) Triglycerides ≥1.7 mmol/L and/or HDL-cholesterol <0.9 mmol/L in male or <1.0 mmol/L in female, or treatment for this lipid abnormality. Subjects with the fasting glucose ≥7.0 mmol/L or currently undergoing drug treatment for blood glucose control were regarded as DM patients, and subjects with the systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or currently undertaking antihypertensive medication were regarded as HP patients. HU was defined as uric acid ≥416 μmol/L for male and ≥360 μmol/L for female or currently undergoing drug treatment for uric acid control.

Statistical analysis

The continuous data are expressed as mean (standard deviation), and the category data

are expressed in percentage. Differences in continuous data were evaluated by one-way classification ANOVA (normally distributed data) or Kruskal-Wallis H test (non-normally distributed data), while differences in category data were assessed by the $\chi 2$ test. The serum Mg was classified into five categories based on the quintile distribution: ≤0.85, 0.86-0.89, 0.90-0.92, 0.93-0.96 and ≥0.97 mmol/L. Logistic regression was conducted in two models in order to calculate the adjusted odds ratios (ORs) with 95% confidence intervals (95%CI) for the associations of serum Mg with MetS, DM, HP and HU. Three models were adjusted for the association. Model 1 were adjusted for age and sex. Then, model 2, a multivariable model was adopted. Covariates were chosen based on previous similar studies.[27 33 50 51] Model 2 for the association between serum Mg and MetS was adjusted for age (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data) and alcohol drinking status (yes, no). Model 2 for the association between serum Mg and DM was adjusted for age (continuous data), BMI (≥25 kg/m^2, <25 kg/m^2), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), HP (yes, no), and dyslipidemia (yes, no). Dyslipidemia was defined by triglycerides ≥1.7 mmol/L and/or HDL-cholesterol <0.9 mmol/L in male or <1.0 mmol/L in female, or treatment for this lipid abnormality. Model 2 for the association between serum Mg and HP was adjusted for age (continuous data), BMI (≥25 kg/m^2, <25 kg/m^2), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), DM (yes, no), and dyslipidemia (yes, no). Model 2 for the association between serum Mg and HU was adjusted for age (continuous data), BMI (≥25 kg/m^2, <25 kg/m^2), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), HP (yes, no), DM (yes, no) and dyslipidemia (yes, no). Model 3 for all associations were adjusted based on model 2, with additional factor of estimated glomerular filtration rate (eGFR). eGFR was calculated by serum creatinine (Scr), sex,

and patients' age. The Modification of Diet in Renal Disease (MDRD) of eGFR calculation formula was: 186×Scr−1.154×age−0.203×1.210 (if black)×0.742 (if female).[52] Tests for linear trends were conducted based on logistic regression using a median variable of Mg concentration in each category. All data analyses were performed using SPSS 17.0; $P \leq 0.05$ was considered to be statistically significant. All tests were two tailed.

Results

A total of 962 subjects (377 females, accounting for 39.2%) were included in the present cross-sectional study. The characteristics of the study population according to quintiles of serum Mg were presented in Table 1. The mean age of the subjects was 54.9±7.6 years old. The overall prevalence of MetS, DM, HP and HU in OA patients were 21.4%, 12.0%, 38.5% and 18.3% respectively. Significant differences were observed across the quintiles of serum Mg for fasting glucose, as well as the prevalence of DM and HU.

The outcomes of multivariable adjusted associations between MetS and serum Mg concentration were shown in Table 2. Compared with the lowest quintile, the age-sex adjusted ORs (Model 1) suggested significant decreased prevalence of MetS in the second (OR=0.61, 95%CI 0.38-0.97, P=0.038) and the highest (OR=0.59, 95%CI 0.36-0.96, P=0.035) quintiles of serum Mg (P for trend =0.090); the multivariable adjusted ORs (Model 2) also suggested significant decreased prevalence of MetS in the second (OR=0.60, 95%CI 0.37-0.96, P=0.035) and the highest (OR=0.61, 95%CI 0.37-0.99, P=0.047) quintiles, and the P for trend was 0.120. The sensitivity analysis, by adding eGFR into model 2, also reached similar results - significant lower prevalence of MetS in the second (OR=0.58, 95%CI 0.36-0.94, P=0.026) and the highest quintiles (OR=0.56, 95%CI 0.34-0.93, P=0.024) compared with the reference quintile of serum Mg, and the P for trend was 0.066.

Table 3 illustrated the multivariable adjusted relations between serum Mg and DM in OA patients. Both the age-sex adjusted OR values (Model 1) and the multivariable adjusted OR values (Model 2) suggested a strong inverse association

between serum Mg and DM. The age-sex adjusted ORs for the prevalence of DM were 0.38 (95%CI 0.22-0.66, P=0.001), 0.34 (95%CI 0.19-0.61, P<0.001), 0.29 (95%CI 0.15-0.55, P<0.001), and 0.20 (95%CI 0.10-0.40, P<0.001) in the second, third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was <0.001. The multivariable adjusted ORs for the prevalence of DM were 0.38 (95%CI 0.22-0.66, P=0.001), 0.34 (95%CI 0.19-0.62, P<0.001), 0.27 (95%CI 0.14-0.52, P<0.001), and 0.20 (95%CI 0.10-0.40, P<0.001) in the second, third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was <0.001. The sensitivity analysis, by adding eGFR into model 2, showed similar results - significant lower prevalence of DM in the second (OR=0.38, 95%CI 0.22-0.67, P=0.001), third (OR=0.35, 95%CI 0.19-0.64, P=0.001), fourth (OR=0.27, 95%CI 0.14-0.53, P<0.001), and highest quintiles (OR=0.21, 95%CI 0.10-0.41, P<0.001) compared with the reference quintile of serum Mg, and the P for trend was <0.001.

The multivariable-adjusted relations between serum Mg and HP in OA patients were illustrated in Table 4. According to both the age-sex adjusted ORs (Model 1) and the multivariable adjusted ORs (Model 2), there was no significant association between serum Mg and HP, and the P for trend were 0.929 and 0.423, respectively. The sensitivity analysis, by adding eGFR into model 2, reached the same results.

The multivariable-adjusted relations between serum Mg and HU in OA patients were illustrated in Table 5. Both the age-sex adjusted OR values (Model 1) and the multivariable adjusted OR values (Model 2) suggested significant decreased prevalence of HU in the third quintile (age-sex adjusted OR=0.44, 95%CI 0.26-0.75, P=0.002; multivariable adjusted OR=0.42, 95%CI 0.24-0.73, P=0.002) and fifth quintile (age-sex adjusted OR=0.51, 95%CI 0.30-0.85, P=0.010; multivariable adjusted OR=0.50, 95%CI 0.29-0.86, P=0.012) compared with the lowest quintile of serum Mg, and the P for trend were 0.008 and 0.007, respectively. The sensitivity analysis, by adding eGFR into model 2, showed similar outcomes - significant lower prevalence of HU in the third (OR=0.36, 0.20-0.63, P<0.001), fourth (OR=0.54, 95%CI 0.31-0.93, P=0.026), and highest quintiles (OR=0.39, 95%CI 0.22-0.68, P=0.001) compared with the reference quintile of serum Mg, and the P for trend was

<0.001.

Discussion

The results of this study suggested that the serum Mg concentration was negatively associated with the prevalence of MetS, DM and HU in subjects with radiographic knee OA. In order to control potential confounders, several covariates including characteristics, living habits and underlying diseases were selected, and even the eGFR was added into the multivariable logistic regression models to eliminate the influence of renal function on Mg excretion. The reverse associations mentioned above remained significant after adjustments of these confounders. However, the negative association between serum Mg and the prevalence of HP was not observed in radiographic knee OA patients. Moreover, the linear associations were only observed between serum Mg with DM and HU, but not between serum Mg and MetS.

Mg, the fourth most abundant cation in human body and the second most profuse intracellular cation, is a metallic cofactor for over 300 enzymatic reactions. It appears to play an important role in glucose metabolism and insulin homeostasis, which are both highly correlated with metabolic diseases, especially MetS and DM. The mechanisms involved in Mg deficiency in patients with MetS, DM and HU are probably multifactorial. The most important factor may be insulin resistance, as Mg is essential for insulin action and is a critical cofactor for several enzymes in carbohydrate metabolism, which is important for the phosphorylation reactions of tyrosine-kinase in the insulin receptor.[31 53-57] Of course, it is necessary to highlight the fact that insulin can also induce Mg excretion[58] and produce a significant decline of plasma Mg through ion exchange.[59] Thus, there seems to be a vicious circle between Mg deficiency and insulin resistance.

Other potential mechanisms include glucose transportation,[56] oxidative stress[56] and inflammatory cytokines,[60-62] and cellular calcium homeostasis.[54] Mg is an essential cofactor of the high-energy phosphate-bound enzymatic pathways involved in the modulation of glucose transport across cell membranes.[56] It also plays a role in the mechanisms of cellular antioxidant defense.[63] The oxidative stress, defined as a

persistent imbalance between the excessive production of reactive oxygen species and/or defects in antioxidant defense, has been implicated in the pathogenesis of diabetic complications.[56] Moreover, low serum Mg levels are strongly related to elevated serum concentrations of both tumor necrosis factor alpha and C-reactive protein (CRP),[64] suggesting that Mg deficiency may contribute to the development of low-grade chronic inflammation syndrome and the development of glucose metabolic disorders through the former pathway. In addition, lower Mg concentration can enhance calcium-mediated vasoconstriction, blunt cardiac and smooth muscle relaxation, and thus contribute to BP elevation.[54] However, the decreased serum calcium concentration in radiographic knee OA patients may weaken the association between Mg and HP.[65]

MetS[21 22] and DM[4 23 24] were reported to be the risk factors of OA progression. Moreover, serum Mg level has been proved to be significantly associated with the CRP concentration,[27 66-68] and higher CRP might serve as a prediction factor for OA progression.[69 70] Thus, OA progression may be delayed by elevating the serum Mg level through reducing the prevalence of MetS and DM and decreasing the level of CRP. Above all, the present study indicated that the elevation of serum Mg level has the potential to reduce the prevalence of MetS, DM and HU in knee OA patients and thereby may delay the progression of knee OA. However, the specific mechanism needs to be further explored.

The present study has several strengths. Firstly, this is the first study examining the associations between serum Mg and the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. The results of this study will provide a new insight into the treatment of knee OA. Secondly, the multivariable logistical regression models were adjusted for a considerable number of potential confounding factors, which greatly improved the reliability of the results. Thirdly, the kidney is the key organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by adding eGFR into multivariable logistic regression models which showed that the reverse associations remained significant.

Limitations of the present study should also be admitted. The cross-sectional

design precludes causal correlations, so further prospective studies and intervention trials should be undertaken to establish a causal association between serum Mg with the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. Since no previous research investigated such associations in knee OA patients, the value of this study should not be blotted out by the cross-sectional nature. Another limitation of this study lies in the relatively small sample size, and thus, extensive high-quality researches based on a larger sample are needed. Moreover, the dietary intake of Mg in relation to the prevalence of MetS, DM, HP and HU were not assessed in the present study. Last but not the least, it is important to highlight that Mg is an intracellular ion; therefore, the serum Mg concentration must be considered as a poor indicator of body Mg content,[71] even though it has been used in many studies. However, blood Mg level is the second best indicator of body status.[72]

Conclusions

The present study concluded that the serum Mg concentration was inversely associated with the prevalence of MetS, DM and HU in radiographic knee OA patients.

Contributors

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. GHL, YLW and JW conceived the study. GHL, YLW and JW were responsible for conception and design of the study and drafted the manuscript. CZ, TY, HL, YC and DXX contributed to data collection. WJ contributed to preparation and data analysis. BX, ZCL, JTL, and SDJ contributed to study retrieval. GHL contributed to revision of the manuscript. All the authors contributed to the interpretation of the data and critically reviewed the manuscript for publication.

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Competing interests

The authors declare that they have no conflict of interest.

Ethics approval

The protocol of this study was reviewed and approved by the Ethics Committee at Xiangya Hospital.

Data sharing statement

The datasets during the current study available from the corresponding author on reasonable request.

REFERENCES

1. Zhuo Q, Yang W, Chen J, *et al*. Metabolic syndrome meets osteoarthritis. *Nat Rev Rheumatol* 2012;8:729-37.
2. Katz JD, Agrawal S, Velasquez M. Getting to the heart of the matter: osteoarthritis takes its place as part of the metabolic syndrome. *Curr Opin Rheumatol* 2010;22:512-9.
3. Berenbaum F. Diabetes-induced osteoarthritis: from a new paradigm to a new phenotype. *Ann Rheum Dis* 2011;70:1354-6.
4. King KB, Rosenthal AK. The adverse effects of diabetes on osteoarthritis: update on clinical evidence and molecular mechanisms. *Osteoarthritis Cartilage* 2015;23:841-50.
5. Kirkman MS. Osteoarthritis progression: is diabetes a culprit? *Osteoarthritis Cartilage* 2015;23:839-40.
6. Sellam J, Berenbaum F. Is osteoarthritis a metabolic disease? *Joint Bone Spine* 2013;80:568-73.
7. Puenpatom RA, Victor TW. Increased prevalence of metabolic syndrome in individuals with osteoarthritis: an analysis of NHANES III data. *Postgrad Med* 2009;121:9-20.
8. Shin D. Association between metabolic syndrome, radiographic knee osteoarthritis, and intensity of knee pain: results of a national survey. *J Clin Endocrinol Metab* 2014;99:3177-83.
9. Calvet J, Orellana C, Larrosa M, *et al*. High prevalence of cardiovascular co-morbidities in patients with symptomatic knee or hand osteoarthritis. *Scand J Rheumatol* 2015;1-4.
10. Rahman MM, Kopec JA, Cibere J, *et al*. The relationship between osteoarthritis and cardiovascular disease in a population health survey: a cross-sectional study. *BMJ Open* 2013;3:
11. Inoue R, Ishibashi Y, Tsuda E, *et al*. Medical problems and risk factors of metabolic syndrome among radiographic knee osteoarthritis patients in the Japanese general population. *J Orthop Sci* 2011;16:704-9.
12. Hart DJ, Doyle DV, Spector TD. Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. *J Rheumatol* 1995;22:1118-23.
13. Jungmann PM, Kraus MS, Alizai H, *et al*. Association of metabolic risk factors with cartilage degradation assessed by T2 relaxation time at the knee: data from the osteoarthritis initiative. *Arthritis Care Res (Hoboken)* 2013;65:1942-50.
14. Anagnostopoulos I, Zinzaras E, Alexiou I, *et al*. The prevalence of rheumatic diseases in central Greece: a population survey. *BMC Musculoskelet Disord* 2010;11:98.
15. Massengale M, Reichmann WM, Losina E, *et al*. The relationship between hand osteoarthritis and serum leptin concentration in participants of the Third National Health and Nutrition Examination Survey. *Arthritis Res Ther* 2012;14:R132.
16. Nieves-Plaza M, Castro-Santana LE, Font YM, *et al*. Association of hand or knee osteoarthritis with diabetes mellitus in a population of Hispanics from Puerto Rico. *J Clin Rheumatol* 2013;19:1-6.
17. Greiver M, Williamson T, Barber D, *et al*. Prevalence and epidemiology of diabetes in Canadian primary care practices: a report from the Canadian Primary Care Sentinel Surveillance Network. *Can J Diabetes* 2014;38:179-85.
18. Rahman MM, Cibere J, Anis AH, *et al*. Risk of Type 2 Diabetes among Osteoarthritis Patients in a Prospective Longitudinal Study. *Int J Rheumatol* 2014;2014:620920.
19. Reid JL, Morton DJ, Wingard DL, *et al*. Obesity and other cardiovascular disease risk factors and their association with osteoarthritis in Southern California American Indians, 2002-2006. *Ethn Dis* 2010;20:416-22.

20. Birtwhistle R, Morkem R, Peat G, *et al*. Prevalence and management of osteoarthritis in primary care: an epidemiologic cohort study from the Canadian Primary Care Sentinel Surveillance Network. *CMAJ Open* 2015;3:E270-5.
21. Yoshimura N, Muraki S, Oka H, *et al*. Accumulation of metabolic risk factors such as overweight, hypertension, dyslipidaemia, and impaired glucose tolerance raises the risk of occurrence and progression of knee osteoarthritis: a 3-year follow-up of the ROAD study. *Osteoarthritis Cartilage* 2012;20:1217-26.
22. Monira HS, Wang Y, Cicuttini FM, *et al*. Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: a prospective cohort study. *Semin Arthritis Rheum* 2014;43:429-36.
23. Schett G, Kleyer A, Perricone C, *et al*. Diabetes is an independent predictor for severe osteoarthritis: results from a longitudinal cohort study. *Diabetes Care* 2013;36:403-9.
24. Eymard F, Parsons C, Edwards MH, *et al*. Diabetes is a risk factor for knee osteoarthritis progression. *Osteoarthritis Cartilage* 2015;23:851-9.
25. Guerrero-Romero F, Rodriguez-Moran M. Low serum magnesium levels and metabolic syndrome. *Acta Diabetol* 2002;39:209-13.
26. Guerrero-Romero F, Rodriguez-Moran M. Hypomagnesemia, oxidative stress, inflammation, and metabolic syndrome. *Diabetes Metab Res Rev* 2006;22:471-6.
27. Evangelopoulos AA, Vallianou NG, Panagiotakos DB, *et al*. An inverse relationship between cumulating components of the metabolic syndrome and serum magnesium levels. *Nutr Res* 2008;28:659-63.
28. Hjelmesaeth J, Hofso D, Aasheim ET, *et al*. Parathyroid hormone, but not vitamin D, is associated with the metabolic syndrome in morbidly obese women and men: a cross-sectional study. *Cardiovasc Diabetol* 2009;8:7.
29. Lima ML, Cruz T, Rodrigues LE, *et al*. Serum and intracellular magnesium deficiency in patients with metabolic syndrome--evidences for its relation to insulin resistance. *Diabetes Res Clin Pract* 2009;83:257-62.
30. Ma J, Folsom AR, Melnick SL, *et al*. Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin, and carotid arterial wall thickness: the ARIC study. Atherosclerosis Risk in Communities Study. *J Clin Epidemiol* 1995;48:927-40.
31. Kao WH, Folsom AR, Nieto FJ, *et al*. Serum and dietary magnesium and the risk for type 2 diabetes mellitus: the Atherosclerosis Risk in Communities Study. *Arch Intern Med* 1999;159:2151-9.
32. Wang JL, Shaw NS, Yeh HY, *et al*. Magnesium status and association with diabetes in the Taiwanese elderly. *Asia Pac J Clin Nutr* 2005;14:263-9.
33. Chambers EC, Heshka S, Gallagher D, *et al*. Serum magnesium and type-2 diabetes in African Americans and Hispanics: a New York cohort. *J Am Coll Nutr* 2006;25:509-13.
34. Simmons D, Joshi S, Shaw J. Hypomagnesaemia is associated with diabetes: Not pre-diabetes, obesity or the metabolic syndrome. *Diabetes Res Clin Pract* 2010;87:261-6.
35. Sales CH, Pedrosa LF, Lima JG, *et al*. Influence of magnesium status and magnesium intake on the blood glucose control in patients with type 2 diabetes. *Clin Nutr* 2011;30:359-64.
36. Lecube A, Baena-Fustegueras JA, Fort JM, *et al*. Diabetes is the main factor accounting for hypomagnesemia in obese subjects. *PLoS One* 2012;7:e30599.
37. Xu J, Xu W, Yao H, *et al*. Associations of serum and urinary magnesium with the pre-diabetes,

diabetes and diabetic complications in the Chinese Northeast population. *PLoS One* 2013;8:e56750.

