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

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Manuscripts

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3 **1 Association between Serum Magnesium Concentration with Metabolic**
4 **2 Syndrome, Diabetes, Hypertension and Hyperuricemia in Knee Osteoarthritis**
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23 Abstract

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

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

32 Results: A total of 962 radiographic knee OA patients were included. The
33 multivariable-adjusted OR (95% CI) showed a significant lower prevalence of MetS
34 in the second (OR=0.58, 0.36-0.94, P=0.026) and highest quintile (OR=0.56, 95CI%
35 0.34-0.93, P=0.024) compared with the reference quintile of serum Mg. Meanwhile, a
36 significant lower prevalence of DM was observed in the second (OR=0.38, 0.22-0.67,
37 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)
38 and highest quintile (OR=0.21, 95CI% 0.10-0.41, P<0.001). A significant lower
39 prevalence of HU was observed in the third (OR=0.36, 0.20-0.63, P<0.001), fourth
40 (OR=0.54, 0.31-0.93, P=0.026) and highest quintile (OR=0.39, 95CI% 0.22-0.68,
41 P=0.001). However, there was no significant association between serum Mg and HP
42 in OA patients.

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

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

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49 **Key words:** osteoarthritis, magnesium, metabolic syndrome, diabetes, hypertension,
50 hyperuricemia

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3 **69 Strengths and limitations of this study**
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6 70 1. This is the first study examining the associations between serum magnesium (Mg)
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8 71 and the prevalence of metabolic syndrome, diabetes mellitus, hypertension and
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10 72 hyperuricemia in radiographic knee osteoarthritis patients.
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12 73 2. The multivariable logistical regression models in this study were adjusted by a
13
14 74 considerable number of potential confounding factors, which greatly improved the
15
16 75 reliability of the results.
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18 76 3. Kidney is the key organ in maintaining Mg homeostasis. This study conducted a
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20 77 sensitivity analysis by adding estimated glomerular filtration rate into
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22 78 multivariable logistic regression models, and the reverse associations remained
23
24 79 significant.
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26 80 4. This study adopted cross-sectional design which precluded causal correlations.
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29 81 5. Serum Mg concentration was adopted as the indicator of body Mg content in this
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31 82 study which was not the best indicator of body status.
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92 Introduction

93 The association between metabolic diseases, especially metabolic syndrome (MetS)^{1 2}
94 and diabetes mellitus (DM),³⁻⁵ with osteoarthritis (OA) has drawn increasing attention
95 in the past few years, and OA has also been classified into three specific phenotypes
96 including metabolic OA, age-related OA and injure-related OA.⁶ A large number of
97 researches have indicated that the prevalence of MetS,⁷⁻⁹ DM¹⁰⁻¹⁸ and hypertension
98 (HP)^{7 9-13 19 20} are either higher in OA patients or associated with OA. In addition,
99 some other studies reported that MetS,^{21 22} DM^{23 24} and HP^{21 22} are the risk factors of
100 OA progression. Thus, it appears necessary to pay more attention to the high
101 prevalence of metabolic diseases in OA patients and even take measures to reduce
102 their prevalence, which also seems to be beneficial in delaying OA progression.

103 Serum magnesium (Mg), one of the most important micronutrients for human health,
104 has been reported to be negatively associated with MetS,²⁵⁻²⁹ DM³⁰⁻³⁸ and HP^{30 39-41} by
105 lots of studies. Furthermore, our previous study showed an inverse association
106 between serum Mg with hyperuricemia (HU).⁴² However, to our best knowledge,
107 there is not yet a study examined the association between the serum Mg concentration
108 with the aforementioned metabolic diseases (MetS, DM, HP and HU) in OA patients.
109 In addition, another study of ours indicated that the serum Mg concentration may be
110 inversely associated with radiographic knee OA.⁴³ Therefore, it is reasonably
111 speculated that the prevalence of MetS, DM, HP and HU in OA patients may be
112 reduced by elevating the level of serum Mg, which can in turn delay OA progression.
113 Thus, the objective of the present study was to examine the associations between the
114 serum Mg concentration with the prevalence of MetS, DM, HP and HU in
115 radiographic knee OA patients. It was hypothesized that serum Mg concentration was
116 inversely associated with these diseases.