38. Yang SJ, Hwang SY, Baik SH, *et al*. Serum magnesium level is associated with type 2 diabetes in women with a history of gestational diabetes mellitus: the Korea National Diabetes Program study. *J Korean Med Sci* 2014;29:84-9.
39. Singh RB, Rastogi V, Niaz MA, *et al*. Epidemiological study of magnesium status and risk of hypertension in a rural population of north India. *Magnes Res* 1996;9:173-81.
40. Peacock JM, Folsom AR, Arnett DK, *et al*. Relationship of serum and dietary magnesium to incident hypertension: the Atherosclerosis Risk in Communities (ARIC) Study. *Ann Epidemiol* 1999;9:159-65.
41. Guerrero-Romero F, Rodriguez-Moran M, Hernandez-Ronquillo G, *et al*. Low Serum Magnesium Levels and Its Association with High Blood Pressure in Children. *J Pediatr* 2016;168:93-8.
42. Zeng C, Wang YL, Wei J, *et al*. Association between low serum magnesium concentration and hyperuricemia. *Magnes Res* 2015;28:56-63.
43. Zeng C, Wei J, Li H, *et al*. Relationship between Serum Magnesium Concentration and Radiographic Knee Osteoarthritis. *J Rheumatol* 2015;42:1231-6.
44. Wei J, Zeng C, Gong QY, *et al*. Associations between Dietary Antioxidant Intake and Metabolic Syndrome. *PLoS One* 2015;10:e130876.
45. Xie DX, Xiong YL, Zeng C, *et al*. Association between low dietary zinc and hyperuricaemia in middle-aged and older males in China: a cross-sectional study. *BMJ Open* 2015;5:e8637.
46. Wei J, Zeng C, Gong QY, *et al*. The association between dietary selenium intake and diabetes: a cross-sectional study among middle-aged and older adults. *Nutr J* 2015;14:18.
47. Expert Panel on Metabolic Syndrome of Chinese Diabetes Society: Recommendations on metabolic syndrome of Chinese Diabetes Society (Chinese). *Chin J Diabetes* 2004;14:156-61.
48. Pang C, Jia L, Hou X, *et al*. The significance of screening for microvascular diseases in Chinese community-based subjects with various metabolic abnormalities. *PLoS One* 2014;9:e97928.
49. Zhou H, Guo ZR, Yu LG, *et al*. Evidence on the applicability of the ATPIII, IDF and CDS metabolic syndrome diagnostic criteria to identify CVD and T2DM in the Chinese population from a 6.3-year cohort study in mid-eastern China. *Diabetes Res Clin Pract* 2010;90:319-25.
50. Joosten MM, Gansevoort RT, Mukamal KJ, *et al*. Urinary magnesium excretion and risk of hypertension: the prevention of renal and vascular end-stage disease study. *Hypertension* 2013;61:1161-7.
51. Choi MK, Bae YJ. Association of Magnesium Intake with High Blood Pressure in Korean Adults: Korea National Health and Nutrition Examination Survey 2007-2009. *PLoS One* 2015;10:e130405.
52. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002;39:S1-266.
53. Grundy SM, Cleeman JI, Daniels SR, *et al*. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112:2735-52.
54. Barbagallo M, Dominguez LJ, Galioto A, *et al*. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. *Mol Aspects Med* 2003;24:39-52.
55. Song Y, Ridker PM, Manson JE, *et al*. Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* 2005;28:1438-44.
56. Guerrero-Romero F, Rodriguez-Moran M. Complementary therapies for diabetes: the case for

chromium, magnesium, and antioxidants. *Arch Med Res* 2005;36:250-7.
57. Huerta MG, Roemmich JN, Kington ML, *et al*. Magnesium deficiency is associated with insulin resistance in obese children. *Diabetes Care* 2005;28:1175-81.
58. Djurhuus MS, Skott P, Hother-Nielson O, *et al*. Insulin increases renal magnesium excretion: a possible cause of magnesium depletion in hyperinsulinaemic states. *Diabet Med* 1995;12:664-9.
59. Paolisso G, Sgambato S, Passariello N, *et al*. Insulin induces opposite changes in plasma and erythrocyte magnesium concentrations in normal man. *Diabetologia* 1986;29:644-7.
60. Bonora E, Targher G, Zenere MB, *et al*. Relationship of uric acid concentration to cardiovascular risk factors in young men. Role of obesity and central fat distribution. The Verona Young Men Atherosclerosis Risk Factors Study. *Int J Obes Relat Metab Disord* 1996;20:975-80.
61. Lyngdoh T, Marques-Vidal P, Paccaud F, *et al*. Elevated serum uric acid is associated with high circulating inflammatory cytokines in the population-based Colaus study. *PLoS One* 2011;6:e19901.
62. Kirilmaz B, Asgun F, Alioglu E, *et al*. High inflammatory activity related to the number of metabolic syndrome components. *J Clin Hypertens (Greenwich)* 2010;12:136-44.
63. Salmonowicz B, Krzystek-Korpacka M, Noczyńska A. Trace elements, magnesium, and the efficacy of antioxidant systems in children with type 1 diabetes mellitus and in their siblings. *Adv Clin Exp Med* 2014;23:259-68.
64. Rodrı´guez-Mora´n M, Guerrero-Romero F. Elevated serum concentration of tumor necrosis factor-alpha is linked to low serum magnesium levels in the obesity-related inflammatory response. *Magnes Res* 2004;17:189–96.
65. Li H, Zeng C, Wei J, *et al*. Serum Calcium Concentration Is Inversely Associated With Radiographic Knee Osteoarthritis: A Cross-Sectional Study. *Medicine (Baltimore)*. 2016;95:e2838.
66. Chacko SA SYNL. Relations of dietary magnesium intake to biomarkers of inflammation and endothelial dysfunction in an ethnically diverse cohort of postmenopausal women. *Diabetes Care* 2010;33:304-10.
67. Bo S DMGS. Dietary magnesium and fiber intakes and inflammatory and metabolic indicators in middle-aged subjects from a population-based cohort. *Am J Clin Nutr* 2006;84:1062-9.
68. Kim DJ XPLK. Magnesium intake in relation to systemic inflammation, insulin resistance, and the incidence of diabetes. *Diabetes Care* 2010;33:2604-10.
69. Spector TD HDND. Low-level increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. *Arthritis Rheum* 1997;40:723-7.
71. Smith JW MTGE. Significance of C-reactive protein in osteoarthritis and total knee arthroplasty outcomes. *Ther Adv Musculoskelet Dis* 2012;4:315-25.
68. Topf JM, Murray PT. Hypomagnesemia and hypermagnesemia. *Rev Endocr Metab Disord* 2003;4:195-206.
72. Jr. Sabatier M PFAM. A compartmental model of magnesium metabolism in healthy men based on two stable isotope tracers. *Am J Physiol Regul Integr Comp Physiol* 2003;285:R656-63.

353 Table 1 Basic characteristics of included subjects according to quintiles of serum Mg (n=962)

	Quintiles of serum Mg					P
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
Age (years)	53.8 (7.3)	54.6 (7.6)	55.2 (7.9)	55.3 (7.1)	56.1 (8.0)	0.062
BMI (kg/m^2)	25.2 (3.2)	24.9 (3.2)	25.0 (3.7)	25.2 (3.4)	24.6 (3.2)	0.464
Female (%)	37.5	42.3	36.8	42.3	37.0	0.627
Smoking (%)	27.5	27.4	21.6	24.4	21.7	0.457
Alcohol drinking (%)	34.5	36.3	40.5	41.1	38.1	0.645
High school diploma (%)	45.0	47.4	45.3	56.5	48.1	0.184
Activity level (h/w)	2.0 (3.5)	2.0 (3.3)	2.3 (3.5)	2.1 (3.1)	2.4 (3.5)	0.457
Fasting glucose (mmol/l)	6.6 (3.0)	5.7 (1.7)	5.7 (1.4)	5.5 (0.9)	5.5 (1.6)	0.009
Systolic pressure (mm Hg)	129.2 (16.9)	128.3 (17.9)	130.4 (16.2)	128.8 (16.3)	129.6 (17.7)	0.837
Diastolic pressure (mm Hg)	81.2 (11.8)	79.8 (12.1)	80.7 (11.0)	80.7 (10.7)	80.3 (10.5)	0.654
HDL-cholesterol (mmol/l)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	1.5 (0.3)	1.5 (0.4)	0.374
Triglyceride (mmol/l)	2.1 (1.9)	1.8 (1.5)	2.0 (2.1)	1.8 (1.0)	2.3 (2.9)	0.620

Uric acid (μmol/l)	337.3 (101.7)	329.0 (80.7)	321.3 (86.3)	331.5 (78.0)	329.4 (81.7)	0.590
eGFR (ml/min/1.73m^2)	80.2 (14.4)	77.7 (10.7)	76.0 (10.6)	75.8 (10.7)	74.3 (12.0)	<0.001
MetS (%)	26.5	17.7	25.8	19.6	17.5	0.059
DM (%)	23.5	10.7	10.0	8.3	6.3	<0.001
HP (%)	40.0	33.5	37.4	42.3	40.2	0.432
HU (%)	25.5	19.1	13.2	18.5	14.8	0.018

Data are mean (Standard Deviation), unless otherwise indicated; Mg, magnesium; OA, osteoarthritis; BMI, body mass index; HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate; MetS, metabolic syndrome; DM, diabetes mellitus; HP, hypertension; HU, hyperuricemia.

P values are for test of difference across all quintiles of serum Mg.

Table 2 Multivariable-adjusted relations of serum Mg and MetS in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
MetS (%)	26.5	17.7	25.8	19.6	17.5	-
Model 1*	1.00 (reference)	0.61 (0.38, 0.97)	0.97 (0.61, 1.52)	0.69 (0.42, 1.14)	0.59 (0.36, 0.96)	0.090
P value	-	0.038	0.881	0.150	0.035	-
Model 2*	1.00 (reference)	0.60 (0.37, 0.96)	1.00 (0.63, 1.57)	0.70 (0.42, 1.15)	0.61 (0.37, 0.99)	0.120
P value	-	0.035	0.99	0.160	0.047	-
Model 3*	1.00 (reference)	0.58 (0.36, 0.94)	0.95 (0.60, 1.50)	0.66 (0.40, 1.10)	0.56 (0.34, 0.93)	0.066
P value	-	0.026	0.818	0.109	0.024	

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; MetS, metabolic syndrome.

*Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

Table 3 Multivariable-adjusted relations of serum Mg and DM in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
DM (%)	23.5	10.7	10.0	8.3	6.3	-
Model 1*	1.00 (reference)	0.38 (0.22, 0.66)	0.34 (0.19, 0.61)	0.29 (0.15, 0.55)	0.20 (0.10, 0.40)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-
Model 2*	1.00 (reference)	0.38 (0.22, 0.66)	0.34 (0.19, 0.62)	0.27 (0.14, 0.52)	0.20 (0.10, 0.40)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-
Model 3*	1.00 (reference)	0.38 (0.22, 0.67)	0.35 (0.19, 0.64)	0.27 (0.14, 0.53)	0.21 (0.10, 0.41)	<0.001
P value	-	0.001	0.001	<0.001	<0.001	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; DM, diabetes mellitus.

*Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (≥25 kg/m^2, <25 kg/m^2), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), hypertension (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

Table 4 Multivariable-adjusted relations of serum Mg and HP in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
HP (%)	40.0	33.5	37.4	42.3	40.2	-
Model 1*	1.00 (reference)	0.71 (0.47, 1.06)	0.83 (0.54, 1.25)	1.00 (0.66, 1.54)	0.89 (0.59, 1.35)	0.929
P value	-	0.095	0.368	0.987	0.582	-
Model 2*	1.00 (reference)	0.78 (0.51, 1.18)	0.92 (0.60, 1.41)	1.16 (0.75, 1.80)	1.03 (0.67, 1.58)	0.423
P value	-	0.242	0.708	0.502	0.896	-
Model 3*	1.00 (reference)	0.77 (0.51, 1.17)	0.90 (0.59, 1.38)	1.13 (0.73, 1.76)	0.99 (0.64, 1.53)	0.524
P value	-	0.218	0.629	0.577	0.978	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; HP, hypertension.

* Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (≥25 kg/m^2, <25 kg/m^2), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

Table 5 Multivariable-adjusted relations of serum Mg and HU in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
HU (%)	25.5	19.1	13.2	18.5	14.8	-
Model 1*	1.00 (reference)	0.71 (0.44, 1.14)	0.44 (0.26, 0.75)	0.68 (0.41, 1.14)	0.51 (0.30, 0.85)	0.008
P value	-	0.157	0.002	0.144	0.010	-
Model 2*	1.00 (reference)	0.73 (0.45, 1.19)	0.42 (0.24, 0.73)	0.62 (0.37, 1.06)	0.50 (0.29, 0.86)	0.007
P value	-	0.205	0.002	0.082	0.012	-
Model 3*	1.00 (reference)	0.67 (0.41, 1.11)	0.36 (0.20, 0.63)	0.54 (0.31, 0.93)	0.39 (0.22, 0.68)	<0.001
P value	-	0.119	<0.001	0.026	0.001	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; HU, hyperuricemia.

* Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (≥25 kg/m^2, <25 kg/m^2), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), hypertension (yes, no), diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Reported on Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	6-7
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	-
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	5-6

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4
		(b) Give reasons for non-participation at each stage	4
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	-
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	-
		Cross-sectional study—Report numbers of outcome events or summary measures	8-9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-9
		(*b*) Report category boundaries when continuous variables were categorized	8-9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	9-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-10
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between Serum Magnesium Concentration with Metabolic Syndrome, Diabetes, Hypertension and Hyperuricemia in Knee Osteoarthritis

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Association between Serum Magnesium Concentration with Metabolic Syndrome, Diabetes, Hypertension and Hyperuricemia in Knee Osteoarthritis

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Abstract

Objectives: To examine the associations between serum magnesium (Mg) concentration with the prevalence of metabolic syndrome (MetS), diabetes mellitus (DM), hypertension (HP) and hyperuricemia (HU) in radiographic knee osteoarthritis (OA) patients.

Methods: The present study was conducted at the Health Management Center of Xiangya Hospital. Radiographic OA was evaluated for patients aged over 40 years with basic characteristics and blood biochemical assessment. Serum Mg concentration was measured using the chemiluminescence method. MetS, DM, HP and HU were diagnosed based on standard protocols. The associations between serum Mg concentration with MetS, DM, HP and HU were evaluated by conducting multivariable adjusted logistic regression.

Results: A total of 962 radiographic knee OA patients were included. Compared with the lowest quintile, the multivariable-adjusted odds ratios (ORs) and related 95% confidence intervals (95%CI) of DM were 0.40 (95%CI 0.23-0.70, P=0.001), 0.33 (95%CI 0.18-0.60, P<0.001), 0.27 (95%CI 0.14-0.52, P<0.001) and 0.22 (95%CI 0.11-0.44, P<0.001) in the second, third, fourth and highest quintiles of serum Mg, respectively (P for trend <0.001); the multivariable-adjusted ORs of HU were 0.33 (95%CI 0.19-0.59, P<0.001), 0.52 (95%CI 0.30-0.91, P=0.022) and 0.39 (95%CI 0.22-0.70, P=0.001) in the third, fourth and highest quintiles of serum Mg respectively (P for trend <0.001); and the multivariable-adjusted ORs of MetS were 0.59 (95%CI 0.36-0.94, P=0.027) in the second and 0.56 (95%CI 0.34-0.93, P=0.024) in the highest quintiles of serum Mg (P for trend =0.067). There was no significant association between serum Mg and HP in OA patients.

Conclusions: The serum Mg concentration was inversely associated with the prevalence of MetS, DM and HU in radiographic knee OA patients.

Level of Evidence: Level Ⅲ, cross-sectional study.

Key words: osteoarthritis, magnesium, metabolic syndrome, diabetes, hypertension, hyperuricemia

Strengths and limitations of this study

1. This is the first study examining the associations between serum magnesium (Mg) and the prevalence of metabolic syndrome, diabetes mellitus, hypertension and hyperuricemia in radiographic knee osteoarthritis patients.
2. The multivariable logistical regression models in this study were adjusted for a considerable number of potential confounding factors, which greatly improved the reliability of the results.
3. The kidney is the key organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by adding estimated glomerular filtration rate into the multivariable logistic regression models, and the reverse associations remained significant.
4. This study adopted cross-sectional design which precluded causal correlations.
5. Serum Mg concentration was adopted as the indicator of body Mg content in this study which may not be the best indicator of body status.

Introduction

The association between osteoarthritis (OA) and metabolic diseases, especially metabolic syndrome (MetS)[1 2] and diabetes mellitus (DM),[3-5] has drawn increasing attention in the past few years. OA includes three specific phenotypes: metabolic OA, age-related OA and injury-related OA.[6] A large number of studies have indicated that the prevalence of MetS,[7-9] DM[10-18] and hypertension (HP)[7 9-13 19 20] is either higher in OA patients or associated with OA. In addition, some other studies reported that MetS,[21 22] DM[23 24] and HP[21 22] are risk factors of OA progression. Thus, it appears necessary to pay more attention and adopt appropriate measures to reduce the high prevalence of metabolic diseases in OA patients, which also seems to be beneficial in delaying OA progression.

Serum magnesium (Mg), one of the most important micronutrients for human health, has been reported to be negatively associated with MetS,[25-29] DM[30-38] and HP[30 39-41] by lots of studies. Meanwhile, our previous study showed an inverse association between serum Mg and hyperuricemia (HU).[42] However, to the best knowledge of the authors, there is not yet a study examining the association between the serum Mg concentration and the aforementioned metabolic diseases (MetS, DM, HP and HU) in OA patients. On the other hand, we have previously shown that the serum Mg concentration may be inversely associated with radiographic knee OA.[43] Therefore, we speculate that the prevalence of MetS, DM, HP and HU in OA patients may be reduced by elevating the level of serum Mg, which can in turn delay OA progression. Thus, the objective of the present study was to examine the associations between the serum Mg concentration with the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. It was hypothesized that serum Mg concentration was inversely associated with these diseases.

Methods

Study population

The present study was conducted at the Health Management Center of Xiangya Hospital between October 2013 and November 2014. The study design has been

published previously.[42-46] The protocol has been reviewed and approved by the Ethics Committee of Xiangya Hospital, Central South University (reference numbers: 201312459), and the methods were developed in "accordance" with the approved guidelines. Informed consent has been obtained from all participants. Registered nurses were engaged to interview all participants during the examination using a standard questionnaire, with the purpose to collect information on demographic characteristics and health-related habits. Participants were selected based on the following inclusion criteria: 1) 40 years old or above; 2) undergoing weight-bearing bilateral anteroposterior radiography of the knee, and diagnosed with knee OA according to the Kellgren-Lawrence (K-L) radiographic atlas (knee joint was graded K-L 2 or above); 3) availability of all basic characteristics, including age, gender, body mass index (BMI) and blood pressure; 4) availability of biochemical test results, including serum Mg concentration; 5) availability of information related to the living habits, including education background, activity level, smoking, drinking and medication status. Initially, the present cross-sectional study retrieved 1820 radiographic knee OA patients aged over 40 years who exhibited sound basic characteristics and required blood biochemical assessment (including serum Mg concentration). Among them, 962 patients offered demographic characteristics and health-related habits and were finally included in this study.

Blood biochemistry

All blood samples were drawn after a 12-hour overnight fast and were kept at 4℃ until analysis. Blood tests were undertaken using the Beckman Coulter AU 5800 (Beckman Coulter Inc., Brea, CA, USA). The inter- and intra-assay coefficients of variation were tested at both low concentrations (2.5 mmol/L for glucose, 118 μmol/L for uric acid and 0.60 mmol/L for serum Mg) and high concentrations (6.7 mmol/L for glucose, 472 μmol/L for uric acid and 1.00 mmol/L for serum Mg) of standard human samples. The intra-assay coefficients of variation were 0.98% (2.5 mmol/L) and 1.72% (6.7 mmol/L) for glucose, 1.39% (118 μmol/L) and 0.41% (472 μmol/L) for uric acid, and 1.86% (0.60 mmol/L) and 1.65% (1.00 mmol/L) for serum Mg respectively. The

inter-assay coefficients of variation were 2.45% (2.5 mmol/L) and 1.46% (6.7 mmol/L) for glucose, 1.40% (118 μmol/L) and 1.23% (472 μmol/L) for uric acid, and 1.87% (0.60 mmol/L) and 1.70% (1.00 mmol/L) for serum Mg respectively.

Assessment of other exposures

Blood pressure was measured by an electronic sphygmomanometer. The weight and height of each subjects was measured respectively to calculate the BMI. Information on the average frequency of physical activity (never, one to two times per week, three to four times per week, five times and above per week) and average duration of physical activity (less than half an hour, half an hour to one hour, one to two hours, more than two hours) was collected through survey questionnaire. The smoking, alcohol drinking and medication status were collected during the face-to-face interview.

Assessment of MetS, DM, HP and HU

MetS was diagnosed based on the Chinese Diabetes Society (CDS) criteria,[47-49] which requires meeting at least 3 of the following 4 items: (1) BMI ≥25 kg/m^2; (2) Fasting plasma glucose (FPG) ≥6.1 mmol/L, or diagnosed DM; (3) Systolic blood pressure (BP) ≥140 mmHg or diastolic BP≥90 mmHg, or treatment of previously diagnosed HP; (4) Triglycerides ≥1.7 mmol/L and/or HDL-cholesterol <0.9 mmol/L in male or <1.0 mmol/L in female, or treatment for this lipid abnormality. Subjects with the fasting glucose ≥7.0 mmol/L or currently undergoing drug treatment for blood glucose control were regarded as DM patients, and subjects with the systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or currently undertaking antihypertensive medication were regarded as HP patients. HU was defined as uric acid ≥416 μmol/L for male and ≥360 μmol/L for female or currently undergoing drug treatment for uric acid control.

Statistical analysis

The continuous data are expressed as mean with standard deviation, and the category

data are expressed in percentage. Differences in continuous data were evaluated by one-way classification ANOVA (normally distributed data) or Kruskal-Wallis H test (non-normally distributed data), while differences in category data were assessed by the $\chi 2$ test. The serum Mg was classified into five categories based on the quintile distribution: ≤0.85, 0.86-0.89, 0.90-0.92, 0.93-0.96 and ≥0.97 mmol/L. The prevalence of MetS, DM, HP and HU in each quintile of serum Mg in OA patients were assessed by scatter plots.