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118 Methods

119 **Study population**

120 The present study was conducted at the Health Management Center of Xiangya
121 Hospital between October 2013 and November 2014. The study design has been
122 published previously.⁴²⁻⁴⁶ The protocol of this study was reviewed and approved by
123 the local Ethics and Research Committee, and the methods were carried out in
124 “accordance” with the approved guidelines. Also the study population gave informed
125 consent. Registered nurses interviewed all participants during the examination using a
126 standard questionnaire, with the purpose to collect information on demographic
127 characteristics and health-related habits. Participants were selected according to the
128 following inclusion criteria: 1) 40 years old or above; 2) undergoing weight-bearing
129 bilateral anteroposterior radiography of the knee, and diagnosed with knee OA
130 according to Kellgren-Lawrence (K-L) radiographic atlas (knee joint was graded K-L
131 2 or above); 3) availability of all basic characteristics, including age, gender, body
132 mass index (BMI) and blood pressure; 4) availability of biochemical test results,
133 including serum Mg concentration; 5) availability of information related to the living
134 habits, including education background, activity level, smoking, drinking and
135 medication status. Initially, this cross-sectional study included 1820 radiographic knee
136 OA patients aged over than 40 years with sound basic characteristics and needed
137 blood biochemical assessment (including serum Mg concentration). Among them, 962
138 patients offered demographic characteristics and health-related habits and they were
139 finally included in this study.

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141 **Blood biochemistry**

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

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3 146 uric acid and 0.60 mmol/L for serum Mg) and high concentrations (6.7 mmol/L for
4 147 glucose, 472 μ mol/L for uric acid and 1.00 mmol/L for serum Mg) of standard human
5 148 samples. The intra-assay coefficients of variation were 0.98% (2.5 mmol/L) and 1.72%
6 149 (6.7 mmol/L) for glucose, 1.39% (118 μ mol/L) and 0.41% (472 μ mol/L) for uric acid,
7 150 and 1.86% (0.60 mmol/L) and 1.65% (1.00 mmol/L) for serum Mg. The inter-assay
8 151 coefficients of variation were 2.45% (2.5 mmol/L) and 1.46% (6.7 mmol/L) for
9 152 glucose, 1.40% (118 μ mol/L) and 1.23% (472 μ mol/L) for uric acid, and 1.87% (0.60
10 153 mmol/L) and 1.70% (1.00 mmol/L) for serum Mg.
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21 155 **Assessment of other exposures**

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24 156 Blood pressure was measured by an electronic sphygmomanometer. The weight and
25 157 height of each subjects was measured respectively to calculate the BMI. Participants
26 158 were asked about their average frequency of physical activity (never, one to two times
27 159 per week, three to four times per week, five times and above per week) and average
28 160 duration of physical activity (within half an hour, half an hour to one hour, one to two
29 161 hours, more than two hours). The smoking, alcohol drinking and medication status
30 162 were asked face to face.
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40 164 **Assessment of MetS, DM, HP and HU**

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42 165 MetS was diagnosed according to the Chinese Diabetes Society (CDS) criteria.⁴⁷⁻⁴⁹
43 166 CDS criteria for metabolic syndrome requires 3 items or all the four items: (1) BMI
44 167 ≥ 25 kg/m²; (2) Fasting plasma glucose (FPG) ≥ 6.1 mmol/L, or diagnosed DM; (3)
45 168 Systolic blood pressure (BP) ≥ 140 mmHg or diastolic BP ≥ 90 mmHg, or treatment of
46 169 previously diagnosed HP; (4) Triglycerides ≥ 1.7 mmol/L and/or HDL-cholesterol
47 170 < 0.9 mmol/L in male or < 1.0 mmol/L in female, or treatment for this lipid
48 171 abnormality. Subjects with the fasting glucose ≥ 7.0 mmol/L or currently undergoing
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3 172 drug treatment for blood glucose control were regarded as DM patients, and subjects
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5 173 with the systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg
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7 174 or currently using antihypertensive medication were regarded as HP patients. HU was
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9 175 defined as uric acid ≥ 416 $\mu\text{mol/L}$ for male and ≥ 360 $\mu\text{mol/L}$ for female or currently
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11 176 undergoing drug treatment for uric acid control.
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14 15 16 178 **Statistical analysis**