Logistic regression was conducted to calculate the odds ratios (ORs) with 95% confidence intervals (95%CI) for the associations between serum Mg and MetS, DM, HP and HU. Specifically, model 1 was adjusted by covariates of age (continuous data) and gender (male, female). Then, model 2 was adjusted by additional covariates of BMI (continuous data), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), HP (yes, no), DM (yes, no), and dyslipidemia (yes, no) on the basis of model 1. Dyslipidemia was defined as triglycerides ⩾ 1.7 mmol/L and/or HDL-cholesterol <0.9 mmol/L in male or <1.0 mmol/L in female, or treatment for this lipid abnormality. Notably, the selection of covariates in model 2 varied slightly for examining different associations (between serum Mg and MetS, DM, HP or HU). For example, BMI, HP and dyslipidemia were adjusted for the association between serum Mg and DM, but not for the association between serum Mg and MetS, simply because MetS was diagnosed based on BMI, HP and dyslipidemia status. Model 3 was established based on model 2, with adjustment of an additional covariate, estimated glomerular filtration rate (eGFR). eGFR (continuous data) was calculated from the Chronic Kidney Disease Epidemiology Collaboration equation.[50] All covariates in the present study were chosen referring to some of the previous similar studies.[27 33 51 52] Tests for linear trends were conducted based on logistic regression using a median variable of Mg concentration in each category.

Scatter plots were plotted using R 3.4.4.[53] Other data analyses were performed using SPSS 17.0; P ≤0.05 was considered to be statistically significant. All tests were two tailed.

Patient and public involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in the design or implementation of the study. There are no plans to disseminate the results of the research to study participants

Results

A total of 962 subjects (377 females, accounting for 39.2%) were included in the present cross-sectional study. The characteristics of the study population according to quintiles of serum Mg were presented in Table 1. The mean age of the subjects was 54.9±7.6 years old. The overall prevalence of MetS, DM, HP and HU in OA patients were 21.4%, 12.0%, 38.5% and 18.3% respectively. Significant differences were observed across the quintiles of serum Mg for fasting glucose, as well as the prevalence of DM and HU.

The prevalence of MetS in each quintile of serum Mg in OA patients was shown in Figure 1 (A). The outcomes of multivariable adjusted associations between MetS and serum Mg concentration were shown in Table 2. Compared with the lowest quintile, the age-gender adjusted ORs (Model 1) suggested significant decreased prevalence of MetS in the second (OR=0.61, 95%CI 0.38-0.97, P=0.038) and the highest (OR=0.59, 95%CI 0.36-0.96, P=0.035) quintiles of serum Mg (P for trend =0.090); the multivariable adjusted ORs (Model 2) also suggested significant decreased prevalence of MetS in the second (OR=0.60, 95%CI 0.37-0.96, P=0.035) and the highest (OR=0.61, 95%CI 0.37-0.99, P=0.047) quintiles, and the P for trend was 0.120. The sensitivity analysis, by adding eGFR into model 2, also reached similar results - significant lower prevalence of MetS in the second (OR=0.59, 95%CI 0.36-0.94, P=0.027) and the highest quintiles (OR=0.56, 95%CI 0.34-0.93, P=0.024) compared with the reference quintile of serum Mg, and the P for trend was 0.067.

Figure 1 (B) showed the prevalence of DM in each category of serum Mg in OA patients. Table 3 illustrated the multivariable adjusted relations between serum Mg and DM in OA patients. Both the age-gender adjusted OR values (Model 1) and the

multivariable adjusted OR values (Model 2) suggested a strong inverse association between serum Mg and DM. The age-gender adjusted ORs for the prevalence of DM were 0.38 (95%CI 0.22-0.66, P=0.001), 0.34 (95%CI 0.19-0.61, P<0.001), 0.29 (95%CI 0.15-0.55, P<0.001), and 0.20 (95%CI 0.10-0.40, P<0.001) in the second, third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was <0.001. The multivariable adjusted ORs for the prevalence of DM were 0.40 (95%CI 0.23-0.70, P=0.001), 0.32 (95%CI 0.18-0.59, P<0.001), 0.26 (95%CI 0.13-0.50, P<0.001), and 0.21 (95%CI 0.11-0.42, P<0.001) in the second, third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was <0.001. The sensitivity analysis, by adding eGFR into model 2, showed similar results - significant lower prevalence of DM in the second (OR=0.40, 95%CI 0.23-0.70, P=0.001), third (OR=0.33, 95%CI 0.18-0.60, P<0.001), fourth (OR=0.27, 95%CI 0.14-0.52, P<0.001), and highest quintiles (OR=0.22, 95%CI 0.11-0.44, P<0.001) compared with the reference quintile of serum Mg, and the P for trend was <0.001.

The prevalence of HP in each quintile of serum Mg in OA patients was depicted in Figure 1 (C). The multivariable-adjusted relations between serum Mg and HP in OA patients were illustrated in Table 4. According to both the age-gender adjusted ORs (Model 1) and the multivariable adjusted ORs (Model 2), there was no significant association between serum Mg and HP, and the P for trend were 0.929 and 0.377, respectively. The sensitivity analysis, by adding eGFR into model 2, reached the same results.

The prevalence of HU in each category of serum Mg in OA patients was shown in Figure 1 (D). The multivariable-adjusted relations between serum Mg and HU in OA patients were illustrated in Table 5. Both the age-gender adjusted OR values (Model 1) and the multivariable adjusted OR values (Model 2) suggested significant decreased prevalence of HU in the third quintile (age-gender adjusted OR=0.44, 95%CI 0.26-0.75, P=0.002; multivariable adjusted OR=0.38, 95%CI 0.22-0.67, P=0.001) and fifth quintile (age-gender adjusted OR=0.51, 95%CI 0.30-0.85, P=0.010; multivariable adjusted OR=0.50, 95%CI 0.29-0.87, P=0.013) compared with the lowest quintile of serum Mg, and the P for trend were 0.008 and 0.006, respectively.

The sensitivity analysis, by adding eGFR into model 2, showed similar outcomes - significant lower prevalence of HU in the third (OR=0.33, 0.19-0.59, P<0.001), fourth (OR=0.52, 95%CI 0.30-0.91, P=0.022), and highest quintiles (OR=0.39, 95%CI 0.22-0.70, P=0.001) compared with the reference quintile of serum Mg, and the P for trend was <0.001.

Discussion

The results of this study suggested that the serum Mg concentration was negatively associated with the prevalence of MetS, DM and HU in subjects with radiographic knee OA. In order to control potential confounders, several covariates including characteristics, living habits and underlying diseases were selected, and even the eGFR was added into the multivariable logistic regression models to eliminate the influence of renal function on Mg excretion. The reverse associations mentioned above remained significant after adjustments of these confounders. However, the negative association between serum Mg and the prevalence of HP was not observed in radiographic knee OA patients. Moreover, the linear associations were only observed between serum Mg with DM and HU, but not between serum Mg and MetS.

Mg, the fourth most abundant cation in human body and the second most profuse intracellular cation, is a metallic cofactor for over 300 enzymatic reactions. It appears to play an important role in glucose metabolism and insulin homeostasis, which are both highly correlated with metabolic diseases, especially MetS and DM. The mechanisms involved in Mg deficiency in patients with MetS, DM and HU are probably multifactorial. The most important factor may be insulin resistance, as Mg is essential for insulin action and is a critical cofactor for several enzymes in carbohydrate metabolism, which is important for the phosphorylation reactions of tyrosine-kinase in the insulin receptor.[31 54-58] Of course, it is necessary to highlight the fact that insulin can also induce Mg excretion[59] and produce a significant decline of plasma Mg through ion exchange.[60] Thus, there seems to be a vicious circle between Mg deficiency and insulin resistance.

Other potential mechanisms include glucose transportation,[57] oxidative stress[57]

and inflammatory cytokines,[61-63] and cellular calcium homeostasis.[55] Mg is an essential cofactor of the high-energy phosphate-bound enzymatic pathways involved in the modulation of glucose transport across cell membranes.[57] It also plays a role in the mechanisms of cellular antioxidant defense.[64] The oxidative stress, defined as a persistent imbalance between the excessive production of reactive oxygen species and/or defects in antioxidant defense, has been implicated in the pathogenesis of diabetic complications.[57] Moreover, low serum Mg levels are strongly related to elevated serum concentrations of both tumor necrosis factor alpha and C-reactive protein (CRP),[65] suggesting that Mg deficiency may contribute to the development of low-grade chronic inflammation syndrome and the development of glucose metabolic disorders through the former pathway. In addition, lower Mg concentration can enhance calcium-mediated vasoconstriction, blunt cardiac and smooth muscle relaxation, and thus contribute to BP elevation.[55] However, the decreased serum calcium concentration in radiographic knee OA patients may weaken the association between Mg and HP.[66]

MetS[21 22] and DM[4 23 24] were reported to be the risk factors of OA progression. Moreover, serum Mg level has been proved to be significantly associated with the CRP concentration,[27 67-69] and higher CRP might serve as a prediction factor for OA progression.[70 71] Thus, OA progression may be delayed by elevating the serum Mg level through reducing the prevalence of MetS and DM and decreasing the level of CRP. Above all, the present study indicated that the elevation of serum Mg level has the potential to reduce the prevalence of MetS, DM and HU in knee OA patients and thereby may delay the progression of knee OA. However, the specific mechanism needs to be further explored.

The present study has several strengths. Firstly, this is the first study examining the associations between serum Mg and the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. The results of this study will provide a new insight into the treatment of knee OA. Secondly, the multivariable logistical regression models were adjusted for a considerable number of potential confounding factors, which greatly improved the reliability of the results. Thirdly, the kidney is the key

organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by adding eGFR into multivariable logistic regression models which showed that the reverse associations remained significant.

Limitations of the present study should also be admitted. The cross-sectional design precludes causal correlations, so further prospective studies and intervention trials should be undertaken to establish a causal association between serum Mg with the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. Since no previous research investigated such associations in knee OA patients, the value of this study should not be blotted out by the cross-sectional nature. Another limitation of this study lies in the relatively small sample size, and thus, extensive high-quality researches based on a larger sample are needed. Moreover, the dietary intake of Mg in relation to the prevalence of MetS, DM, HP and HU were not assessed in the present study. Last but not the least, it is important to highlight that Mg is an intracellular ion; therefore, the serum Mg concentration must be considered as a poor indicator of body Mg content,[72] even though it has been used in many studies. However, blood Mg level is the second best indicator of body status.[73]

Conclusions

The present study concluded that the serum Mg concentration was inversely associated with the prevalence of MetS, DM and HU in radiographic knee OA patients.

Contributors

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. GHL, YLW and JW conceived the study. GHL, YLW and JW were responsible for conception and design of the study and drafted the manuscript. CZ, TY, HL, YC and DXX contributed to data collection. WJ contributed to preparation and data analysis. BX, ZCL, JTL, and SDJ contributed to study retrieval. GHL and YLW contributed to revision of the manuscript. All the authors contributed to the interpretation of the data and critically reviewed the manuscript for publication.

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Competing interests

The authors declare that they have no conflict of interest.

Ethics approval

The protocol of this study was reviewed and approved by the Ethics Committee at Xiangya Hospital.

Data sharing statement

The datasets during the current study available from the corresponding author on reasonable request.

REFERENCE

1 Zhuo Q, Yang W, Chen J, *et al*. Metabolic syndrome meets osteoarthritis. *Nat Rev Rheumatol* 2012;8(12):729-37.

2 Katz JD, Agrawal S, Velasquez M. Getting to the heart of the matter: osteoarthritis takes its place as part of the metabolic syndrome. *Curr Opin Rheumatol* 2010;22(5):512-9.

3 Berenbaum F. Diabetes-induced osteoarthritis: from a new paradigm to a new phenotype. *Ann Rheum Dis* 2011;70(8):1354-6.

4 King KB, Rosenthal AK. The adverse effects of diabetes on osteoarthritis: update on clinical evidence and molecular mechanisms. *Osteoarthritis Cartilage* 2015;23(6):841-50.

5 Kirkman MS. Osteoarthritis progression: is diabetes a culprit? *Osteoarthritis Cartilage* 2015;23(6):839-40.

6 Sellam J, Berenbaum F. Is osteoarthritis a metabolic disease? *Joint Bone Spine* 2013;80(6):568-73.

7 Puenpatom RA, Victor TW. Increased prevalence of metabolic syndrome in individuals with osteoarthritis: an analysis of NHANES III data. *Postgrad Med* 2009;121(6):9-20.

8 Shin D. Association between metabolic syndrome, radiographic knee osteoarthritis, and intensity of knee pain: results of a national survey. *J Clin Endocrinol Metab* 2014;99(9):3177-83.

9 Calvet J, Orellana C, Larrosa M, *et al*. High prevalence of cardiovascular co-morbidities in patients with symptomatic knee or hand osteoarthritis. *Scand J Rheumatol* 2015:1-4.

10 Rahman MM, Kopec JA, Cibere J, *et al*. The relationship between osteoarthritis and cardiovascular disease in a population health survey: a cross-sectional study. *BMJ Open* 2013;3(5):e2624.

11 Inoue R, Ishibashi Y, Tsuda E, *et al*. Medical problems and risk factors of metabolic syndrome among radiographic knee osteoarthritis patients in the

Japanese general population. *J Orthop Sci* 2011;16(6):704-9.

12 Hart DJ, Doyle DV, Spector TD. Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. *J Rheumatol* 1995;22(6):1118-23.

13 Jungmann PM, Kraus MS, Alizai H, *et al*. Association of metabolic risk factors with cartilage degradation assessed by T2 relaxation time at the knee: data from the osteoarthritis initiative. *Arthritis Care Res (Hoboken)* 2013;65(12):1942-50.

14 Anagnostopoulos I, Zinzaras E, Alexiou I, *et al*. The prevalence of rheumatic diseases in central Greece: a population survey. *BMC Musculoskelet Disord* 2010;11:98.

15 Massengale M, Reichmann WM, Losina E, *et al*. The relationship between hand osteoarthritis and serum leptin concentration in participants of the Third National Health and Nutrition Examination Survey. *Arthritis Res Ther* 2012;14(3):R132.

16 Nieves-Plaza M, Castro-Santana LE, Font YM, *et al*. Association of hand or knee osteoarthritis with diabetes mellitus in a population of Hispanics from Puerto Rico. *J Clin Rheumatol* 2013;19(1):1-6.

17 Greiver M, Williamson T, Barber D, *et al*. Prevalence and epidemiology of diabetes in Canadian primary care practices: a report from the Canadian Primary Care Sentinel Surveillance Network. *Can J Diabetes* 2014;38(3):179-85.

18 Rahman MM, Cibere J, Anis AH, *et al*. Risk of Type 2 Diabetes among Osteoarthritis Patients in a Prospective Longitudinal Study. *Int J Rheumatol* 2014;2014:620920.

19 Reid JL, Morton DJ, Wingard DL, *et al*. Obesity and other cardiovascular disease risk factors and their association with osteoarthritis in Southern California American Indians, 2002-2006. *Ethn Dis* 2010;20(4):416-22.

20 Birtwhistle R, Morkem R, Peat G, *et al*. Prevalence and management of osteoarthritis in primary care: an epidemiologic cohort study from the Canadian Primary Care Sentinel Surveillance Network. *CMAJ Open* 2015;3(3):E270-5.

21 Yoshimura N, Muraki S, Oka H, *et al*. Accumulation of metabolic risk factors such as overweight, hypertension, dyslipidaemia, and impaired glucose tolerance raises the risk of occurrence and progression of knee osteoarthritis: a 3-year

follow-up of the ROAD study. *Osteoarthritis Cartilage* 2012;20(11):1217-26.

22 Monira HS, Wang Y, Cicuttini FM, *et al*. Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: a prospective cohort study. *Semin Arthritis Rheum* 2014;43(4):429-36.

23 Schett G, Kleyer A, Perricone C, *et al*. Diabetes is an independent predictor for severe osteoarthritis: results from a longitudinal cohort study. *Diabetes Care* 2013;36(2):403-9.

24 Eymard F, Parsons C, Edwards MH, *et al*. Diabetes is a risk factor for knee osteoarthritis progression. *Osteoarthritis Cartilage* 2015;23(6):851-9.

25 Guerrero-Romero F, Rodriguez-Moran M. Low serum magnesium levels and metabolic syndrome. *Acta Diabetol* 2002;39(4):209-13.

26 Guerrero-Romero F, Rodriguez-Moran M. Hypomagnesemia, oxidative stress, inflammation, and metabolic syndrome. *Diabetes Metab Res Rev* 2006;22(6):471-6.

27 Evangelopoulos AA, Vallianou NG, Panagiotakos DB, *et al*. An inverse relationship between cumulating components of the metabolic syndrome and serum magnesium levels. *Nutr Res* 2008;28(10):659-63.

28 Hjelmesaeth J, Hofso D, Aasheim ET, *et al*. Parathyroid hormone, but not vitamin D, is associated with the metabolic syndrome in morbidly obese women and men: a cross-sectional study. *Cardiovasc Diabetol* 2009;8:7.

29 Lima ML, Cruz T, Rodrigues LE, *et al*. Serum and intracellular magnesium deficiency in patients with metabolic syndrome--evidences for its relation to insulin resistance. *Diabetes Res Clin Pract* 2009;83(2):257-62.

30 Ma J, Folsom AR, Melnick SL, *et al*. Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin, and carotid arterial wall thickness: the ARIC study. Atherosclerosis Risk in Communities Study. *J Clin Epidemiol* 1995;48(7):927-40.

31 Kao WH, Folsom AR, Nieto FJ, *et al*. Serum and dietary magnesium and the risk for type 2 diabetes mellitus: the Atherosclerosis Risk in Communities Study.

Arch Intern Med 1999;159(18):2151-9.

32 Wang JL, Shaw NS, Yeh HY, *et al*. Magnesium status and association with diabetes in the Taiwanese elderly. *Asia Pac J Clin Nutr* 2005;14(3):263-9.

33 Chambers EC, Heshka S, Gallagher D, *et al*. Serum magnesium and type-2 diabetes in African Americans and Hispanics: a New York cohort. *J Am Coll Nutr* 2006;25(6):509-13.

34 Simmons D, Joshi S, Shaw J. Hypomagnesaemia is associated with diabetes: Not pre-diabetes, obesity or the metabolic syndrome. *Diabetes Res Clin Pract* 2010;87(2):261-6.

35 Sales CH, Pedrosa LF, Lima JG, *et al*. Influence of magnesium status and magnesium intake on the blood glucose control in patients with type 2 diabetes. *Clin Nutr* 2011;30(3):359-64.

36 Lecube A, Baena-Fustegueras JA, Fort JM, *et al*. Diabetes is the main factor accounting for hypomagnesemia in obese subjects. *PLoS One* 2012;7(1):e30599.

37 Xu J, Xu W, Yao H, *et al*. Associations of serum and urinary magnesium with the pre-diabetes, diabetes and diabetic complications in the Chinese Northeast population. *PLoS One* 2013;8(2):e56750.

38 Yang SJ, Hwang SY, Baik SH, *et al*. Serum magnesium level is associated with type 2 diabetes in women with a history of gestational diabetes mellitus: the Korea National Diabetes Program study. *J Korean Med Sci* 2014;29(1):84-9.

39 Singh RB, Rastogi V, Niaz MA, *et al*. Epidemiological study of magnesium status and risk of hypertension in a rural population of north India. *Magnes Res* 1996;9(3):173-81.

40 Peacock JM, Folsom AR, Arnett DK, *et al*. Relationship of serum and dietary magnesium to incident hypertension: the Atherosclerosis Risk in Communities (ARIC) Study. *Ann Epidemiol* 1999;9(3):159-65.

41 Guerrero-Romero F, Rodriguez-Moran M, Hernandez-Ronquillo G, *et al*. Low Serum Magnesium Levels and Its Association with High Blood Pressure in Children. *J Pediatr* 2016;168:93-8.

42 Zeng C, Wang YL, Wei J, *et al*. Association between low serum magnesium

concentration and hyperuricemia. *Magnes Res* 2015;28(2):56-63.

43 Zeng C, Wei J, Li H, *et al*. Relationship between Serum Magnesium Concentration and Radiographic Knee Osteoarthritis. *J Rheumatol* 2015;42(7):1231-6.

44 Wei J, Zeng C, Gong QY, *et al*. Associations between Dietary Antioxidant Intake and Metabolic Syndrome. *PLoS One* 2015;10(6):e130876.

45 Xie DX, Xiong YL, Zeng C, *et al*. Association between low dietary zinc and hyperuricaemia in middle-aged and older males in China: a cross-sectional study. *BMJ Open* 2015;5(10):e8637.

46 Wei J, Zeng C, Gong QY, *et al*. The association between dietary selenium intake and diabetes: a cross-sectional study among middle-aged and older adults. *Nutr J* 2015;14:18.

47 Expert Panel on Metabolic Syndrome of Chinese Diabetes Society: Recommendations on metabolic syndrome of Chinese Diabetes Society (Chinese). *Chin J Diabetes* 2004;14:156-61.