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18 179 The continuous data are expressed as mean (standard deviation), and the category data
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20 180 are expressed in percentage. Differences in continuous data were evaluated by
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22 181 one-way classification ANOVA (normally distributed data) or Kruskal-Wallis H test
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24 182 (non-normally distributed data), while differences in category data were assessed by
25
26 183 the χ^2 test. The serum Mg was classified into five categories based on the quintile
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28 184 distribution: ≤ 0.85 , 0.86-0.89, 0.90-0.92, 0.93-0.96 and ≥ 0.97 mmol/L. Logistic
29
30 185 regression was conducted in two models in order to calculate the adjusted ORs with
31
32 186 95% CIs for the associations of serum Mg with MetS, DM, HP and HU. Three models
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34 187 were adjusted for the association. Model 1 were adjusted for age and sex. Then,
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36 188 model 2, a multivariable model was adopted. Covariates were chosen based on
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38 189 previous similar studies.^{27 33 50 51} Model 2 for the association between serum Mg and
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40 190 MetS was adjusted by age (continuous data), gender (male, female), educational level
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42 191 (high school or above, lower than high school), smoking status (yes, no), activity level
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44 192 (continuous data) and alcohol drinking status (yes, no). Model 2 for the association
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46 193 between serum Mg and diabetes was adjusted by age (continuous data), BMI (≥ 25
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48 194 kg/m², < 25 kg/m²), gender (male, female), educational level (high school or above,
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50 195 lower than high school), smoking status (yes, no), activity level (continuous data),
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52 196 alcohol drinking status (yes, no), HP (yes, no), and dyslipidemia (yes, no).
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54 197 Dyslipidemia was defined by triglycerides ≥ 1.7 mmol/L and/or HDL-cholesterol < 0.9
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56 198 mmol/L in male or < 1.0 mmol/L in female, or treatment for this lipid abnormality.
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3 199 Model 2 for the association between serum Mg and hypertension was adjusted by age
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5 200 (continuous data), BMI (≥ 25 kg/m², < 25 kg/m²), gender (male, female), educational
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7 201 level (high school or above, lower than high school), smoking status (yes, no), activity
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9 202 level (continuous data), alcohol drinking status (yes, no), diabetes (yes, no), and
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11 203 dyslipidemia (yes, no). Model 2 for the association between serum Mg and HU was
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13 204 adjusted by age (continuous data), BMI (≥ 25 kg/m², < 25 kg/m²), gender (male,
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15 205 female), educational level (high school or above, lower than high school), smoking
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17 206 status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), HP
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19 207 (yes, no), DM (yes, no) and dyslipidemia (yes, no). Model 3 for all associations were
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21 208 adjusted based on model 2, with additional factor of estimated glomerular filtration
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23 209 rate (eGFR). eGFR was calculated by serum creatinine (Scr), sex, and patients' age.
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25 210 The calculation formula was: $186 \times \text{Scr}^{-1.154} \times \text{age}^{-0.203} \times 1.210$ (if black) $\times 0.742$
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27 211 (if female).⁵² Tests for linear trends were conducted based on logistic regression using
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29 212 a median variable of Mg concentration in each category. All data analyses were
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31 213 performed using SPSS 17.0; $P \leq 0.05$ was considered to be statistically significant. All
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33 214 test were two tailed.

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36 **Results**

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38 217 A total of 962 subjects were included in the present cross-sectional study. The
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40 218 characteristics of the study population according to quintiles of serum Mg were
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42 219 illustrated in Table 1. The mean age of the subjects was 54.9 ± 7.6 years old, and there
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44 220 were 377 females (39.2%). The overall prevalence of MetS, DM, HP and HU in OA
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46 221 patients were 21.4%, 12.0%, 38.5% and 18.3% respectively. Significant differences
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48 222 were observed across quintiles of serum Mg for fasting glucose, the prevalence of DM
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50 223 and HU.

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52 224 Outcomes of multivariable adjusted associations between MetS and serum Mg
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54 225 concentration were shown in Table 2. The age-sex adjusted OR values (Model 1)

226 suggested a significant lower prevalence of MetS in the second (OR=0.61, 95CI%
227 0.38-0.97, P=0.038) and highest quintile (OR=0.59, 95CI% 0.36-0.96, P=0.035)
228 compared with the reference quintile of serum Mg in OA patients, and the P for trend
229 was 0.090. The multivariable adjusted OR values (Model 2) showed similar outcomes
230 (OR=0.60, 95CI% 0.37-0.96, P=0.035 in the second quintile; OR=0.61, 95CI%
231 0.37-0.99, P=0.047 in the fifth quintile), and the P for trend was 0.120. The sensitivity
232 analysis, by adding eGFR into model 2, also reached similar outcomes - a significant
233 lower prevalence of MetS in the second (OR=0.58, 0.36-0.94, P=0.026) and highest
234 quintile (OR=0.56, 95CI% 0.34-0.93, P=0.024) compared with the reference quintile
235 of serum Mg, and the P for trend was 0.066.