48 Pang C, Jia L, Hou X, *et al*. The significance of screening for microvascular diseases in Chinese community-based subjects with various metabolic abnormalities. *PLoS One* 2014;9(5):e97928.

49 Zhou H, Guo ZR, Yu LG, *et al*. Evidence on the applicability of the ATPIII, IDF and CDS metabolic syndrome diagnostic criteria to identify CVD and T2DM in the Chinese population from a 6.3-year cohort study in mid-eastern China. *Diabetes Res Clin Pract* 2010;90(3):319-25.

50 Levey AS, Stevens LA, Schmid CH, *et al*. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150(9):604-12.

51 Joosten MM, Gansevoort RT, Mukamal KJ, *et al*. Urinary magnesium excretion and risk of hypertension: the prevention of renal and vascular end-stage disease study. *Hypertension* 2013;61(6):1161-7.

52 Choi MK, Bae YJ. Association of Magnesium Intake with High Blood Pressure in Korean Adults: Korea National Health and Nutrition Examination Survey 2007-2009. *PLoS One* 2015;10(6):e130405.

53 R Development Core Team. R: A Language and Environment for Statistical Computing, Vienna, Austria: R Foundation for Statistical Computing. 2016.

54 Grundy SM, Cleeman JI, Daniels SR, *et al*. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112(17):2735-52.

55 Barbagallo M, Dominguez LJ, Galioto A, *et al*. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. *Mol Aspects Med* 2003;24(1-3):39-52.

56 Song Y, Ridker PM, Manson JE, *et al*. Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* 2005;28(6):1438-44.

57 Guerrero-Romero F, Rodriguez-Moran M. Complementary therapies for diabetes: the case for chromium, magnesium, and antioxidants. *Arch Med Res* 2005;36(3):250-7.

58 Huerta MG, Roemmich JN, Kington ML, *et al*. Magnesium deficiency is associated with insulin resistance in obese children. *Diabetes Care* 2005;28(5):1175-81.

59 Djurhuus MS, Skott P, Hother-Nielson O, *et al*. Insulin increases renal magnesium excretion: a possible cause of magnesium depletion in hyperinsulinaemic states. *Diabet Med* 1995;12(8):664-9.

60 Paolisso G, Sgambato S, Passariello N, *et al*. Insulin induces opposite changes in plasma and erythrocyte magnesium concentrations in normal man. *Diabetologia* 1986;29(9):644-7.

61 Bonora E, Targher G, Zenere MB, *et al*. Relationship of uric acid concentration to cardiovascular risk factors in young men. Role of obesity and central fat distribution. The Verona Young Men Atherosclerosis Risk Factors Study. *Int J Obes Relat Metab Disord* 1996;20(11):975-80.

62 Lyngdoh T, Marques-Vidal P, Paccaud F, *et al*. Elevated serum uric acid is associated with high circulating inflammatory cytokines in the population-based Colaus study. *PLoS One* 2011;6(5):e19901.

63 Kirilmaz B, Asgun F, Alioglu E, *et al*. High inflammatory activity related to the number of metabolic syndrome components. *J Clin Hypertens (Greenwich)* 2010;12(2):136-44.

64 Salmonowicz B, Krzystek-Korpacka M, Noczynska A. Trace elements, magnesium, and the efficacy of antioxidant systems in children with type 1 diabetes mellitus and in their siblings. *Adv Clin Exp Med* 2014;23(2):259-68.

65 M RGN, F G. Elevated serum concentration of tumor necrosis factor-alpha is linked to low serum magnesium levels in the obesity-related inflammatory response. *Magnes Res* 2004;17:189-96.

66 Li H, Zeng C, Wei J, *et al*. Serum Calcium Concentration Is Inversely Associated With Radiographic Knee Osteoarthritis: A Cross-Sectional Study. *Medicine (Baltimore)* 2016;95(6):e2838.

67 Chacko SA, Song Y, Nathan L, *et al*. Relations of dietary magnesium intake to biomarkers of inflammation and endothelial dysfunction in an ethnically diverse cohort of postmenopausal women. *Diabetes Care* 2010;33(2):304-10.

68 Bo S, Durazzo M, Guidi S, *et al*. Dietary magnesium and fiber intakes and inflammatory and metabolic indicators in middle-aged subjects from a population-based cohort. *Am J Clin Nutr* 2006;84(5):1062-9.

69 Kim DJ, Xun P, Liu K, *et al*. Magnesium intake in relation to systemic inflammation, insulin resistance, and the incidence of diabetes. *Diabetes Care* 2010;33(12):2604-10.

70 Spector TD, Hart DJ, Nandra D, *et al*. Low-level increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. *Arthritis Rheum* 1997;40(4):723-7.

71 Smith JW, Martins TB, Gopez E, *et al*. Significance of C-reactive protein in osteoarthritis and total knee arthroplasty outcomes. *Ther Adv Musculoskelet Dis* 2012;4(5):315-25.

72 Topf JM, Murray PT. Hypomagnesemia and hypermagnesemia. *Rev Endocr Metab Disord* 2003;4(2):195-206.

73 Sabatier M, Pont F, Arnaud MJ, *et al*. A compartmental model of magnesium

metabolism in healthy men based on two stable isotope tracers. *Am J Physiol Regul Integr Comp Physiol* 2003;285(3):R656-63.

568 Table 1 Basic characteristics of included subjects according to quintiles of serum Mg (n=962)

	Quintiles of serum Mg					P
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
Age (years)	53.8 (7.3)	54.6 (7.6)	55.2 (7.9)	55.3 (7.1)	56.1 (8.0)	0.062
BMI (kg/m^2)	25.2 (3.2)	24.9 (3.2)	25.0 (3.7)	25.2 (3.4)	24.6 (3.2)	0.464
Female (%)	37.5	42.3	36.8	42.3	37.0	0.627
Smoking (%)	27.5	27.4	21.6	24.4	21.7	0.457
Alcohol drinking (%)	34.5	36.3	40.5	41.1	38.1	0.645
High school diploma (%)	45.0	47.4	45.3	56.5	48.1	0.184
Activity level (h/w)	2.0 (3.5)	2.0 (3.3)	2.3 (3.5)	2.1 (3.1)	2.4 (3.5)	0.457
Fasting glucose (mmol/l)	6.6 (3.0)	5.7 (1.7)	5.7 (1.4)	5.5 (0.9)	5.5 (1.6)	0.009
Systolic pressure (mm Hg)	129.2 (16.9)	128.3 (17.9)	130.4 (16.2)	128.8 (16.3)	129.6 (17.7)	0.837
Diastolic pressure (mm Hg)	81.2 (11.8)	79.8 (12.1)	80.7 (11.0)	80.7 (10.7)	80.3 (10.5)	0.654
HDL-cholesterol (mmol/l)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	1.5 (0.3)	1.5 (0.4)	0.374
Triglyceride (mmol/l)	2.1 (1.9)	1.8 (1.5)	2.0 (2.1)	1.8 (1.0)	2.3 (2.9)	0.620

Uric acid (μmol/l)	337.3 (101.7)	329.0 (80.7)	321.3 (86.3)	331.5 (78.0)	329.4 (81.7)	0.590
eGFR (ml/min/1.73m^2)	80.2 (14.4)	77.7 (10.7)	76.0 (10.6)	75.8 (10.7)	74.3 (12.0)	<0.001
MetS (%)	26.5	17.7	25.8	19.6	17.5	0.059
DM (%)	23.5	10.7	10.0	8.3	6.3	<0.001
HP (%)	40.0	33.5	37.4	42.3	40.2	0.432
HU (%)	25.5	19.1	13.2	18.5	14.8	0.018

Data are mean (Standard Deviation), unless otherwise indicated; Mg, magnesium; OA, osteoarthritis; BMI, body mass index; HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate; MetS, metabolic syndrome; DM, diabetes mellitus; HP, hypertension; HU, hyperuricemia.

P values are for test of difference across all quintiles of serum Mg.

Figure 1 The prevalence of MetS (A), DM (B), HP (C) and HU (D) in each quintile of serum Mg in OA patients

Table 2 Multivariable-adjusted relations of serum Mg and MetS in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
MetS (%)	26.5	17.7	25.8	19.6	17.5	-
Model 1*	1.00 (reference)	0.61 (0.38, 0.97)	0.97 (0.61, 1.52)	0.69 (0.42, 1.14)	0.59 (0.36, 0.96)	0.090
P value	-	0.038	0.881	0.150	0.035	-
Model 2*	1.00 (reference)	0.60 (0.37, 0.96)	1.00 (0.63, 1.57)	0.70 (0.42, 1.15)	0.61 (0.37, 0.99)	0.120
P value	-	0.035	0.99	0.160	0.047	-
Model 3*	1.00 (reference)	0.59 (0.36, 0.94)	0.95 (0.60, 1.51)	0.67 (0.40, 1.10)	0.56 (0.34, 0.93)	0.067
P value	-	0.027	0.830	0.114	0.024	

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; MetS, metabolic syndrome.

*Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

Table 3 Multivariable-adjusted relations of serum Mg and DM in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
DM (%)	23.5	10.7	10.0	8.3	6.3	-
Model 1*	1.00 (reference)	0.38 (0.22, 0.66)	0.34 (0.19, 0.61)	0.29 (0.15, 0.55)	0.20 (0.10, 0.40)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-
Model 2*	1.00 (reference)	0.40 (0.23, 0.70)	0.32 (0.18, 0.59)	0.26 (0.13, 0.50)	0.21 (0.11, 0.42)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-
Model 3*	1.00 (reference)	0.40 (0.23, 0.70)	0.33 (0.18, 0.60)	0.27 (0.14, 0.52)	0.22 (0.11, 0.44)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; DM, diabetes mellitus.

*Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), hypertension (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

Table 4 Multivariable-adjusted relations of serum Mg and HP in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
HP (%)	40.0	33.5	37.4	42.3	40.2	-
Model 1*	1.00 (reference)	0.71 (0.47, 1.06)	0.83 (0.54, 1.25)	1.00 (0.66, 1.54)	0.89 (0.59, 1.35)	0.929
P value	-	0.095	0.368	0.987	0.582	-
Model 2*	1.00 (reference)	0.77 (0.50, 1.19)	0.89 (0.57, 1.39)	1.10 (0.70, 1.74)	1.08 (0.69, 1.68)	0.377
P value	-	0.245	0.608	0.686	0.744	-
Model 3*	1.00 (reference)	0.77 (0.50, 1.19)	0.88 (0.56, 1.38)	1.09 (0.68, 1.72)	1.05 (0.67, 1.65)	0.434
P value	-	0.235	0.574	0.727	0.818	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; HP, hypertension.

* Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

593 Table 5 Multivariable-adjusted relations of serum Mg and HU in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
HU (%)	25.5	19.1	13.2	18.5	14.8	-
Model 1*	1.00 (reference)	0.71 (0.44, 1.14)	0.44 (0.26, 0.75)	0.68 (0.41, 1.14)	0.51 (0.30, 0.85)	0.008
P value	-	0.157	0.002	0.144	0.010	-
Model 2*	1.00 (reference)	0.73 (0.45, 1.20)	0.38 (0.22, 0.67)	0.59 (0.35, 1.02)	0.50 (0.29, 0.87)	0.006
P value	-	0.210	0.001	0.058	0.013	-
Model 3*	1.00 (reference)	0.68 (0.41, 1.14)	0.33 (0.19, 0.59)	0.52 (0.30, 0.91)	0.39 (0.22, 0.70)	<0.001
P value	-	0.142	<0.001	0.022	0.001	-

594 Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; HU, hyperuricemia.

595 * Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (continuous data), gender (male,
596 female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no),
597 hypertension (yes, no), diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data)

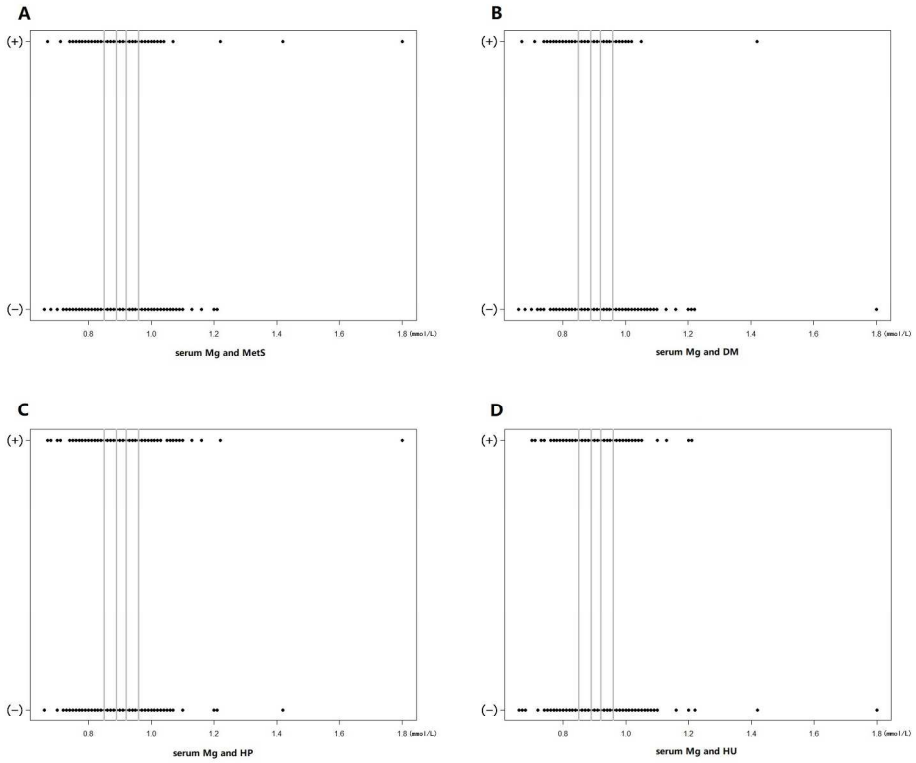

Figure 1 The prevalence of MetS (A), DM (B), HP (C) and HU (D) in each quintile of serum Mg in OA patients.

576x474mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Reported on Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	~~5~~4-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	6-7
Study size	10	Explain how the study size was arrived at	4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	-
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4-5
		(*e*) Describe any sensitivity analyses	~~5~~-6-7

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4-5
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		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
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Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	-
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	-
		Cross-sectional study—Report numbers of outcome events or summary measures	8-108-9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-108-9
		(*b*) Report category boundaries when continuous variables were categorized	8-108-9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-108-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	9-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-1010-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4-5
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Secondary Subject Heading:	Rheumatology, Public health, Epidemiology
Keywords:	osteoarthritis, magnesium, metabolic syndrome, diabetes, Hypertension < CARDIOLOGY, hyperuricemia

Association between Serum Magnesium Concentration with Metabolic Syndrome, Diabetes, Hypertension and Hyperuricemia in Knee Osteoarthritis

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Abstract

Objectives: To examine the associations between serum magnesium (Mg) concentration with the prevalence of metabolic syndrome (MetS), diabetes mellitus (DM), hypertension (HP) and hyperuricemia (HU) in radiographic knee osteoarthritis (OA) patients.

Methods: The present study was conducted at the Health Management Center of Xiangya Hospital. Radiographic OA was evaluated for patients aged over 40 years with basic characteristics and blood biochemical assessment. Serum Mg concentration was measured using the chemiluminescence method. MetS, DM, HP and HU were diagnosed based on standard protocols. The associations between serum Mg concentration with MetS, DM, HP and HU were evaluated by conducting multivariable adjusted logistic regression.

Results: A total of 962 radiographic knee OA patients were included. Compared with the lowest quintile, the multivariable-adjusted odds ratios (ORs) and related 95% confidence intervals (95%CI) of DM were 0.40 (95%CI 0.23-0.70, P=0.001), 0.33 (95%CI 0.18-0.60, P<0.001), 0.27 (95%CI 0.14-0.52, P<0.001) and 0.22 (95%CI 0.11-0.44, P<0.001) in the second, third, fourth and highest quintiles of serum Mg, respectively (P for trend <0.001); the multivariable-adjusted ORs of HU were 0.33 (95%CI 0.19-0.59, P<0.001), 0.52 (95%CI 0.30-0.91, P=0.022) and 0.39 (95%CI 0.22-0.70, P=0.001) in the third, fourth and highest quintiles of serum Mg respectively (P for trend <0.001); and the multivariable-adjusted ORs of MetS were 0.59 (95%CI 0.36-0.94, P=0.027) in the second and 0.56 (95%CI 0.34-0.93, P=0.024) in the highest quintiles of serum Mg. However, the inverse association between serum Mg and the prevalence of MetS was nonlinear (P for trend =0.067). There was no significant association between serum Mg and HP in OA patients.

Conclusions: The serum Mg concentration was inversely associated with the prevalence of MetS, DM and HU in radiographic knee OA patients.

Level of Evidence: Level Ⅲ, cross-sectional study.

Key words: osteoarthritis, magnesium, metabolic syndrome, diabetes, hypertension, hyperuricemia

Strengths and limitations of this study

1. This is the first study examining the associations between serum magnesium (Mg) and the prevalence of metabolic syndrome, diabetes mellitus, hypertension and hyperuricemia in radiographic knee osteoarthritis patients.
2. The multivariable logistical regression models in this study were adjusted for a considerable number of potential confounding factors, which greatly improved the reliability of the results.
3. The kidney is the key organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by adding estimated glomerular filtration rate into the multivariable logistic regression models, and the reverse associations remained significant.
4. This study adopted cross-sectional design which precluded causal correlations.
5. Serum Mg concentration was adopted as the indicator of body Mg content in this study which may not be the best indicator of body status.

Introduction

The association between osteoarthritis (OA) and metabolic diseases, especially metabolic syndrome (MetS)[1 2] and diabetes mellitus (DM),[3-5] has drawn increasing attention in the past few years. OA includes three specific phenotypes: metabolic OA, age-related OA and injury-related OA.[6] A large number of studies have indicated that the prevalence of MetS,[7-9] DM[10-18] and hypertension (HP)[7 9-13 19 20] is either higher in OA patients or associated with OA. In addition, some other studies reported that MetS,[21 22] DM[23 24] and HP[21 22] are risk factors of OA progression. Thus, it appears necessary to pay more attention and adopt appropriate measures to reduce the high prevalence of metabolic diseases in OA patients, which also seems to be beneficial in delaying OA progression.

Serum magnesium (Mg), one of the most important micronutrients for human health, has been reported to be negatively associated with MetS,[25-29] DM[30-38] and HP[30 39-41] by lots of studies. Meanwhile, our previous study showed an inverse association between serum Mg and hyperuricemia (HU).[42] However, to the best knowledge of the authors, there is not yet a study examining the association between the serum Mg concentration and the aforementioned metabolic diseases (MetS, DM, HP and HU) in OA patients. On the other hand, we have previously shown that the serum Mg concentration may be inversely associated with radiographic knee OA.[43] Therefore, we speculate that the prevalence of MetS, DM, HP and HU in OA patients may be reduced by elevating the level of serum Mg, which can in turn delay OA progression. Thus, the objective of the present study was to examine the associations between the serum Mg concentration with the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. It was hypothesized that serum Mg concentration was inversely associated with these diseases.

Methods

Study population

The present study was conducted at the Health Management Center of Xiangya Hospital between October 2013 and November 2014. The study design has been

published previously.[42-46] The protocol has been reviewed and approved by the Ethics Committee of Xiangya Hospital, Central South University (reference numbers: 201312459), and the methods were developed in "accordance" with the approved guidelines. Informed consent has been obtained from all participants. Registered nurses were engaged to interview all participants during the examination using a standard questionnaire, with the purpose to collect information on demographic characteristics and health-related habits. Participants were selected based on the following inclusion criteria: 1) 40 years old or above; 2) undergoing weight-bearing bilateral anteroposterior radiography of the knee, and diagnosed with knee OA according to the Kellgren-Lawrence (K-L) radiographic atlas (knee joint was graded K-L 2 or above); 3) availability of all basic characteristics, including age, gender, body mass index (BMI) and blood pressure; 4) availability of biochemical test results, including serum Mg concentration; 5) availability of information related to the living habits, including education background, activity level, smoking, drinking and medication status. Initially, the present cross-sectional study retrieved 1820 radiographic knee OA patients aged over 40 years who exhibited sound basic characteristics and required blood biochemical assessment (including serum Mg concentration). Among them, 962 patients offered demographic characteristics and health-related habits and were finally included in this study.

Blood biochemistry

All blood samples were drawn after a 12-hour overnight fast and were kept at 4℃ until analysis. Blood tests were undertaken using the Beckman Coulter AU 5800 (Beckman Coulter Inc., Brea, CA, USA). The inter- and intra-assay coefficients of variation were tested at both low concentrations (2.5 mmol/L for glucose, 118 μmol/L for uric acid and 0.60 mmol/L for serum Mg) and high concentrations (6.7 mmol/L for glucose, 472 μmol/L for uric acid and 1.00 mmol/L for serum Mg) of standard human samples. The intra-assay coefficients of variation were 0.98% (2.5 mmol/L) and 1.72% (6.7 mmol/L) for glucose, 1.39% (118 μmol/L) and 0.41% (472 μmol/L) for uric acid, and 1.86% (0.60 mmol/L) and 1.65% (1.00 mmol/L) for serum Mg respectively. The

inter-assay coefficients of variation were 2.45% (2.5 mmol/L) and 1.46% (6.7 mmol/L) for glucose, 1.40% (118 μmol/L) and 1.23% (472 μmol/L) for uric acid, and 1.87% (0.60 mmol/L) and 1.70% (1.00 mmol/L) for serum Mg respectively.