236 Table 3 indicated the multivariable adjusted relations of serum Mg and DM in OA
237 patients. Both age-sex adjusted OR values (Model 1) and multivariable adjusted OR
238 values (Model 2) suggested a strong inverse association between serum Mg and
239 diabetes. The age-sex adjusted ORs for the prevalence of diabetes were 0.38 (95CI%
240 0.22-0.66, P=0.001), 0.34 (95CI% 0.19-0.61, P<0.001), 0.29 (95CI% 0.15-0.55,
241 P<0.001), and 0.20 (95CI% 0.10-0.40, P<0.001) in the second, third, fourth and fifth
242 quintiles of serum Mg respectively, and the P for trend was smaller than 0.0001. The
243 multivariable adjusted ORs for the prevalence of diabetes were 0.38 (95CI%
244 0.22-0.66, P=0.001), 0.34 (95CI% 0.19-0.62, P<0.001), 0.27 (95CI% 0.14-0.52,
245 P<0.001), and 0.20 (95CI% 0.10-0.40, P<0.001) in the second, third, fourth and fifth
246 quintiles of serum Mg respectively, and the P for trend was smaller than 0.0001. The
247 sensitivity analysis, by adding eGFR into model 2, showed similar outcomes - a
248 significant lower prevalence of DM in the second (OR=0.38, 0.22-0.67, P=0.001),
249 third (OR=0.35, 0.19-0.64, P=0.001), fourth (OR=0.27, 0.14-0.53, P<0.001), and
250 highest quintile (OR=0.21, 95CI% 0.10-0.41, P<0.001) compared with the reference
251 quintile of serum Mg, and the P for trend was <0.001.

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

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

257 The multivariable-adjusted relations of serum Mg and HU in OA patients were
258 illustrated in Table 5. Both the age-sex adjusted OR values (Model 1) and the
259 multivariable adjusted OR values (Model 2) suggested significant decreased
260 prevalence of HU in the third quintile (age-sex adjusted OR=0.44, 95CI% 0.26-0.75,
261 P=0.002; multivariable adjusted OR=0.42, 95CI% 0.24-0.73, P=0.002) and fifth
262 quintile (age-sex adjusted OR=0.51, 95CI% 0.30-0.85, P=0.010; multivariable
263 adjusted OR=0.50, 95CI% 0.29-0.86, P=0.012) compared with the lowest quintile of
264 serum Mg, and the P for trend was 0.008 and 0.007, respectively. The sensitivity
265 analysis, by adding eGFR into model 2, showed similar outcomes - a significant lower
266 prevalence of HU in the third (OR=0.36, 0.20-0.63, P<0.001), fourth (OR=0.54,
267 0.31-0.93, P=0.026), and highest quintile (OR=0.39, 95CI% 0.22-0.68, P=0.001)
268 compared with the reference quintile of serum Mg, and the P for trend was <0.001.

269

270 Discussion

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

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3 280 Mg, the fourth most abundant cation in human body and the second most profuse
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5 281 intracellular cation, is a metallic cofactor for over 300 enzymatic reactions. It appears
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7 282 to play an important role in glucose metabolism and insulin homeostasis, which are
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9 283 highly correlated with metabolic diseases, especially MetS and DM. The mechanisms
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11 284 involved in the Mg deficiency with MetS, DM and HU are probably multifactorial.
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13 285 The most important one may be insulin resistance, as Mg is essential for insulin action
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15 286 and is a critical cofactor for several enzymes in carbohydrate metabolism, which is
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17 287 important for phosphorylation reactions of tyrosine-kinase in the insulin receptor.³¹
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19 288⁵³⁻⁵⁷ Incidentally, our previous prospective study involving 62897 person-years of
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21 289 follow-up showed that hematocrit was independently associated with the incidence of
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23 290 HU through, with a high possibility, the insulin resistance mechanism.⁵⁸ Other
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25 291 potential mechanisms included cellular calcium homeostasis,⁵⁴ glucose
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27 292 transportation,⁵⁶ oxidative stress⁵⁶ and inflammatory cytokines.⁵⁹⁻⁶¹ Of course, it is
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29 293 necessary to highlight the fact that insulin can also induce Mg excretion⁶² and produce
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31 294 a significant decline of plasma Mg through ion exchange.⁶³ Thus, there seems to be a
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33 295 vicious circle between Mg deficiency and insulin resistance.