Assessment of other exposures

Blood pressure was measured by an electronic sphygmomanometer. The weight and height of each subject were measured respectively to calculate the BMI. Information on the average frequency of physical activity (never, one to two times per week, three to four times per week, five times and above per week) and average duration of physical activity (less than half an hour, half an hour to one hour, one to two hours, more than two hours) were collected through survey questionnaire. The smoking, alcohol drinking and medication status were collected during the face-to-face interview.

Assessment of MetS, DM, HP and HU

MetS was diagnosed based on the Chinese Diabetes Society (CDS) criteria,[47-49] which requires meeting at least 3 of the following 4 items: (1) BMI ≥25 kg/m^2; (2) Fasting plasma glucose (FPG) ≥6.1 mmol/L, or diagnosed DM; (3) Systolic blood pressure (BP) ≥140 mmHg or diastolic BP≥90 mmHg, or treatment of previously diagnosed HP; (4) Triglycerides ≥1.7 mmol/L and/or HDL-cholesterol <0.9 mmol/L in male or <1.0 mmol/L in female, or treatment for this lipid abnormality. Subjects with the fasting glucose ≥7.0 mmol/L or currently undergoing drug treatment for blood glucose control were regarded as DM patients, and subjects with the systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or currently undertaking antihypertensive medication were regarded as HP patients. HU was defined as uric acid ≥416 μmol/L for male and ≥360 μmol/L for female or currently undergoing drug treatment for uric acid control.

Statistical analysis

The continuous data were expressed as mean with standard deviation, and the

category data were expressed in percentage. Differences in continuous data were evaluated by one-way classification ANOVA (normally distributed data) or Kruskal-Wallis H test (non-normally distributed data), while differences in category data were assessed by the $\chi 2$ test. The serum Mg was classified into five categories based on the quintile distribution: ≤0.85, 0.86-0.89, 0.90-0.92, 0.93-0.96 and ≥0.97 mmol/L. The prevalence of MetS, DM, HP and HU in each quintile of serum Mg in OA patients were assessed by scatter plots.

Logistic regression was conducted to calculate the odds ratios (ORs) with 95% confidence intervals (95%CI) for the associations between serum Mg and MetS, DM, HP and HU. Specifically, model 1 was adjusted by covariates of age (continuous data) and gender (male, female). Then, model 2 was adjusted by additional covariates of BMI (continuous data), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), HP (yes, no), DM (yes, no), and dyslipidemia (yes, no) on the basis of model 1. Dyslipidemia was defined as triglycerides ≥1.7 mmol/L and/or HDL-cholesterol <0.9 mmol/L in male or <1.0 mmol/L in female, or treatment for this lipid abnormality. Notably, the selection of covariates in model 2 varied slightly for examining different associations (between serum Mg and MetS, DM, HP or HU). For example, BMI, HP and dyslipidemia were adjusted for the association between serum Mg and DM, but not for the association between serum Mg and MetS, simply because MetS was diagnosed based on BMI, HP and dyslipidemia status. Model 3 was established based on model 2, with adjustment of an additional covariate, estimated glomerular filtration rate (eGFR). eGFR (continuous data) was calculated from the Chronic Kidney Disease Epidemiology Collaboration equation.[50] All covariates in the present study were chosen referring to some of the previous similar studies.[27 33 51 52] Tests for linear trends were conducted based on logistic regression using a median variable of Mg concentration in each category.

Scatter plots were plotted using R 3.4.4.[53] Other data analyses were performed using SPSS 17.0; $P \leq 0.05$ was considered to be statistically significant. All tests were two tailed.

Patient and public involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in the design or implementation of the study. There were no plans to disseminate the results of the research to study participants.

Results

A total of 962 subjects (377 females, accounting for 39.2%) were included in the present cross-sectional study. The characteristics of the study population according to quintiles of serum Mg were presented in Table 1. The mean age of the subjects was 54.9±7.6 years old. The overall prevalence of MetS, DM, HP and HU in OA patients were 21.4%, 12.0%, 38.5% and 18.3% respectively. Significant differences were observed across the quintiles of serum Mg for fasting glucose, as well as the prevalence of DM and HU.

The prevalence of MetS in each quintile of serum Mg in OA patients was shown in Figure 1 (A). The outcomes of multivariable adjusted associations between MetS and serum Mg concentration were shown in Table 2. Compared with the lowest quintile, the age-gender adjusted ORs (Model 1) suggested significant decreased prevalence of MetS in the second (OR=0.61, 95%CI 0.38-0.97, P=0.038) and the highest (OR=0.59, 95%CI 0.36-0.96, P=0.035) quintiles of serum Mg; the multivariable adjusted ORs (Model 2) also suggested significant decreased prevalence of MetS in the second (OR=0.60, 95%CI 0.37-0.96, P=0.035) and the highest (OR=0.61, 95%CI 0.37-0.99, P=0.047) quintiles. The sensitivity analysis, by adding eGFR into model 2, also reached similar results - significant lower prevalence of MetS in the second (OR=0.59, 95%CI 0.36-0.94, P=0.027) and the highest quintiles (OR=0.56, 95%CI 0.34-0.93, P=0.024) compared with the reference quintile of serum Mg. No clear trend was evident in the third and fourth quintiles of serum Mg. The P for trend were 0.090 (Model 1), 0.120 (Model 2), 0.067 (Model 3), respectively.

Figure 1 (B) showed the prevalence of DM in each category of serum Mg in OA patients. Table 3 illustrated the multivariable adjusted relations between serum Mg

and DM in OA patients. Both the age-gender adjusted OR values (Model 1) and the multivariable adjusted OR values (Model 2) suggested a strong inverse association between serum Mg and DM. The age-gender adjusted ORs for the prevalence of DM were 0.38 (95%CI 0.22-0.66, P=0.001), 0.34 (95%CI 0.19-0.61, P<0.001), 0.29 (95%CI 0.15-0.55, P<0.001), and 0.20 (95%CI 0.10-0.40, P<0.001) in the second, third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was <0.001. The multivariable adjusted ORs for the prevalence of DM were 0.40 (95%CI 0.23-0.70, P=0.001), 0.32 (95%CI 0.18-0.59, P<0.001), 0.26 (95%CI 0.13-0.50, P<0.001), and 0.21 (95%CI 0.11-0.42, P<0.001) in the second, third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was <0.001. The sensitivity analysis, by adding eGFR into model 2, showed similar results - significant lower prevalence of DM in the second (OR=0.40, 95%CI 0.23-0.70, P=0.001), third (OR=0.33, 95%CI 0.18-0.60, P<0.001), fourth (OR=0.27, 95%CI 0.14-0.52, P<0.001), and highest quintiles (OR=0.22, 95%CI 0.11-0.44, P<0.001) compared with the reference quintile of serum Mg, and the P for trend was <0.001.

The prevalence of HP in each quintile of serum Mg in OA patients was depicted in Figure 1 (C). The multivariable-adjusted relations between serum Mg and HP in OA patients were illustrated in Table 4. According to both the age-gender adjusted ORs (Model 1) and the multivariable adjusted ORs (Model 2), there was no significant association between serum Mg and HP, and the P for trend were 0.929 and 0.377, respectively. The sensitivity analysis, by adding eGFR into model 2, reached the same results.

The prevalence of HU in each category of serum Mg in OA patients was shown in Figure 1 (D). The multivariable-adjusted relations between serum Mg and HU in OA patients were illustrated in Table 5. Both the age-gender adjusted OR values (Model 1) and the multivariable adjusted OR values (Model 2) suggested significant decreased prevalence of HU in the third quintile (age-gender adjusted OR=0.44, 95%CI 0.26-0.75, P=0.002; multivariable adjusted OR=0.38, 95%CI 0.22-0.67, P=0.001) and fifth quintile (age-gender adjusted OR=0.51, 95%CI 0.30-0.85, P=0.010; multivariable adjusted OR=0.50, 95%CI 0.29-0.87, P=0.013) compared with the

lowest quintile of serum Mg, and the P for trend were 0.008 and 0.006, respectively. The sensitivity analysis, by adding eGFR into model 2, showed similar outcomes - significant lower prevalence of HU in the third (OR=0.33, 0.19-0.59, P<0.001), fourth (OR=0.52, 95%CI 0.30-0.91, P=0.022), and highest quintiles (OR=0.39, 95%CI 0.22-0.70, P=0.001) compared with the reference quintile of serum Mg, and the P for trend was <0.001.

Discussion

The results of this study suggested that the serum Mg concentration was negatively associated with the prevalence of MetS, DM and HU in subjects with radiographic knee OA. To control potential confounders, several covariates including characteristics, living habits and underlying diseases were selected, and even the eGFR was added into the multivariable logistic regression models to eliminate the influence of renal function on Mg excretion. The reverse associations mentioned above remained significant after adjustments of these confounders. However, the association between serum Mg and the prevalence of MetS was nonlinear, with no clear trend in the third and fourth quintiles of serum Mg. Moreover, the negative association between serum Mg and the prevalence of HP was not observed in radiographic knee OA patients.

Mg, the fourth most abundant cation in human body and the second most profuse intracellular cation, is a metallic cofactor for over 300 enzymatic reactions. It appears to play an important role in glucose metabolism and insulin homeostasis, which are both highly correlated with metabolic diseases, especially MetS and DM. The mechanisms involved in Mg deficiency in patients with MetS, DM and HU are probably multifactorial. The most important factor may be insulin resistance, as Mg is essential for insulin action and is a critical cofactor for several enzymes in carbohydrate metabolism, which is important for the phosphorylation reactions of tyrosine-kinase in the insulin receptor.[31 54-58] Of course, it is necessary to highlight the fact that insulin can also induce Mg excretion[59] and produce a significant decline of plasma Mg through ion exchange.[60] Thus, there seems to be a vicious circle between

Mg deficiency and insulin resistance.

Other potential mechanisms include glucose transportation,[57] oxidative stress[57] and inflammatory cytokines,[61-63] and cellular calcium homeostasis.[55] Mg is an essential cofactor of the high-energy phosphate-bound enzymatic pathways involved in the modulation of glucose transport across cell membranes.[57] It also plays a role in the mechanisms of cellular antioxidant defense.[64] The oxidative stress, defined as a persistent imbalance between the excessive production of reactive oxygen species and/or defects in antioxidant defense, has been implicated in the pathogenesis of diabetic complications.[57] Moreover, low serum Mg levels are strongly related to elevated serum concentrations of both tumor necrosis factor alpha and C-reactive protein (CRP),[65] suggesting that Mg deficiency may contribute to the development of low-grade chronic inflammation syndrome and the development of glucose metabolic disorders through the former pathway. In addition, lower Mg concentration can enhance calcium-mediated vasoconstriction, blunt cardiac and smooth muscle relaxation, and thus contribute to BP elevation.[55] However, the decreased serum calcium concentration in radiographic knee OA patients may weaken the association between Mg and HP.[66]

MetS[21 22] and DM[4 23 24] were reported to be the risk factors of OA progression. Moreover, serum Mg level has been proved to be significantly associated with the CRP concentration,[27 67-69] and higher CRP might serve as a prediction factor for OA progression.[70 71] Thus, OA progression may be delayed by elevating the serum Mg level through reducing the prevalence of MetS and DM and decreasing the level of CRP. Above all, the present study indicated that the elevation of serum Mg level has the potential to reduce the prevalence of MetS, DM and HU in knee OA patients and thereby may delay the progression of knee OA. However, the specific mechanism needs to be further explored.

The present study has several strengths. Firstly, this is the first study examining the associations between serum Mg and the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. The results of this study will provide a new insight into the treatment of knee OA. Secondly, the multivariable logistical regression

models were adjusted for a considerable number of potential confounding factors, which greatly improved the reliability of the results. Thirdly, the kidney is the key organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by adding eGFR into multivariable logistic regression models which showed that the reverse associations remained significant.

Limitations of the present study should also be admitted. The cross-sectional design precludes causal correlations, so further prospective studies and intervention trials should be undertaken to establish a causal association between serum Mg with the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. Since no previous research investigated such associations in knee OA patients, the value of this study should not be blotted out by the cross-sectional nature. Another limitation of this study lies in the relatively small sample size, and thus, extensive high-quality researches based on a larger sample are needed. Moreover, the dietary intake of Mg in relation to the prevalence of MetS, DM, HP and HU were not assessed in the present study. Last but not the least, it is important to highlight that Mg is an intracellular ion; therefore, the serum Mg concentration must be considered as a poor indicator of body Mg content,[72] even though it has been used in many studies. However, blood Mg level is the second best indicator of body status.[73]

Conclusions

The present study concluded that the serum Mg concentration was inversely associated with the prevalence of MetS, DM and HU in radiographic knee OA patients.

Contributors

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. GHL, YLW and JW conceived the study. GHL, YLW and JW were responsible for conception and design of the study and drafted the manuscript. CZ, TY, HL, YC and DXX contributed to data collection. WJ contributed to preparation and data analysis. BX, ZCL, JTL, and SDJ contributed to study retrieval. GHL and YLW contributed to revision of the manuscript. All the authors contributed to the interpretation of the data and critically reviewed the manuscript for publication.

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Competing interests

The authors declare that they have no conflict of interest.

Ethics approval

The protocol of this study was reviewed and approved by the Ethics Committee at Xiangya Hospital.

Data sharing statement

The datasets during the current study available from the corresponding author on reasonable request.

REFERENCE

1 Zhuo Q, Yang W, Chen J, *et al*. Metabolic syndrome meets osteoarthritis. *Nat Rev Rheumatol* 2012;8(12):729-37.

2 Katz JD, Agrawal S, Velasquez M. Getting to the heart of the matter: osteoarthritis takes its place as part of the metabolic syndrome. *Curr Opin Rheumatol* 2010;22(5):512-9.

3 Berenbaum F. Diabetes-induced osteoarthritis: from a new paradigm to a new phenotype. *Ann Rheum Dis* 2011;70(8):1354-6.

4 King KB, Rosenthal AK. The adverse effects of diabetes on osteoarthritis: update on clinical evidence and molecular mechanisms. *Osteoarthritis Cartilage* 2015;23(6):841-50.

5 Kirkman MS. Osteoarthritis progression: is diabetes a culprit? *Osteoarthritis Cartilage* 2015;23(6):839-40.

6 Sellam J, Berenbaum F. Is osteoarthritis a metabolic disease? *Joint Bone Spine* 2013;80(6):568-73.

7 Puenpatom RA, Victor TW. Increased prevalence of metabolic syndrome in individuals with osteoarthritis: an analysis of NHANES III data. *Postgrad Med* 2009;121(6):9-20.

8 Shin D. Association between metabolic syndrome, radiographic knee osteoarthritis, and intensity of knee pain: results of a national survey. *J Clin Endocrinol Metab* 2014;99(9):3177-83.

9 Calvet J, Orellana C, Larrosa M, *et al*. High prevalence of cardiovascular co-morbidities in patients with symptomatic knee or hand osteoarthritis. *Scand J Rheumatol* 2015:1-4.

10 Rahman MM, Kopec JA, Cibere J, *et al*. The relationship between osteoarthritis and cardiovascular disease in a population health survey: a cross-sectional study. *BMJ Open* 2013;3(5):e2624.

11 Inoue R, Ishibashi Y, Tsuda E, *et al*. Medical problems and risk factors of metabolic syndrome among radiographic knee osteoarthritis patients in the

Japanese general population. *J Orthop Sci* 2011;16(6):704-9.

12 Hart DJ, Doyle DV, Spector TD. Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. *J Rheumatol* 1995;22(6):1118-23.

13 Jungmann PM, Kraus MS, Alizai H, *et al*. Association of metabolic risk factors with cartilage degradation assessed by T2 relaxation time at the knee: data from the osteoarthritis initiative. *Arthritis Care Res (Hoboken)* 2013;65(12):1942-50.

14 Anagnostopoulos I, Zinzaras E, Alexiou I, *et al*. The prevalence of rheumatic diseases in central Greece: a population survey. *BMC Musculoskelet Disord* 2010;11:98.

15 Massengale M, Reichmann WM, Losina E, *et al*. The relationship between hand osteoarthritis and serum leptin concentration in participants of the Third National Health and Nutrition Examination Survey. *Arthritis Res Ther* 2012;14(3):R132.

16 Nieves-Plaza M, Castro-Santana LE, Font YM, *et al*. Association of hand or knee osteoarthritis with diabetes mellitus in a population of Hispanics from Puerto Rico. *J Clin Rheumatol* 2013;19(1):1-6.

17 Greiver M, Williamson T, Barber D, *et al*. Prevalence and epidemiology of diabetes in Canadian primary care practices: a report from the Canadian Primary Care Sentinel Surveillance Network. *Can J Diabetes* 2014;38(3):179-85.

18 Rahman MM, Cibere J, Anis AH, *et al*. Risk of Type 2 Diabetes among Osteoarthritis Patients in a Prospective Longitudinal Study. *Int J Rheumatol* 2014;2014:620920.

19 Reid JL, Morton DJ, Wingard DL, *et al*. Obesity and other cardiovascular disease risk factors and their association with osteoarthritis in Southern California American Indians, 2002-2006. *Ethn Dis* 2010;20(4):416-22.

20 Birtwhistle R, Morkem R, Peat G, *et al*. Prevalence and management of osteoarthritis in primary care: an epidemiologic cohort study from the Canadian Primary Care Sentinel Surveillance Network. *CMAJ Open* 2015;3(3):E270-5.

21 Yoshimura N, Muraki S, Oka H, *et al*. Accumulation of metabolic risk factors such as overweight, hypertension, dyslipidaemia, and impaired glucose tolerance raises the risk of occurrence and progression of knee osteoarthritis: a 3-year

follow-up of the ROAD study. *Osteoarthritis Cartilage* 2012;20(11):1217-26.

22 Monira HS, Wang Y, Cicuttini FM, *et al*. Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: a prospective cohort study. *Semin Arthritis Rheum* 2014;43(4):429-36.

23 Schett G, Kleyer A, Perricone C, *et al*. Diabetes is an independent predictor for severe osteoarthritis: results from a longitudinal cohort study. *Diabetes Care* 2013;36(2):403-9.

24 Eymard F, Parsons C, Edwards MH, *et al*. Diabetes is a risk factor for knee osteoarthritis progression. *Osteoarthritis Cartilage* 2015;23(6):851-9.

25 Guerrero-Romero F, Rodriguez-Moran M. Low serum magnesium levels and metabolic syndrome. *Acta Diabetol* 2002;39(4):209-13.

26 Guerrero-Romero F, Rodriguez-Moran M. Hypomagnesemia, oxidative stress, inflammation, and metabolic syndrome. *Diabetes Metab Res Rev* 2006;22(6):471-6.

27 Evangelopoulos AA, Vallianou NG, Panagiotakos DB, *et al*. An inverse relationship between cumulating components of the metabolic syndrome and serum magnesium levels. *Nutr Res* 2008;28(10):659-63.

28 Hjelmesaeth J, Hofso D, Aasheim ET, *et al*. Parathyroid hormone, but not vitamin D, is associated with the metabolic syndrome in morbidly obese women and men: a cross-sectional study. *Cardiovasc Diabetol* 2009;8:7.

29 Lima ML, Cruz T, Rodrigues LE, *et al*. Serum and intracellular magnesium deficiency in patients with metabolic syndrome--evidences for its relation to insulin resistance. *Diabetes Res Clin Pract* 2009;83(2):257-62.

30 Ma J, Folsom AR, Melnick SL, *et al*. Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin, and carotid arterial wall thickness: the ARIC study. Atherosclerosis Risk in Communities Study. *J Clin Epidemiol* 1995;48(7):927-40.

31 Kao WH, Folsom AR, Nieto FJ, *et al*. Serum and dietary magnesium and the risk for type 2 diabetes mellitus: the Atherosclerosis Risk in Communities Study.

Arch Intern Med 1999;159(18):2151-9.

32 Wang JL, Shaw NS, Yeh HY, *et al*. Magnesium status and association with diabetes in the Taiwanese elderly. *Asia Pac J Clin Nutr* 2005;14(3):263-9.

33 Chambers EC, Heshka S, Gallagher D, *et al*. Serum magnesium and type-2 diabetes in African Americans and Hispanics: a New York cohort. *J Am Coll Nutr* 2006;25(6):509-13.

34 Simmons D, Joshi S, Shaw J. Hypomagnesaemia is associated with diabetes: Not pre-diabetes, obesity or the metabolic syndrome. *Diabetes Res Clin Pract* 2010;87(2):261-6.

35 Sales CH, Pedrosa LF, Lima JG, *et al*. Influence of magnesium status and magnesium intake on the blood glucose control in patients with type 2 diabetes. *Clin Nutr* 2011;30(3):359-64.

36 Lecube A, Baena-Fustegueras JA, Fort JM, *et al*. Diabetes is the main factor accounting for hypomagnesemia in obese subjects. *PLoS One* 2012;7(1):e30599.

37 Xu J, Xu W, Yao H, *et al*. Associations of serum and urinary magnesium with the pre-diabetes, diabetes and diabetic complications in the Chinese Northeast population. *PLoS One* 2013;8(2):e56750.

38 Yang SJ, Hwang SY, Baik SH, *et al*. Serum magnesium level is associated with type 2 diabetes in women with a history of gestational diabetes mellitus: the Korea National Diabetes Program study. *J Korean Med Sci* 2014;29(1):84-9.

39 Singh RB, Rastogi V, Niaz MA, *et al*. Epidemiological study of magnesium status and risk of hypertension in a rural population of north India. *Magnes Res* 1996;9(3):173-81.

40 Peacock JM, Folsom AR, Arnett DK, *et al*. Relationship of serum and dietary magnesium to incident hypertension: the Atherosclerosis Risk in Communities (ARIC) Study. *Ann Epidemiol* 1999;9(3):159-65.

41 Guerrero-Romero F, Rodriguez-Moran M, Hernandez-Ronquillo G, *et al*. Low Serum Magnesium Levels and Its Association with High Blood Pressure in Children. *J Pediatr* 2016;168:93-8.

42 Zeng C, Wang YL, Wei J, *et al*. Association between low serum magnesium

concentration and hyperuricemia. *Magnes Res* 2015;28(2):56-63.

43 Zeng C, Wei J, Li H, *et al*. Relationship between Serum Magnesium Concentration and Radiographic Knee Osteoarthritis. *J Rheumatol* 2015;42(7):1231-6.

44 Wei J, Zeng C, Gong QY, *et al*. Associations between Dietary Antioxidant Intake and Metabolic Syndrome. *PLoS One* 2015;10(6):e130876.

45 Xie DX, Xiong YL, Zeng C, *et al*. Association between low dietary zinc and hyperuricaemia in middle-aged and older males in China: a cross-sectional study. *BMJ Open* 2015;5(10):e8637.

46 Wei J, Zeng C, Gong QY, *et al*. The association between dietary selenium intake and diabetes: a cross-sectional study among middle-aged and older adults. *Nutr J* 2015;14:18.

47 Expert Panel on Metabolic Syndrome of Chinese Diabetes Society: Recommendations on metabolic syndrome of Chinese Diabetes Society (Chinese). *Chin J Diabetes* 2004;14:156-61.

48 Pang C, Jia L, Hou X, *et al*. The significance of screening for microvascular diseases in Chinese community-based subjects with various metabolic abnormalities. *PLoS One* 2014;9(5):e97928.

49 Zhou H, Guo ZR, Yu LG, *et al*. Evidence on the applicability of the ATPIII, IDF and CDS metabolic syndrome diagnostic criteria to identify CVD and T2DM in the Chinese population from a 6.3-year cohort study in mid-eastern China. *Diabetes Res Clin Pract* 2010;90(3):319-25.

50 Levey AS, Stevens LA, Schmid CH, *et al*. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150(9):604-12.

51 Joosten MM, Gansevoort RT, Mukamal KJ, *et al*. Urinary magnesium excretion and risk of hypertension: the prevention of renal and vascular end-stage disease study. *Hypertension* 2013;61(6):1161-7.

52 Choi MK, Bae YJ. Association of Magnesium Intake with High Blood Pressure in Korean Adults: Korea National Health and Nutrition Examination Survey 2007-2009. *PLoS One* 2015;10(6):e130405.

53 R Development Core Team. R: A Language and Environment for Statistical Computing, Vienna, Austria: R Foundation for Statistical Computing. 2016.

54 Grundy SM, Cleeman JI, Daniels SR, *et al*. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112(17):2735-52.

55 Barbagallo M, Dominguez LJ, Galioto A, *et al*. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. *Mol Aspects Med* 2003;24(1-3):39-52.

56 Song Y, Ridker PM, Manson JE, *et al*. Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* 2005;28(6):1438-44.

57 Guerrero-Romero F, Rodriguez-Moran M. Complementary therapies for diabetes: the case for chromium, magnesium, and antioxidants. *Arch Med Res* 2005;36(3):250-7.

58 Huerta MG, Roemmich JN, Kington ML, *et al*. Magnesium deficiency is associated with insulin resistance in obese children. *Diabetes Care* 2005;28(5):1175-81.

59 Djurhuus MS, Skott P, Hother-Nielson O, *et al*. Insulin increases renal magnesium excretion: a possible cause of magnesium depletion in hyperinsulinaemic states. *Diabet Med* 1995;12(8):664-9.

60 Paolisso G, Sgambato S, Passariello N, *et al*. Insulin induces opposite changes in plasma and erythrocyte magnesium concentrations in normal man. *Diabetologia* 1986;29(9):644-7.

61 Bonora E, Targher G, Zenere MB, *et al*. Relationship of uric acid concentration to cardiovascular risk factors in young men. Role of obesity and central fat distribution. The Verona Young Men Atherosclerosis Risk Factors Study. *Int J Obes Relat Metab Disord* 1996;20(11):975-80.

62 Lyngdoh T, Marques-Vidal P, Paccaud F, *et al*. Elevated serum uric acid is associated with high circulating inflammatory cytokines in the population-based Colaus study. *PLoS One* 2011;6(5):e19901.

63 Kirilmaz B, Asgun F, Alioglu E, *et al*. High inflammatory activity related to the number of metabolic syndrome components. *J Clin Hypertens (Greenwich)* 2010;12(2):136-44.

64 Salmonowicz B, Krzystek-Korpacka M, Noczynska A. Trace elements, magnesium, and the efficacy of antioxidant systems in children with type 1 diabetes mellitus and in their siblings. *Adv Clin Exp Med* 2014;23(2):259-68.

65 Rodríguez-Morán M, Guerrero-Romero F. Elevated serum concentration of tumor necrosis factor-alpha is linked to low serum magnesium levels in the obesity-related inflammatory response. *Magnes Res* 2004;17:189-96.

66 Li H, Zeng C, Wei J, *et al*. Serum Calcium Concentration Is Inversely Associated With Radiographic Knee Osteoarthritis: A Cross-Sectional Study. *Medicine (Baltimore)* 2016;95(6):e2838.

67 Chacko SA, Song Y, Nathan L, *et al*. Relations of dietary magnesium intake to biomarkers of inflammation and endothelial dysfunction in an ethnically diverse cohort of postmenopausal women. *Diabetes Care* 2010;33(2):304-10.

68 Bo S, Durazzo M, Guidi S, *et al*. Dietary magnesium and fiber intakes and inflammatory and metabolic indicators in middle-aged subjects from a population-based cohort. *Am J Clin Nutr* 2006;84(5):1062-9.

69 Kim DJ, Xun P, Liu K, *et al*. Magnesium intake in relation to systemic inflammation, insulin resistance, and the incidence of diabetes. *Diabetes Care* 2010;33(12):2604-10.

70 Spector TD, Hart DJ, Nandra D, *et al*. Low-level increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. *Arthritis Rheum* 1997;40(4):723-7.

71 Smith JW, Martins TB, Gopez E, *et al*. Significance of C-reactive protein in osteoarthritis and total knee arthroplasty outcomes. *Ther Adv Musculoskelet Dis* 2012;4(5):315-25.

72 Topf JM, Murray PT. Hypomagnesemia and hypermagnesemia. *Rev Endocr Metab Disord* 2003;4(2):195-206.

73 Sabatier M, Pont F, Arnaud MJ, *et al*. A compartmental model of magnesium

metabolism in healthy men based on two stable isotope tracers. *Am J Physiol Regul Integr Comp Physiol* 2003;285(3):R656-63.

570 Table 1 Basic characteristics of included subjects according to quintiles of serum Mg (n=962)

	Quintiles of serum Mg					P
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
Age (years)	53.8 (7.3)	54.6 (7.6)	55.2 (7.9)	55.3 (7.1)	56.1 (8.0)	0.062
BMI (kg/m^2)	25.2 (3.2)	24.9 (3.2)	25.0 (3.7)	25.2 (3.4)	24.6 (3.2)	0.464
Female (%)	37.5	42.3	36.8	42.3	37.0	0.627
Smoking (%)	27.5	27.4	21.6	24.4	21.7	0.457
Alcohol drinking (%)	34.5	36.3	40.5	41.1	38.1	0.645
High school diploma (%)	45.0	47.4	45.3	56.5	48.1	0.184
Activity level (h/w)	2.0 (3.5)	2.0 (3.3)	2.3 (3.5)	2.1 (3.1)	2.4 (3.5)	0.457
Fasting glucose (mmol/l)	6.6 (3.0)	5.7 (1.7)	5.7 (1.4)	5.5 (0.9)	5.5 (1.6)	0.009
Systolic pressure (mm Hg)	129.2 (16.9)	128.3 (17.9)	130.4 (16.2)	128.8 (16.3)	129.6 (17.7)	0.837
Diastolic pressure (mm Hg)	81.2 (11.8)	79.8 (12.1)	80.7 (11.0)	80.7 (10.7)	80.3 (10.5)	0.654
HDL-cholesterol (mmol/l)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	1.5 (0.3)	1.5 (0.4)	0.374
Triglyceride (mmol/l)	2.1 (1.9)	1.8 (1.5)	2.0 (2.1)	1.8 (1.0)	2.3 (2.9)	0.620

Uric acid (μmol/l)	337.3 (101.7)	329.0 (80.7)	321.3 (86.3)	331.5 (78.0)	329.4 (81.7)	0.590
eGFR (ml/min/1.73m^2)	80.2 (14.4)	77.7 (10.7)	76.0 (10.6)	75.8 (10.7)	74.3 (12.0)	<0.001
MetS (%)	26.5	17.7	25.8	19.6	17.5	0.059
DM (%)	23.5	10.7	10.0	8.3	6.3	<0.001
HP (%)	40.0	33.5	37.4	42.3	40.2	0.432
HU (%)	25.5	19.1	13.2	18.5	14.8	0.018

Data are mean (Standard Deviation), unless otherwise indicated; Mg, magnesium; OA, osteoarthritis; BMI, body mass index; HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate; MetS, metabolic syndrome; DM, diabetes mellitus; HP, hypertension; HU, hyperuricemia.

P values are for test of difference across all quintiles of serum Mg.

Figure 1 The prevalence of MetS (A), DM (B), HP (C) and HU (D) in each quintile of serum Mg in radiographic knee OA patients

The figures above present the prevalence of MetS (A), DM (B), HP (C) and HU (D) among the 962 OA patients under different quintiles of serum Mg levels. The horizontal axis denotes the serum Mg level, and the vertical axis indicates whether a subject is diagnosed with the specific disease: (+) - disease; (-) - no disease. The solid gray lines represent the boundaries in between the five quintiles of serum Mg levels. The red and black spots represent the prevalence of diseases and no diseases at each serum Mg level, respectively. The darker the color of a spot, the more OA patients there are at the corresponding concentration.

Table 2 Multivariable-adjusted relations of serum Mg and MetS in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
MetS (%)	26.5	17.7	25.8	19.6	17.5	-
Model 1*	1.00 (reference)	0.61 (0.38, 0.97)	0.97 (0.61, 1.52)	0.69 (0.42, 1.14)	0.59 (0.36, 0.96)	0.090
P value	-	0.038	0.881	0.150	0.035	-
Model 2*	1.00 (reference)	0.60 (0.37, 0.96)	1.00 (0.63, 1.57)	0.70 (0.42, 1.15)	0.61 (0.37, 0.99)	0.120
P value	-	0.035	0.99	0.160	0.047	-
Model 3*	1.00 (reference)	0.59 (0.36, 0.94)	0.95 (0.60, 1.51)	0.67 (0.40, 1.10)	0.56 (0.34, 0.93)	0.067
P value	-	0.027	0.830	0.114	0.024	

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; MetS, metabolic syndrome.

*Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

Table 3 Multivariable-adjusted relations of serum Mg and DM in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
DM (%)	23.5	10.7	10.0	8.3	6.3	-
Model 1*	1.00 (reference)	0.38 (0.22, 0.66)	0.34 (0.19, 0.61)	0.29 (0.15, 0.55)	0.20 (0.10, 0.40)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-
Model 2*	1.00 (reference)	0.40 (0.23, 0.70)	0.32 (0.18, 0.59)	0.26 (0.13, 0.50)	0.21 (0.11, 0.42)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-
Model 3*	1.00 (reference)	0.40 (0.23, 0.70)	0.33 (0.18, 0.60)	0.27 (0.14, 0.52)	0.22 (0.11, 0.44)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; DM, diabetes mellitus.

*Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), hypertension (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

Table 4 Multivariable-adjusted relations of serum Mg and HP in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
HP (%)	40.0	33.5	37.4	42.3	40.2	-
Model 1*	1.00 (reference)	0.71 (0.47, 1.06)	0.83 (0.54, 1.25)	1.00 (0.66, 1.54)	0.89 (0.59, 1.35)	0.929
P value	-	0.095	0.368	0.987	0.582	-
Model 2*	1.00 (reference)	0.77 (0.50, 1.19)	0.89 (0.57, 1.39)	1.10 (0.70, 1.74)	1.08 (0.69, 1.68)	0.377
P value	-	0.245	0.608	0.686	0.744	-
Model 3*	1.00 (reference)	0.77 (0.50, 1.19)	0.88 (0.56, 1.38)	1.09 (0.68, 1.72)	1.05 (0.67, 1.65)	0.434
P value	-	0.235	0.574	0.727	0.818	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; HP, hypertension.

* Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

Table 5 Multivariable-adjusted relations of serum Mg and HU in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
HU (%)	25.5	19.1	13.2	18.5	14.8	-
Model 1*	1.00 (reference)	0.71 (0.44, 1.14)	0.44 (0.26, 0.75)	0.68 (0.41, 1.14)	0.51 (0.30, 0.85)	0.008
P value	-	0.157	0.002	0.144	0.010	-
Model 2*	1.00 (reference)	0.73 (0.45, 1.20)	0.38 (0.22, 0.67)	0.59 (0.35, 1.02)	0.50 (0.29, 0.87)	0.006
P value	-	0.210	0.001	0.058	0.013	-
Model 3*	1.00 (reference)	0.68 (0.41, 1.14)	0.33 (0.19, 0.59)	0.52 (0.30, 0.91)	0.39 (0.22, 0.70)	<0.001
P value	-	0.142	<0.001	0.022	0.001	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; HU, hyperuricemia.

* Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), hypertension (yes, no), diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data)

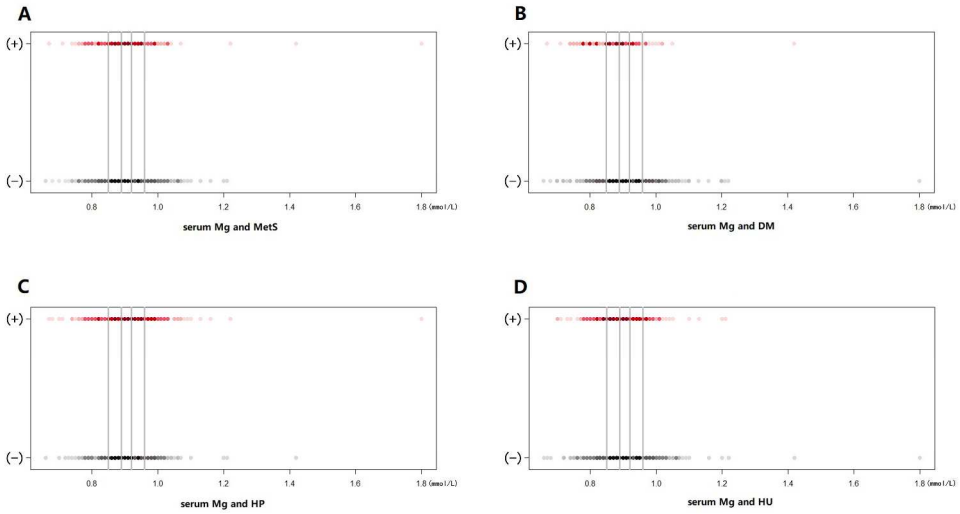

Figure 1 The prevalence of MetS (A), DM (B), HP (C) and HU (D) in each quintile of serum Mg in radiographic knee OA patients

The figures above present the prevalence of MetS (A), DM (B), HP (C) and HU (D) among the 962 OA patients under different quintiles of serum Mg levels. The horizontal axis denotes the serum Mg level, and the vertical axis indicates whether a subject is diagnosed with the specific disease: (+) - disease; (-) - no disease. The solid gray lines represent the boundaries in between the five quintiles of serum Mg levels. The red and black spots represent the prevalence of diseases and no diseases at each serum Mg level, respectively. The darker the color of a spot, the more OA patients there are at the corresponding concentration.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Reported on Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	6-7
Study size	10	Explain how the study size was arrived at	4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	-
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4-5
		(*e*) Describe any sensitivity analyses	6-7

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4-5
		(b) Give reasons for non-participation at each stage	4-5
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	-
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	-
		Cross-sectional study—Report numbers of outcome events or summary measures	8-10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	8-10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Association between serum magnesium concentration and metabolic syndrome, diabetes, hypertension and hyperuricemia in knee osteoarthritis: a cross-sectional study in Hunan Province, China

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Association between serum magnesium concentration and metabolic syndrome, diabetes, hypertension and hyperuricemia in knee osteoarthritis: a cross-sectional study in Hunan Province, China

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Abstract

Objectives: To examine the associations between serum magnesium (Mg) concentration with the prevalence of metabolic syndrome (MetS), diabetes mellitus (DM), hypertension (HP) and hyperuricemia (HU) in radiographic knee osteoarthritis (OA) patients.

Methods: The present study was conducted at the Health Management Center of Xiangya Hospital. Radiographic OA was evaluated for patients aged over 40 years with basic characteristics and blood biochemical assessment. Serum Mg concentration was measured using the chemiluminescence method. MetS, DM, HP and HU were diagnosed based on standard protocols. The associations between serum Mg concentration with MetS, DM, HP and HU were evaluated by conducting multivariable adjusted logistic regression.

Results: A total of 962 radiographic knee OA patients were included. Compared with the lowest quintile, the multivariable-adjusted odds ratios (ORs) and related 95% confidence intervals (95%CI) of DM were 0.40 (95%CI 0.23-0.70, P=0.001), 0.33 (95%CI 0.18-0.60, P<0.001), 0.27 (95%CI 0.14-0.52, P<0.001) and 0.22 (95%CI 0.11-0.44, P<0.001) in the second, third, fourth and highest quintiles of serum Mg, respectively (P for trend <0.001); the multivariable-adjusted ORs of HU were 0.33 (95%CI 0.19-0.59, P<0.001), 0.52 (95%CI 0.30-0.91, P=0.022) and 0.39 (95%CI 0.22-0.70, P=0.001) in the third, fourth and highest quintiles of serum Mg respectively (P for trend <0.001); and the multivariable-adjusted ORs of MetS were 0.59 (95%CI 0.36-0.94, P=0.027) in the second and 0.56 (95%CI 0.34-0.93, P=0.024) in the highest quintiles of serum Mg. However, the inverse association between serum Mg and the prevalence of MetS was nonlinear (P for trend =0.067). There was no significant association between serum Mg and HP in OA patients.

Conclusions: The serum Mg concentration was inversely associated with the prevalence of MetS, DM and HU in radiographic knee OA patients.

Level of Evidence: Level Ⅲ, cross-sectional study.

Key words: osteoarthritis, magnesium, metabolic syndrome, diabetes, hypertension, hyperuricemia

Strengths and limitations of this study

1. This is the first study examining the associations between serum magnesium (Mg) and the prevalence of metabolic syndrome, diabetes mellitus, hypertension and hyperuricemia in radiographic knee osteoarthritis patients.
2. The multivariable logistical regression models in this study were adjusted for a considerable number of potential confounding factors, which greatly improved the reliability of the results.
3. The kidney is the key organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by adding estimated glomerular filtration rate into the multivariable logistic regression models, and the reverse associations remained significant.
4. This study adopted cross-sectional design which precluded causal correlations.
5. Serum Mg concentration was adopted as the indicator of body Mg content in this study which may not be the best indicator of body status.

Introduction

The association between osteoarthritis (OA) and metabolic diseases, especially metabolic syndrome (MetS)[1 2] and diabetes mellitus (DM),[3-5] has drawn increasing attention in the past few years. OA includes three specific phenotypes: metabolic OA, age-related OA and injury-related OA.[6] A large number of studies have indicated that the prevalence of MetS,[7-9] DM[10-18] and hypertension (HP)[7 9-13 19 20] is either higher in OA patients or associated with OA. In addition, some other studies reported that MetS,[21 22] DM[23 24] and HP[21 22] are risk factors of OA progression. Thus, it appears necessary to pay more attention and adopt appropriate measures to reduce the high prevalence of metabolic diseases in OA patients, which also seems to be beneficial in delaying OA progression.