33 296 MetS^{21 22} and DM^{4 23 24} were reported to be the risk factors of OA progression. It
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35 297 seems that OA progression may be delayed by elevating the serum Mg level through
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37 298 decreasing the prevalence of MetS and DM. Some other studies proved that the serum
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39 299 Mg level was significantly associated with the high-sensitive C-reactive protein (CRP)
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41 300 concentration,^{27 64-66} and higher CRP might serve as a prediction factor for OA
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43 301 progression.^{67 68} Thus, OA progression may also be delayed by elevating the serum
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45 302 Mg level through decreasing the level of CRP. Above all, the present study indicated
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47 303 that elevating serum Mg level has the potential to reduce the prevalence of MetS, DM
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49 304 and HU in knee OA patients and may delay the progression of knee OA (Figure 1).
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51 305 However, the specific mechanism needs to be further explored.

52 306 The present study has several strengths. Firstly, this is the first study examining the
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54 307 associations between serum Mg and the prevalence of MetS, DM, HP and HU in

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3 308 radiographic knee OA patients. The results of this study will provide a new insight
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5 309 into the treatment of knee OA. Secondly, the multivariable logistical regression
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7 310 models were adjusted by a considerable number of potential confounding factors,
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9 311 which greatly improved the reliability of the results. Thirdly, kidney is the key organ
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11 312 in maintaining Mg homeostasis. This study conducted a sensitivity analysis by adding
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13 313 eGFR into multivariable logistic regression models, and the reverse associations
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15 314 remained significant.

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17 315 Limitations of the present study should also be admitted. The cross-sectional design
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19 316 precludes causal correlations, so further prospective studies and intervention trials
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21 317 should be undertaken to establish a causal association between serum Mg with the
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23 318 prevalence of MetS, DM, HP and HU in radiographic knee OA patients. Since no
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25 319 previous research investigated such associations in knee OA patients, the value of this
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27 320 study should not be blotted out by the cross-sectional nature. Another limitation of
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29 321 this study lies in the relatively small sample size, and thus, extensive high-quality
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31 322 researches based on a larger sample are needed. Last but not the least, it is important
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33 323 to highlight that Mg is an intracellular ion; therefore, the serum Mg concentration
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35 324 must be considered as a poor indicator of body magnesium content,⁶⁹ even though this
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37 325 parameter has been used in many studies. However, blood magnesium level is the
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39 326 second best indicator of body status.⁷⁰

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41 42 328 **Conclusions**

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45 329 The present study indicated that the serum Mg concentration was inversely associated
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47 330 with the prevalence of MetS, DM and HU in radiographic knee OA patients. Thus,
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49 331 elevating serum Mg level is more likely to be associated with the decreasing
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51 332 prevalence of MetS, DM and HU among subjects with knee OA.

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334 **Contributors**

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

343

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353

354 **Competing interests**

355 The authors declare that they have no conflict of interest.

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357 **Ethics approval**

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3 358 The protocol of this study was reviewed and approved by the Ethics Committee at
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5 359 Xiangya Hospital.

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10 361 **Data sharing statement**

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13 362 The datasets during the current study available from the corresponding author on
14
15 363 reasonable request.

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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

7	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
9	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
11	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
14	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
17	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
20	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
22	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
25	MetS (%)	26.5	17.7	25.8	19.6	17.5	0.059
28	DM (%)	23.5	10.7	10.0	8.3	6.3	<0.001
30	HP (%)	40.0	33.5	37.4	42.3	40.2	0.432
33	HU (%)	25.5	19.1	13.2	18.5	14.8	0.018

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

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

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.026	0.818	0.109	0.024	-

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

381 *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
382 (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
383 based on model 2, with additional factor of eGFR (continuous data).

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.001	0.001	<0.001	<0.001	-

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

395 *Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (≥ 25 kg/m², < 25 kg/m²), gender
396 (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status
397 (yes, no), hypertension (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.218	0.629	0.577	0.978	-

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

409 * Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (≥ 25 kg/m², < 25 kg/m²), gender
410 (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status
411 (yes, no), diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.119	<0.001	0.026	0.001	-

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

423 * Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (≥ 25 kg/m², < 25 kg/m²), gender
424 (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status
425 (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
426 data).