Serum magnesium (Mg), one of the most important micronutrients for human health, has been reported to be negatively associated with MetS,[25-29] DM[30-38] and HP[30 39-41] by lots of studies. Meanwhile, our previous study showed an inverse association between serum Mg and hyperuricemia (HU).[42] However, to the best knowledge of the authors, there is not yet a study examining the association between the serum Mg concentration and the aforementioned metabolic diseases (MetS, DM, HP and HU) in OA patients. On the other hand, we have previously shown that the serum Mg concentration may be inversely associated with radiographic knee OA.[43] Therefore, we speculate that the prevalence of MetS, DM, HP and HU in OA patients may be reduced by elevating the level of serum Mg, which can in turn delay OA progression. Thus, the objective of the present study was to examine the associations between the serum Mg concentration with the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. It was hypothesized that serum Mg concentration was inversely associated with these diseases.

Methods

Study population

The present study was conducted at the Health Management Center of Xiangya Hospital between October 2013 and November 2014. The study design has been

published previously.[42-46] The protocol has been reviewed and approved by the Ethics Committee of Xiangya Hospital, Central South University (reference numbers: 201312459), and the methods were developed in "accordance" with the approved guidelines. Informed consent has been obtained from all participants. Registered nurses were engaged to interview all participants during the examination using a standard questionnaire, with the purpose to collect information on demographic characteristics and health-related habits. Participants were selected based on the following inclusion criteria: 1) 40 years old or above; 2) undergoing weight-bearing bilateral anteroposterior radiography of the knee, and diagnosed with knee OA according to the Kellgren-Lawrence (K-L) radiographic atlas (knee joint was graded K-L 2 or above); 3) availability of all basic characteristics, including age, gender, body mass index (BMI) and blood pressure; 4) availability of biochemical test results, including serum Mg concentration; 5) availability of information related to the living habits, including education background, activity level, smoking, drinking and medication status. Initially, the present cross-sectional study retrieved 1820 radiographic knee OA patients aged over 40 years who exhibited sound basic characteristics and required blood biochemical assessment (including serum Mg concentration). Among them, 962 patients offered demographic characteristics and health-related habits and were finally included in this study.

Blood biochemistry

All blood samples were drawn after a 12-hour overnight fast and were kept at 4℃ until analysis. Blood tests were undertaken using the Beckman Coulter AU 5800 (Beckman Coulter Inc., Brea, CA, USA). The inter- and intra-assay coefficients of variation were tested at both low concentrations (2.5 mmol/L for glucose, 118 μmol/L for uric acid and 0.60 mmol/L for serum Mg) and high concentrations (6.7 mmol/L for glucose, 472 μmol/L for uric acid and 1.00 mmol/L for serum Mg) of standard human samples. The intra-assay coefficients of variation were 0.98% (2.5 mmol/L) and 1.72% (6.7 mmol/L) for glucose, 1.39% (118 μmol/L) and 0.41% (472 μmol/L) for uric acid, and 1.86% (0.60 mmol/L) and 1.65% (1.00 mmol/L) for serum Mg respectively. The

inter-assay coefficients of variation were 2.45% (2.5 mmol/L) and 1.46% (6.7 mmol/L) for glucose, 1.40% (118 μmol/L) and 1.23% (472 μmol/L) for uric acid, and 1.87% (0.60 mmol/L) and 1.70% (1.00 mmol/L) for serum Mg respectively.

Assessment of other exposures

Blood pressure was measured by an electronic sphygmomanometer. The weight and height of each subject were measured respectively to calculate the BMI. Information on the average frequency of physical activity (never, one to two times per week, three to four times per week, five times and above per week) and average duration of physical activity (less than half an hour, half an hour to one hour, one to two hours, more than two hours) were collected through survey questionnaire. The smoking, alcohol drinking and medication status were collected during the face-to-face interview.

Assessment of MetS, DM, HP and HU

MetS was diagnosed based on the Chinese Diabetes Society (CDS) criteria,[47-49] which requires meeting at least 3 of the following 4 items: (1) BMI ≥25 kg/m^2; (2) Fasting plasma glucose (FPG) ≥6.1 mmol/L, or diagnosed DM; (3) Systolic blood pressure (BP) ≥140 mmHg or diastolic BP≥90 mmHg, or treatment of previously diagnosed HP; (4) Triglycerides ≥1.7 mmol/L and/or HDL-cholesterol <0.9 mmol/L in male or <1.0 mmol/L in female, or treatment for this lipid abnormality. Subjects with the fasting glucose ≥7.0 mmol/L or currently undergoing drug treatment for blood glucose control were regarded as DM patients, and subjects with the systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or currently undertaking antihypertensive medication were regarded as HP patients. HU was defined as uric acid ≥416 μmol/L for male and ≥360 μmol/L for female or currently undergoing drug treatment for uric acid control.

Statistical analysis

The continuous data were expressed as mean with standard deviation, and the

category data were expressed in percentage. Differences in continuous data were evaluated by one-way classification ANOVA (normally distributed data) or Kruskal-Wallis H test (non-normally distributed data), while differences in category data were assessed by the $\chi 2$ test. The serum Mg was classified into five categories based on the quintile distribution: ≤0.85, 0.86-0.89, 0.90-0.92, 0.93-0.96 and ≥0.97 mmol/L. The prevalence of MetS, DM, HP and HU in each quintile of serum Mg in OA patients were assessed by scatter plots.

Logistic regression was conducted to calculate the odds ratios (ORs) with 95% confidence intervals (95%CI) for the associations between serum Mg and MetS, DM, HP and HU. Specifically, model 1 was adjusted by covariates of age (continuous data) and gender (male, female). Then, model 2 was adjusted by additional covariates of BMI (continuous data), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), HP (yes, no), DM (yes, no), and dyslipidemia (yes, no) on the basis of model 1. Dyslipidemia was defined as triglycerides ≥1.7 mmol/L and/or HDL-cholesterol <0.9 mmol/L in male or <1.0 mmol/L in female, or treatment for this lipid abnormality. Notably, the selection of covariates in model 2 varied slightly for examining different associations (between serum Mg and MetS, DM, HP or HU). For example, BMI, HP and dyslipidemia were adjusted for the association between serum Mg and DM, but not for the association between serum Mg and MetS, simply because MetS was diagnosed based on BMI, HP and dyslipidemia status. Model 3 was established based on model 2, with adjustment of an additional covariate, estimated glomerular filtration rate (eGFR). eGFR (continuous data) was calculated from the Chronic Kidney Disease Epidemiology Collaboration equation.[50] All covariates in the present study were chosen referring to some of the previous similar studies.[27 33 51 52] Tests for linear trends were conducted based on logistic regression using a median variable of Mg concentration in each category.

Scatter plots were plotted using R 3.4.4.[53] Other data analyses were performed using SPSS 17.0; $P \leq 0.05$ was considered to be statistically significant. All tests were two tailed.

Patient and public involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in the design or implementation of the study. There were no plans to disseminate the results of the research to study participants.

Results

A total of 962 subjects (377 females, accounting for 39.2%) were included in the present cross-sectional study. The characteristics of the study population according to quintiles of serum Mg were presented in Table 1. The mean age of the subjects was 54.9±7.6 years old. The overall prevalence of MetS, DM, HP and HU in OA patients were 21.4%, 12.0%, 38.5% and 18.3% respectively. Significant differences were observed across the quintiles of serum Mg for fasting glucose, as well as the prevalence of DM and HU.

The prevalence of MetS in each quintile of serum Mg in OA patients was shown in Figure 1 (A). The outcomes of multivariable adjusted associations between MetS and serum Mg concentration were shown in Table 2. Compared with the lowest quintile, the age-gender adjusted ORs (Model 1) suggested significant decreased prevalence of MetS in the second (OR=0.61, 95%CI 0.38-0.97, P=0.038) and the highest (OR=0.59, 95%CI 0.36-0.96, P=0.035) quintiles of serum Mg; the multivariable adjusted ORs (Model 2) also suggested significant decreased prevalence of MetS in the second (OR=0.60, 95%CI 0.37-0.96, P=0.035) and the highest (OR=0.61, 95%CI 0.37-0.99, P=0.047) quintiles. The sensitivity analysis, by adding eGFR into model 2, also reached similar results - significant lower prevalence of MetS in the second (OR=0.59, 95%CI 0.36-0.94, P=0.027) and the highest quintiles (OR=0.56, 95%CI 0.34-0.93, P=0.024) compared with the reference quintile of serum Mg. No clear trend was evident in the third and fourth quintiles of serum Mg. The P for trend were 0.090 (Model 1), 0.120 (Model 2), 0.067 (Model 3), respectively.

Figure 1 (B) showed the prevalence of DM in each category of serum Mg in OA patients. Table 3 illustrated the multivariable adjusted relations between serum Mg

and DM in OA patients. Both the age-gender adjusted OR values (Model 1) and the multivariable adjusted OR values (Model 2) suggested a strong inverse association between serum Mg and DM. The age-gender adjusted ORs for the prevalence of DM were 0.38 (95%CI 0.22-0.66, P=0.001), 0.34 (95%CI 0.19-0.61, P<0.001), 0.29 (95%CI 0.15-0.55, P<0.001), and 0.20 (95%CI 0.10-0.40, P<0.001) in the second, third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was <0.001. The multivariable adjusted ORs for the prevalence of DM were 0.40 (95%CI 0.23-0.70, P=0.001), 0.32 (95%CI 0.18-0.59, P<0.001), 0.26 (95%CI 0.13-0.50, P<0.001), and 0.21 (95%CI 0.11-0.42, P<0.001) in the second, third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was <0.001. The sensitivity analysis, by adding eGFR into model 2, showed similar results - significant lower prevalence of DM in the second (OR=0.40, 95%CI 0.23-0.70, P=0.001), third (OR=0.33, 95%CI 0.18-0.60, P<0.001), fourth (OR=0.27, 95%CI 0.14-0.52, P<0.001), and highest quintiles (OR=0.22, 95%CI 0.11-0.44, P<0.001) compared with the reference quintile of serum Mg, and the P for trend was <0.001.

The prevalence of HP in each quintile of serum Mg in OA patients was depicted in Figure 1 (C). The multivariable-adjusted relations between serum Mg and HP in OA patients were illustrated in Table 4. According to both the age-gender adjusted ORs (Model 1) and the multivariable adjusted ORs (Model 2), there was no significant association between serum Mg and HP, and the P for trend were 0.929 and 0.377, respectively. The sensitivity analysis, by adding eGFR into model 2, reached the same results.

The prevalence of HU in each category of serum Mg in OA patients was shown in Figure 1 (D). The multivariable-adjusted relations between serum Mg and HU in OA patients were illustrated in Table 5. Both the age-gender adjusted OR values (Model 1) and the multivariable adjusted OR values (Model 2) suggested significant decreased prevalence of HU in the third quintile (age-gender adjusted OR=0.44, 95%CI 0.26-0.75, P=0.002; multivariable adjusted OR=0.38, 95%CI 0.22-0.67, P=0.001) and fifth quintile (age-gender adjusted OR=0.51, 95%CI 0.30-0.85, P=0.010; multivariable adjusted OR=0.50, 95%CI 0.29-0.87, P=0.013) compared with the

lowest quintile of serum Mg, and the P for trend were 0.008 and 0.006, respectively. The sensitivity analysis, by adding eGFR into model 2, showed similar outcomes - significant lower prevalence of HU in the third (OR=0.33, 0.19-0.59, P<0.001), fourth (OR=0.52, 95%CI 0.30-0.91, P=0.022), and highest quintiles (OR=0.39, 95%CI 0.22-0.70, P=0.001) compared with the reference quintile of serum Mg, and the P for trend was <0.001.

Discussion

The results of this study suggested that the serum Mg concentration was negatively associated with the prevalence of MetS, DM and HU in subjects with radiographic knee OA. To control potential confounders, several covariates including characteristics, living habits and underlying diseases were selected, and even the eGFR was added into the multivariable logistic regression models to eliminate the influence of renal function on Mg excretion. The reverse associations mentioned above remained significant after adjustments of these confounders. However, the association between serum Mg and the prevalence of MetS was nonlinear, with no clear trend in the third and fourth quintiles of serum Mg. Moreover, the negative association between serum Mg and the prevalence of HP was not observed in radiographic knee OA patients.

Mg, the fourth most abundant cation in human body and the second most profuse intracellular cation, is a metallic cofactor for over 300 enzymatic reactions. It appears to play an important role in glucose metabolism and insulin homeostasis, which are both highly correlated with metabolic diseases, especially MetS and DM. The mechanisms involved in Mg deficiency in patients with MetS, DM and HU are probably multifactorial. The most important factor may be insulin resistance, as Mg is essential for insulin action and is a critical cofactor for several enzymes in carbohydrate metabolism, which is important for the phosphorylation reactions of tyrosine-kinase in the insulin receptor.[31 54-58] Of course, it is necessary to highlight the fact that insulin can also induce Mg excretion[59] and produce a significant decline of plasma Mg through ion exchange.[60] Thus, there seems to be a vicious circle between

Mg deficiency and insulin resistance.

Other potential mechanisms include glucose transportation,[57] oxidative stress[57] and inflammatory cytokines,[61-63] and cellular calcium homeostasis.[55] Mg is an essential cofactor of the high-energy phosphate-bound enzymatic pathways involved in the modulation of glucose transport across cell membranes.[57] It also plays a role in the mechanisms of cellular antioxidant defense.[64] The oxidative stress, defined as a persistent imbalance between the excessive production of reactive oxygen species and/or defects in antioxidant defense, has been implicated in the pathogenesis of diabetic complications.[57] Moreover, low serum Mg levels are strongly related to elevated serum concentrations of both tumor necrosis factor alpha and C-reactive protein (CRP),[65] suggesting that Mg deficiency may contribute to the development of low-grade chronic inflammation syndrome and the development of glucose metabolic disorders through the former pathway. In addition, lower Mg concentration can enhance calcium-mediated vasoconstriction, blunt cardiac and smooth muscle relaxation, and thus contribute to BP elevation.[55] However, the decreased serum calcium concentration in radiographic knee OA patients may weaken the association between Mg and HP.[66]

MetS[21 22] and DM[4 23 24] were reported to be the risk factors of OA progression. Moreover, serum Mg level has been proved to be significantly associated with the CRP concentration,[27 67-69] and higher CRP might serve as a prediction factor for OA progression.[70 71] Thus, OA progression may be delayed by elevating the serum Mg level through reducing the prevalence of MetS and DM and decreasing the level of CRP. Above all, the present study indicated that the elevation of serum Mg level has the potential to reduce the prevalence of MetS, DM and HU in knee OA patients and thereby may delay the progression of knee OA. However, the specific mechanism needs to be further explored.

The present study has several strengths. Firstly, this is the first study examining the associations between serum Mg and the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. The results of this study will provide a new insight into the treatment of knee OA. Secondly, the multivariable logistical regression

models were adjusted for a considerable number of potential confounding factors, which greatly improved the reliability of the results. Thirdly, the kidney is the key organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by adding eGFR into multivariable logistic regression models which showed that the reverse associations remained significant.

Limitations of the present study should also be admitted. The cross-sectional design precludes causal correlations, so further prospective studies and intervention trials should be undertaken to establish a causal association between serum Mg with the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. Since no previous research investigated such associations in knee OA patients, the value of this study should not be blotted out by the cross-sectional nature. Another limitation of this study lies in the relatively small sample size, and thus, extensive high-quality researches based on a larger sample are needed. Moreover, the dietary intake of Mg in relation to the prevalence of MetS, DM, HP and HU were not assessed in the present study. Last but not the least, it is important to highlight that Mg is an intracellular ion; therefore, the serum Mg concentration must be considered as a poor indicator of body Mg content,[72] even though it has been used in many studies. However, blood Mg level is the second best indicator of body status.[73]

Conclusions

The present study concluded that the serum Mg concentration was inversely associated with the prevalence of MetS, DM and HU in radiographic knee OA patients.

Contributors

All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. GHL, YLW and JW conceived the study. GHL, YLW and JW were responsible for conception and design of the study and drafted the manuscript. CZ, TY, HL, YC and DXX contributed to data collection. WJ contributed to preparation and data analysis. BX, ZCL, JTL, and SDJ contributed to study retrieval. GHL and YLW contributed to revision of the manuscript. All the authors contributed to the interpretation of the data and critically reviewed the manuscript for publication.

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Competing interests

The authors declare that they have no conflict of interest.

Ethics approval

The protocol of this study was reviewed and approved by the Ethics Committee at Xiangya Hospital.

Data sharing statement

The datasets during the current study available from the corresponding author on reasonable request.

REFERENCE

1 Zhuo Q, Yang W, Chen J, *et al*. Metabolic syndrome meets osteoarthritis. *Nat Rev Rheumatol* 2012;8(12):729-37.

2 Katz JD, Agrawal S, Velasquez M. Getting to the heart of the matter: osteoarthritis takes its place as part of the metabolic syndrome. *Curr Opin Rheumatol* 2010;22(5):512-9.

3 Berenbaum F. Diabetes-induced osteoarthritis: from a new paradigm to a new phenotype. *Ann Rheum Dis* 2011;70(8):1354-6.

4 King KB, Rosenthal AK. The adverse effects of diabetes on osteoarthritis: update on clinical evidence and molecular mechanisms. *Osteoarthritis Cartilage* 2015;23(6):841-50.

5 Kirkman MS. Osteoarthritis progression: is diabetes a culprit? *Osteoarthritis Cartilage* 2015;23(6):839-40.

6 Sellam J, Berenbaum F. Is osteoarthritis a metabolic disease? *Joint Bone Spine* 2013;80(6):568-73.

7 Puenpatom RA, Victor TW. Increased prevalence of metabolic syndrome in individuals with osteoarthritis: an analysis of NHANES III data. *Postgrad Med* 2009;121(6):9-20.

8 Shin D. Association between metabolic syndrome, radiographic knee osteoarthritis, and intensity of knee pain: results of a national survey. *J Clin Endocrinol Metab* 2014;99(9):3177-83.

9 Calvet J, Orellana C, Larrosa M, *et al*. High prevalence of cardiovascular co-morbidities in patients with symptomatic knee or hand osteoarthritis. *Scand J Rheumatol* 2015:1-4.

10 Rahman MM, Kopec JA, Cibere J, *et al*. The relationship between osteoarthritis and cardiovascular disease in a population health survey: a cross-sectional study. *BMJ Open* 2013;3(5):e2624.

11 Inoue R, Ishibashi Y, Tsuda E, *et al*. Medical problems and risk factors of metabolic syndrome among radiographic knee osteoarthritis patients in the

Japanese general population. *J Orthop Sci* 2011;16(6):704-9.

12 Hart DJ, Doyle DV, Spector TD. Association between metabolic factors and knee osteoarthritis in women: the Chingford Study. *J Rheumatol* 1995;22(6):1118-23.

13 Jungmann PM, Kraus MS, Alizai H, *et al*. Association of metabolic risk factors with cartilage degradation assessed by T2 relaxation time at the knee: data from the osteoarthritis initiative. *Arthritis Care Res (Hoboken)* 2013;65(12):1942-50.

14 Anagnostopoulos I, Zinzaras E, Alexiou I, *et al*. The prevalence of rheumatic diseases in central Greece: a population survey. *BMC Musculoskelet Disord* 2010;11:98.

15 Massengale M, Reichmann WM, Losina E, *et al*. The relationship between hand osteoarthritis and serum leptin concentration in participants of the Third National Health and Nutrition Examination Survey. *Arthritis Res Ther* 2012;14(3):R132.

16 Nieves-Plaza M, Castro-Santana LE, Font YM, *et al*. Association of hand or knee osteoarthritis with diabetes mellitus in a population of Hispanics from Puerto Rico. *J Clin Rheumatol* 2013;19(1):1-6.

17 Greiver M, Williamson T, Barber D, *et al*. Prevalence and epidemiology of diabetes in Canadian primary care practices: a report from the Canadian Primary Care Sentinel Surveillance Network. *Can J Diabetes* 2014;38(3):179-85.

18 Rahman MM, Cibere J, Anis AH, *et al*. Risk of Type 2 Diabetes among Osteoarthritis Patients in a Prospective Longitudinal Study. *Int J Rheumatol* 2014;2014:620920.

19 Reid JL, Morton DJ, Wingard DL, *et al*. Obesity and other cardiovascular disease risk factors and their association with osteoarthritis in Southern California American Indians, 2002-2006. *Ethn Dis* 2010;20(4):416-22.

20 Birtwhistle R, Morkem R, Peat G, *et al*. Prevalence and management of osteoarthritis in primary care: an epidemiologic cohort study from the Canadian Primary Care Sentinel Surveillance Network. *CMAJ Open* 2015;3(3):E270-5.

21 Yoshimura N, Muraki S, Oka H, *et al*. Accumulation of metabolic risk factors such as overweight, hypertension, dyslipidaemia, and impaired glucose tolerance raises the risk of occurrence and progression of knee osteoarthritis: a 3-year

follow-up of the ROAD study. *Osteoarthritis Cartilage* 2012;20(11):1217-26.

22 Monira HS, Wang Y, Cicuttini FM, *et al*. Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: a prospective cohort study. *Semin Arthritis Rheum* 2014;43(4):429-36.

23 Schett G, Kleyer A, Perricone C, *et al*. Diabetes is an independent predictor for severe osteoarthritis: results from a longitudinal cohort study. *Diabetes Care* 2013;36(2):403-9.

24 Eymard F, Parsons C, Edwards MH, *et al*. Diabetes is a risk factor for knee osteoarthritis progression. *Osteoarthritis Cartilage* 2015;23(6):851-9.

25 Guerrero-Romero F, Rodriguez-Moran M. Low serum magnesium levels and metabolic syndrome. *Acta Diabetol* 2002;39(4):209-13.

26 Guerrero-Romero F, Rodriguez-Moran M. Hypomagnesemia, oxidative stress, inflammation, and metabolic syndrome. *Diabetes Metab Res Rev* 2006;22(6):471-6.

27 Evangelopoulos AA, Vallianou NG, Panagiotakos DB, *et al*. An inverse relationship between cumulating components of the metabolic syndrome and serum magnesium levels. *Nutr Res* 2008;28(10):659-63.

28 Hjelmesaeth J, Hofso D, Aasheim ET, *et al*. Parathyroid hormone, but not vitamin D, is associated with the metabolic syndrome in morbidly obese women and men: a cross-sectional study. *Cardiovasc Diabetol* 2009;8:7.

29 Lima ML, Cruz T, Rodrigues LE, *et al*. Serum and intracellular magnesium deficiency in patients with metabolic syndrome--evidences for its relation to insulin resistance. *Diabetes Res Clin Pract* 2009;83(2):257-62.

30 Ma J, Folsom AR, Melnick SL, *et al*. Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin, and carotid arterial wall thickness: the ARIC study. Atherosclerosis Risk in Communities Study. *J Clin Epidemiol* 1995;48(7):927-40.

31 Kao WH, Folsom AR, Nieto FJ, *et al*. Serum and dietary magnesium and the risk for type 2 diabetes mellitus: the Atherosclerosis Risk in Communities Study.

Arch Intern Med 1999;159(18):2151-9.

32 Wang JL, Shaw NS, Yeh HY, *et al*. Magnesium status and association with diabetes in the Taiwanese elderly. *Asia Pac J Clin Nutr* 2005;14(3):263-9.

33 Chambers EC, Heshka S, Gallagher D, *et al*. Serum magnesium and type-2 diabetes in African Americans and Hispanics: a New York cohort. *J Am Coll Nutr* 2006;25(6):509-13.

34 Simmons D, Joshi S, Shaw J. Hypomagnesaemia is associated with diabetes: Not pre-diabetes, obesity or the metabolic syndrome. *Diabetes Res Clin Pract* 2010;87(2):261-6.

35 Sales CH, Pedrosa LF, Lima JG, *et al*. Influence of magnesium status and magnesium intake on the blood glucose control in patients with type 2 diabetes. *Clin Nutr* 2011;30(3):359-64.

36 Lecube A, Baena-Fustegueras JA, Fort JM, *et al*. Diabetes is the main factor accounting for hypomagnesemia in obese subjects. *PLoS One* 2012;7(1):e30599.

37 Xu J, Xu W, Yao H, *et al*. Associations of serum and urinary magnesium with the pre-diabetes, diabetes and diabetic complications in the Chinese Northeast population. *PLoS One* 2013;8(2):e56750.

38 Yang SJ, Hwang SY, Baik SH, *et al*. Serum magnesium level is associated with type 2 diabetes in women with a history of gestational diabetes mellitus: the Korea National Diabetes Program study. *J Korean Med Sci* 2014;29(1):84-9.

39 Singh RB, Rastogi V, Niaz MA, *et al*. Epidemiological study of magnesium status and risk of hypertension in a rural population of north India. *Magnes Res* 1996;9(3):173-81.

40 Peacock JM, Folsom AR, Arnett DK, *et al*. Relationship of serum and dietary magnesium to incident hypertension: the Atherosclerosis Risk in Communities (ARIC) Study. *Ann Epidemiol* 1999;9(3):159-65.

41 Guerrero-Romero F, Rodriguez-Moran M, Hernandez-Ronquillo G, *et al*. Low Serum Magnesium Levels and Its Association with High Blood Pressure in Children. *J Pediatr* 2016;168:93-8.

42 Zeng C, Wang YL, Wei J, *et al*. Association between low serum magnesium

concentration and hyperuricemia. *Magnes Res* 2015;28(2):56-63.

43 Zeng C, Wei J, Li H, *et al*. Relationship between Serum Magnesium Concentration and Radiographic Knee Osteoarthritis. *J Rheumatol* 2015;42(7):1231-6.

44 Wei J, Zeng C, Gong QY, *et al*. Associations between Dietary Antioxidant Intake and Metabolic Syndrome. *PLoS One* 2015;10(6):e130876.

45 Xie DX, Xiong YL, Zeng C, *et al*. Association between low dietary zinc and hyperuricaemia in middle-aged and older males in China: a cross-sectional study. *BMJ Open* 2015;5(10):e8637.

46 Wei J, Zeng C, Gong QY, *et al*. The association between dietary selenium intake and diabetes: a cross-sectional study among middle-aged and older adults. *Nutr J* 2015;14:18.

47 Expert Panel on Metabolic Syndrome of Chinese Diabetes Society: Recommendations on metabolic syndrome of Chinese Diabetes Society (Chinese). *Chin J Diabetes* 2004;14:156-61.

48 Pang C, Jia L, Hou X, *et al*. The significance of screening for microvascular diseases in Chinese community-based subjects with various metabolic abnormalities. *PLoS One* 2014;9(5):e97928.

49 Zhou H, Guo ZR, Yu LG, *et al*. Evidence on the applicability of the ATPIII, IDF and CDS metabolic syndrome diagnostic criteria to identify CVD and T2DM in the Chinese population from a 6.3-year cohort study in mid-eastern China. *Diabetes Res Clin Pract* 2010;90(3):319-25.

50 Levey AS, Stevens LA, Schmid CH, *et al*. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150(9):604-12.

51 Joosten MM, Gansevoort RT, Mukamal KJ, *et al*. Urinary magnesium excretion and risk of hypertension: the prevention of renal and vascular end-stage disease study. *Hypertension* 2013;61(6):1161-7.

52 Choi MK, Bae YJ. Association of Magnesium Intake with High Blood Pressure in Korean Adults: Korea National Health and Nutrition Examination Survey 2007-2009. *PLoS One* 2015;10(6):e130405.

53 R Development Core Team. R: A Language and Environment for Statistical Computing, Vienna, Austria: R Foundation for Statistical Computing. 2016.

54 Grundy SM, Cleeman JI, Daniels SR, *et al*. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112(17):2735-52.

55 Barbagallo M, Dominguez LJ, Galioto A, *et al*. Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. *Mol Aspects Med* 2003;24(1-3):39-52.

56 Song Y, Ridker PM, Manson JE, *et al*. Magnesium intake, C-reactive protein, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* 2005;28(6):1438-44.

57 Guerrero-Romero F, Rodriguez-Moran M. Complementary therapies for diabetes: the case for chromium, magnesium, and antioxidants. *Arch Med Res* 2005;36(3):250-7.

58 Huerta MG, Roemmich JN, Kington ML, *et al*. Magnesium deficiency is associated with insulin resistance in obese children. *Diabetes Care* 2005;28(5):1175-81.

59 Djurhuus MS, Skott P, Hother-Nielson O, *et al*. Insulin increases renal magnesium excretion: a possible cause of magnesium depletion in hyperinsulinaemic states. *Diabet Med* 1995;12(8):664-9.

60 Paolisso G, Sgambato S, Passariello N, *et al*. Insulin induces opposite changes in plasma and erythrocyte magnesium concentrations in normal man. *Diabetologia* 1986;29(9):644-7.

61 Bonora E, Targher G, Zenere MB, *et al*. Relationship of uric acid concentration to cardiovascular risk factors in young men. Role of obesity and central fat distribution. The Verona Young Men Atherosclerosis Risk Factors Study. *Int J Obes Relat Metab Disord* 1996;20(11):975-80.

62 Lyngdoh T, Marques-Vidal P, Paccaud F, *et al*. Elevated serum uric acid is associated with high circulating inflammatory cytokines in the population-based Colaus study. *PLoS One* 2011;6(5):e19901.

63 Kirilmaz B, Asgun F, Alioglu E, *et al*. High inflammatory activity related to the number of metabolic syndrome components. *J Clin Hypertens (Greenwich)* 2010;12(2):136-44.

64 Salmonowicz B, Krzystek-Korpacka M, Noczynska A. Trace elements, magnesium, and the efficacy of antioxidant systems in children with type 1 diabetes mellitus and in their siblings. *Adv Clin Exp Med* 2014;23(2):259-68.

65 Rodríguez-Morán M, Guerrero-Romero F. Elevated serum concentration of tumor necrosis factor-alpha is linked to low serum magnesium levels in the obesity-related inflammatory response. *Magnes Res* 2004;17:189-96.

66 Li H, Zeng C, Wei J, *et al*. Serum Calcium Concentration Is Inversely Associated With Radiographic Knee Osteoarthritis: A Cross-Sectional Study. *Medicine (Baltimore)* 2016;95(6):e2838.

67 Chacko SA, Song Y, Nathan L, *et al*. Relations of dietary magnesium intake to biomarkers of inflammation and endothelial dysfunction in an ethnically diverse cohort of postmenopausal women. *Diabetes Care* 2010;33(2):304-10.

68 Bo S, Durazzo M, Guidi S, *et al*. Dietary magnesium and fiber intakes and inflammatory and metabolic indicators in middle-aged subjects from a population-based cohort. *Am J Clin Nutr* 2006;84(5):1062-9.

69 Kim DJ, Xun P, Liu K, *et al*. Magnesium intake in relation to systemic inflammation, insulin resistance, and the incidence of diabetes. *Diabetes Care* 2010;33(12):2604-10.

70 Spector TD, Hart DJ, Nandra D, *et al*. Low-level increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. *Arthritis Rheum* 1997;40(4):723-7.

71 Smith JW, Martins TB, Gopez E, *et al*. Significance of C-reactive protein in osteoarthritis and total knee arthroplasty outcomes. *Ther Adv Musculoskelet Dis* 2012;4(5):315-25.

72 Topf JM, Murray PT. Hypomagnesemia and hypermagnesemia. *Rev Endocr Metab Disord* 2003;4(2):195-206.

73 Sabatier M, Pont F, Arnaud MJ, *et al*. A compartmental model of magnesium

metabolism in healthy men based on two stable isotope tracers. *Am J Physiol Regul Integr Comp Physiol* 2003;285(3):R656-63.

571 Table 1 Basic characteristics of included subjects according to quintiles of serum Mg (n=962)

	Quintiles of serum Mg					P
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
Age (years)	53.8 (7.3)	54.6 (7.6)	55.2 (7.9)	55.3 (7.1)	56.1 (8.0)	0.062
BMI (kg/m^2)	25.2 (3.2)	24.9 (3.2)	25.0 (3.7)	25.2 (3.4)	24.6 (3.2)	0.464
Female (%)	37.5	42.3	36.8	42.3	37.0	0.627
Smoking (%)	27.5	27.4	21.6	24.4	21.7	0.457
Alcohol drinking (%)	34.5	36.3	40.5	41.1	38.1	0.645
High school diploma (%)	45.0	47.4	45.3	56.5	48.1	0.184
Activity level (h/w)	2.0 (3.5)	2.0 (3.3)	2.3 (3.5)	2.1 (3.1)	2.4 (3.5)	0.457
Fasting glucose (mmol/l)	6.6 (3.0)	5.7 (1.7)	5.7 (1.4)	5.5 (0.9)	5.5 (1.6)	0.009
Systolic pressure (mm Hg)	129.2 (16.9)	128.3 (17.9)	130.4 (16.2)	128.8 (16.3)	129.6 (17.7)	0.837
Diastolic pressure (mm Hg)	81.2 (11.8)	79.8 (12.1)	80.7 (11.0)	80.7 (10.7)	80.3 (10.5)	0.654
HDL-cholesterol (mmol/l)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	1.5 (0.3)	1.5 (0.4)	0.374
Triglyceride (mmol/l)	2.1 (1.9)	1.8 (1.5)	2.0 (2.1)	1.8 (1.0)	2.3 (2.9)	0.620

Uric acid (μmol/l)	337.3 (101.7)	329.0 (80.7)	321.3 (86.3)	331.5 (78.0)	329.4 (81.7)	0.590
eGFR (ml/min/1.73m^2)	80.2 (14.4)	77.7 (10.7)	76.0 (10.6)	75.8 (10.7)	74.3 (12.0)	<0.001
MetS (%)	26.5	17.7	25.8	19.6	17.5	0.059
DM (%)	23.5	10.7	10.0	8.3	6.3	<0.001
HP (%)	40.0	33.5	37.4	42.3	40.2	0.432
HU (%)	25.5	19.1	13.2	18.5	14.8	0.018

572 Data are mean (Standard Deviation), unless otherwise indicated; Mg, magnesium; OA, osteoarthritis; BMI, body mass index; HDL, high density lipoprotein; eGFR,

573 estimated glomerular filtration rate; MetS, metabolic syndrome; DM, diabetes mellitus; HP, hypertension; HU, hyperuricemia.

574 # P values are for test of difference across all quintiles of serum Mg.

575

Figure 1 The prevalence of MetS (A), DM (B), HP (C) and HU (D) in each quintile of serum Mg in radiographic knee OA patients

The figures above present the prevalence of MetS (A), DM (B), HP (C) and HU (D) among the 962 OA patients under different quintiles of serum Mg levels. The horizontal axis denotes the serum Mg level, and the vertical axis indicates whether a subject is diagnosed with the specific disease: (+) - disease; (-) - no disease. The solid gray lines represent the boundaries in between the five quintiles of serum Mg levels. The red and black spots represent the prevalence of diseases and no diseases at each serum Mg level, respectively. The darker the color of a spot, the more OA patients there are at the corresponding concentration.

582 Table 2 Multivariable-adjusted relations of serum Mg and MetS in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
MetS (%)	26.5	17.7	25.8	19.6	17.5	-
Model 1*	1.00 (reference)	0.61 (0.38, 0.97)	0.97 (0.61, 1.52)	0.69 (0.42, 1.14)	0.59 (0.36, 0.96)	0.090
P value	-	0.038	0.881	0.150	0.035	-
Model 2*	1.00 (reference)	0.60 (0.37, 0.96)	1.00 (0.63, 1.57)	0.70 (0.42, 1.15)	0.61 (0.37, 0.99)	0.120
P value	-	0.035	0.99	0.160	0.047	-
Model 3*	1.00 (reference)	0.59 (0.36, 0.94)	0.95 (0.60, 1.51)	0.67 (0.40, 1.10)	0.56 (0.34, 0.93)	0.067
P value	-	0.027	0.830	0.114	0.024	

583 Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; MetS, metabolic syndrome.

584 *Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), gender (male, female), educational
585 level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no); Model 3 was
586 adjusted based on model 2, with additional factor of eGFR (continuous data).

587

Table 3 Multivariable-adjusted relations of serum Mg and DM in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
DM (%)	23.5	10.7	10.0	8.3	6.3	-
Model 1*	1.00 (reference)	0.38 (0.22, 0.66)	0.34 (0.19, 0.61)	0.29 (0.15, 0.55)	0.20 (0.10, 0.40)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-
Model 2*	1.00 (reference)	0.40 (0.23, 0.70)	0.32 (0.18, 0.59)	0.26 (0.13, 0.50)	0.21 (0.11, 0.42)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-
Model 3*	1.00 (reference)	0.40 (0.23, 0.70)	0.33 (0.18, 0.60)	0.27 (0.14, 0.52)	0.22 (0.11, 0.44)	<0.001
P value	-	0.001	<0.001	<0.001	<0.001	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; DM, diabetes mellitus.

*Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), hypertension (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

Table 4 Multivariable-adjusted relations of serum Mg and HP in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
HP (%)	40.0	33.5	37.4	42.3	40.2	-
Model 1*	1.00 (reference)	0.71 (0.47, 1.06)	0.83 (0.54, 1.25)	1.00 (0.66, 1.54)	0.89 (0.59, 1.35)	0.929
P value	-	0.095	0.368	0.987	0.582	-
Model 2*	1.00 (reference)	0.77 (0.50, 1.19)	0.89 (0.57, 1.39)	1.10 (0.70, 1.74)	1.08 (0.69, 1.68)	0.377
P value	-	0.245	0.608	0.686	0.744	-
Model 3*	1.00 (reference)	0.77 (0.50, 1.19)	0.88 (0.56, 1.38)	1.09 (0.68, 1.72)	1.05 (0.67, 1.65)	0.434
P value	-	0.235	0.574	0.727	0.818	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; HP, hypertension.

* Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

Table 5 Multivariable-adjusted relations of serum Mg and HU in OA patients (n = 962)

	Quintiles of serum Mg					
	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	*P* for trend
Median Mg concentration (mmol/L)	0.82	0.87	0.91	0.94	0.99	-
Participants (n)	200	215	190	168	189	-
HU (%)	25.5	19.1	13.2	18.5	14.8	-
Model 1*	1.00 (reference)	0.71 (0.44, 1.14)	0.44 (0.26, 0.75)	0.68 (0.41, 1.14)	0.51 (0.30, 0.85)	0.008
P value	-	0.157	0.002	0.144	0.010	-
Model 2*	1.00 (reference)	0.73 (0.45, 1.20)	0.38 (0.22, 0.67)	0.59 (0.35, 1.02)	0.50 (0.29, 0.87)	0.006
P value	-	0.210	0.001	0.058	0.013	-
Model 3*	1.00 (reference)	0.68 (0.41, 1.14)	0.33 (0.19, 0.59)	0.52 (0.30, 0.91)	0.39 (0.22, 0.70)	<0.001
P value	-	0.142	<0.001	0.022	0.001	-

Data are adjusted OR (95% CI), unless otherwise indicated; Mg, magnesium; n, number; OA, osteoarthritis; HU, hyperuricemia.

* Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (continuous data), gender (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), hypertension (yes, no), diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data)

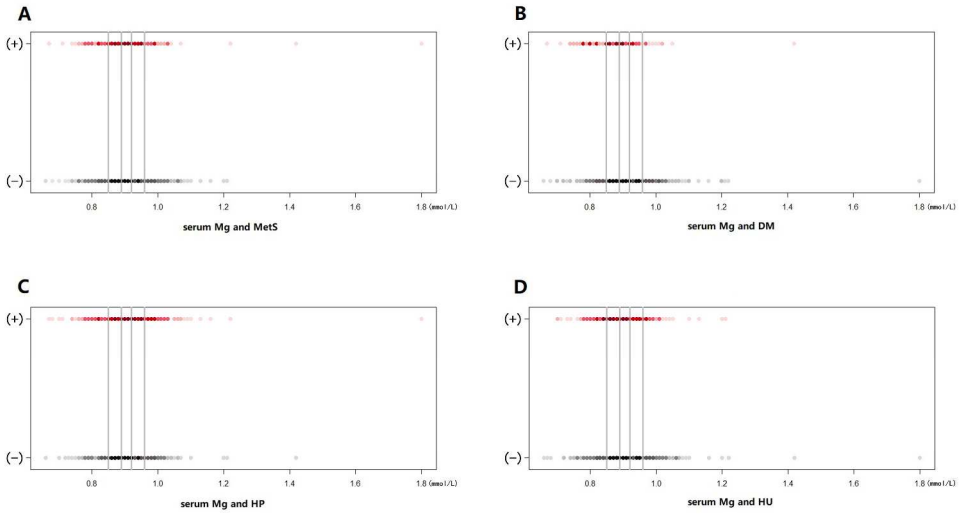

Figure 1 The prevalence of MetS (A), DM (B), HP (C) and HU (D) in each quintile of serum Mg in radiographic knee OA patients

The figures above present the prevalence of MetS (A), DM (B), HP (C) and HU (D) among the 962 OA patients under different quintiles of serum Mg levels. The horizontal axis denotes the serum Mg level, and the vertical axis indicates whether a subject is diagnosed with the specific disease: (+) - disease; (-) - no disease. The solid gray lines represent the boundaries in between the five quintiles of serum Mg levels. The red and black spots represent the prevalence of diseases and no diseases at each serum Mg level, respectively. The darker the color of a spot, the more OA patients there are at the corresponding concentration.

549x304mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Reported on Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	-
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	6-7
Study size	10	Explain how the study size was arrived at	4-5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6-7
		(*b*) Describe any methods used to examine subgroups and interactions	-
		(*c*) Explain how missing data were addressed	-
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	4-5
		(*e*) Describe any sensitivity analyses	6-7

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4-5
		(b) Give reasons for non-participation at each stage	4-5
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	-
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	-
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	-
		Cross-sectional study—Report numbers of outcome events or summary measures	8-10
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8-10
		(*b*) Report category boundaries when continuous variables were categorized	8-10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-10
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	10-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.