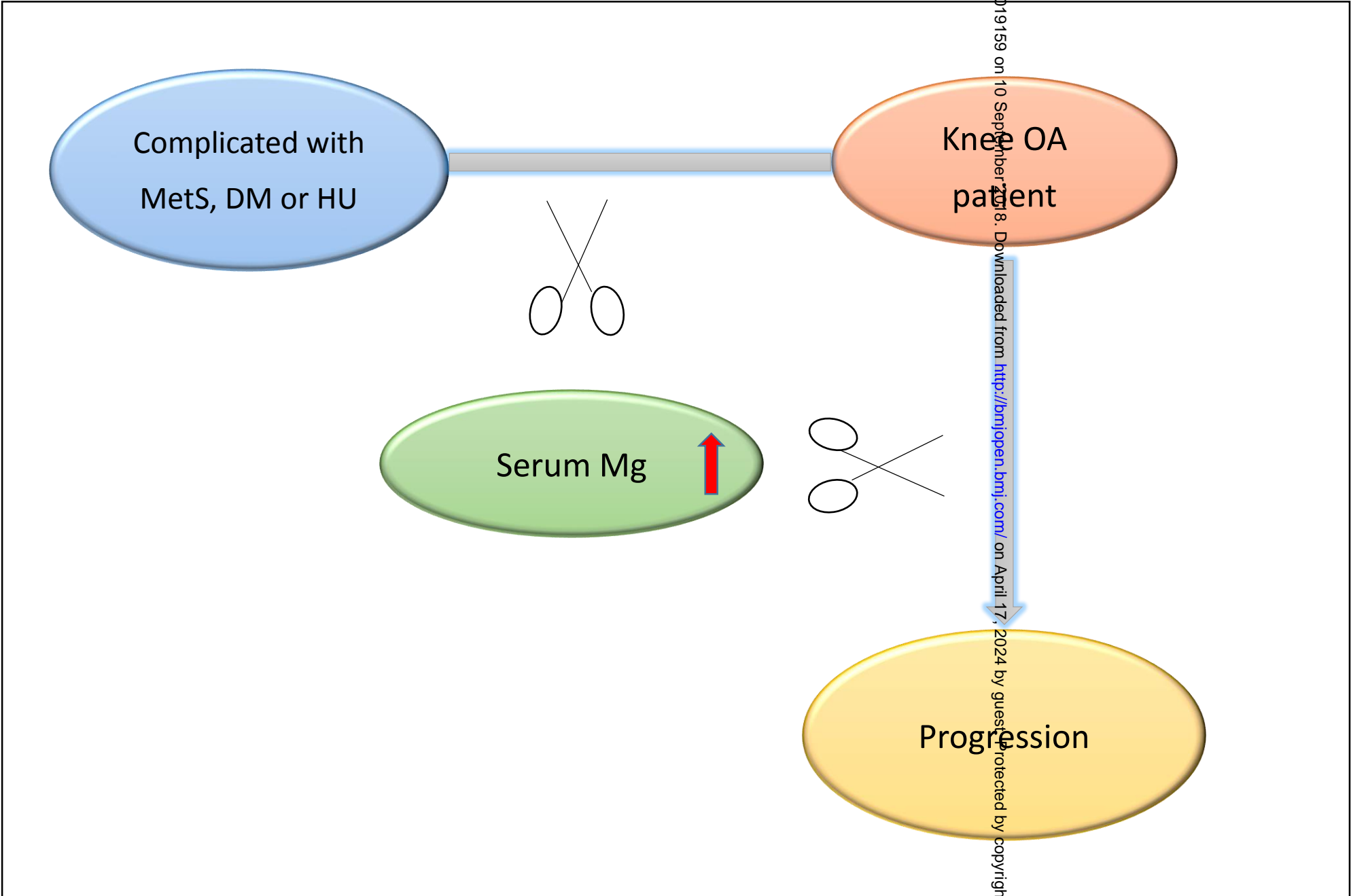
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For peer review only

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17 439 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
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19 440 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
20
21 441 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
22
23 442 and delay the progression of OA. MetS, metabolic syndrome; DM, diabetes mellitus; HU, hyperuricemia; OA, osteoarthritis; Mg, magnesium.

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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	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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	-
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	-
		<i>Cross-sectional study</i> —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.

BMJ Open

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

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Primary Subject Heading:	Rheumatology
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Keywords:	osteoarthritis, magnesium, metabolic syndrome, diabetes, Hypertension < CARDIOLOGY, hyperuricemia

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Manuscripts

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3 **1 Association between Serum Magnesium Concentration with Metabolic**
4 **2 Syndrome, Diabetes, Hypertension and Hyperuricemia in Knee Osteoarthritis**
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9 4 Yi-lun Wang¹, Jie Wei², Chao Zeng¹, Tuo Yang¹, Hui Li¹, Yang Cui³, Dong-xing Xie¹,
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20 **Abstract**

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

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

32 **Results:** A total of 962 radiographic knee OA patients were included. Compared with
33 the lowest quintile, the multivariable-adjusted odds ratios (ORs) and related 95%
34 confidence intervals (95%CI) of DM were 0.38 (95%CI 0.22-0.67, P=0.001), 0.35
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
36 0.10-0.41, P<0.001) in the second, third, fourth and highest quintiles of serum Mg,
37 respectively (P for trend <0.001); the multivariable-adjusted ORs of HU were 0.36
38 (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
39 0.22-0.68, P=0.001) in the third, fourth and highest quintiles of serum Mg
40 respectively (P for trend <0.001); and the multivariable-adjusted ORs of MetS were
41 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)
42 in the highest quintiles of serum Mg (P for trend =0.066). There was no significant
43 association between serum Mg and HP in OA patients.

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

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

47 **Key words:** osteoarthritis, magnesium, metabolic syndrome, diabetes, hypertension,
48 hyperuricemia

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50 **Strengths and limitations of this study**

- 51 1. This is the first study examining the associations between serum magnesium (Mg)
52 and the prevalence of metabolic syndrome, diabetes mellitus, hypertension and
53 hyperuricemia in radiographic knee osteoarthritis patients.
- 54 2. The multivariable logistical regression models in this study were adjusted for a
55 considerable number of potential confounding factors, which greatly improved the
56 reliability of the results.
- 57 3. The kidney is the key organ in maintaining Mg homeostasis. This study conducted
58 a sensitivity analysis by adding estimated glomerular filtration rate into the
59 multivariable logistic regression models, and the reverse associations remained
60 significant.
- 61 4. This study adopted cross-sectional design which precluded causal correlations.
- 62 5. Serum Mg concentration was adopted as the indicator of body Mg content in this
63 study which may not be the best indicator of body status.
64

65 Introduction

66 The association between osteoarthritis (OA) and metabolic diseases, especially
67 metabolic syndrome (MetS)^{1 2} and diabetes mellitus (DM),³⁻⁵ has drawn increasing
68 attention in the past few years. OA includes three specific phenotypes: metabolic OA,
69 age-related OA and injury-related OA.⁶ A large number of studies have indicated that
70 the prevalence of MetS,⁷⁻⁹ DM¹⁰⁻¹⁸ and hypertension (HP)^{7 9-13 19 20} is either higher in
71 OA patients or associated with OA. In addition, some other studies reported that
72 MetS,^{21 22} DM^{23 24} and HP^{21 22} are risk factors of OA progression. Thus, it appears
73 necessary to pay more attention and adopt appropriate measures to reduce the high
74 prevalence of metabolic diseases in OA patients, which also seems to be beneficial in
75 delaying OA progression.

76 Serum magnesium (Mg), one of the most important micronutrients for human
77 health, has been reported to be negatively associated with MetS,²⁵⁻²⁹ DM³⁰⁻³⁸ and HP³⁰
78 ³⁹⁻⁴¹ by lots of studies. Meanwhile, our previous study showed an inverse association
79 between serum Mg and hyperuricemia (HU).⁴² However, to the best knowledge of the
80 authors, there is not yet a study examining the association between the serum Mg
81 concentration and the aforementioned metabolic diseases (MetS, DM, HP and HU) in
82 OA patients. On the other hand, we have previously shown that the serum Mg
83 concentration may be inversely associated with radiographic knee OA.⁴³ Therefore,
84 we speculate that the prevalence of MetS, DM, HP and HU in OA patients may be
85 reduced by elevating the level of serum Mg, which can in turn delay OA progression.
86 Thus, the objective of the present study was to examine the associations between the
87 serum Mg concentration with the prevalence of MetS, DM, HP and HU in
88 radiographic knee OA patients. It was hypothesized that serum Mg concentration was
89 inversely associated with these diseases.

91 Methods

92 Study population

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

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3 95 published previously.⁴²⁻⁴⁶ The protocol has been reviewed and approved by the Ethics
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5 96 Committee of Xiangya Hospital, Central South University (reference numbers:
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7 97 201312459), and the methods were developed in “accordance” with the approved
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9 98 guidelines. Informed consent has been obtained from all participants. Registered
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11 99 nurses were engaged to interview all participants during the examination using a
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13 100 standard questionnaire, with the purpose to collect information on demographic
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15 101 characteristics and health-related habits. Participants were selected based on the
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17 102 following inclusion criteria: 1) 40 years old or above; 2) undergoing weight-bearing
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19 103 bilateral anteroposterior radiography of the knee, and diagnosed with knee OA
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21 104 according to the Kellgren-Lawrence (K-L) radiographic atlas (knee joint was graded
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23 105 K-L 2 or above); 3) availability of all basic characteristics, including age, gender,
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25 106 body mass index (BMI) and blood pressure; 4) availability of biochemical test results,
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27 107 including serum Mg concentration; 5) availability of information related to the living
28
29 108 habits, including education background, activity level, smoking, drinking and
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31 109 medication status. Initially, the present cross-sectional study retrieved 1820
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33 110 radiographic knee OA patients aged over 40 years who exhibited sound basic
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35 111 characteristics and required blood biochemical assessment (including serum Mg
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37 112 concentration). Among them, 962 patients offered demographic characteristics and
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39 113 health-related habits and were finally included in this study.

114

115 **Blood biochemistry**

116 All blood samples were drawn after a 12-hour overnight fast and were kept at 4°C
117 until analysis. Blood tests were undertaken using the Beckman Coulter AU 5800
118 (Beckman Coulter Inc., Brea, CA, USA). The inter- and intra-assay coefficients of
119 variation were tested at both low concentrations (2.5 mmol/L for glucose, 118 µmol/L
120 for uric acid and 0.60 mmol/L for serum Mg) and high concentrations (6.7 mmol/L for
121 glucose, 472 µmol/L for uric acid and 1.00 mmol/L for serum Mg) of standard human
122 samples. The intra-assay coefficients of variation were 0.98% (2.5 mmol/L) and 1.72%
123 (6.7 mmol/L) for glucose, 1.39% (118 µmol/L) and 0.41% (472 µmol/L) for uric acid,
124 and 1.86% (0.60 mmol/L) and 1.65% (1.00 mmol/L) for serum Mg respectively. The

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3 125 inter-assay coefficients of variation were 2.45% (2.5 mmol/L) and 1.46% (6.7 mmol/L)
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5 126 for glucose, 1.40% (118 $\mu\text{mol/L}$) and 1.23% (472 $\mu\text{mol/L}$) for uric acid, and 1.87%
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7 127 (0.60 mmol/L) and 1.70% (1.00 mmol/L) for serum Mg respectively.
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10 129 **Assessment of other exposures**

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12 130 Blood pressure was measured by an electronic sphygmomanometer. The weight and
13
14 131 height of each subjects was measured respectively to calculate the BMI. Information
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16 132 on the average frequency of physical activity (never, one to two times per week, three
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18 133 to four times per week, five times and above per week) and average duration of
19
20 134 physical activity (less than half an hour, half an hour to one hour, one to two hours,
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22 135 more than two hours) was collected through survey questionnaire. The smoking,
23
24 136 alcohol drinking and medication status were collected during the face-to-face
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26 137 interview.
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29 139 **Assessment of MetS, DM, HP and HU**

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31 140 MetS was diagnosed based on the Chinese Diabetes Society (CDS) criteria,⁴⁷⁻⁴⁹ which
32
33 141 requires meeting at least 3 of the following 4 items: (1) BMI ≥ 25 kg/m²; (2) Fasting
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35 142 plasma glucose (FPG) ≥ 6.1 mmol/L, or diagnosed DM; (3) Systolic blood pressure
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37 143 (BP) ≥ 140 mmHg or diastolic BP ≥ 90 mmHg, or treatment of previously diagnosed
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39 144 HP; (4) Triglycerides ≥ 1.7 mmol/L and/or HDL-cholesterol < 0.9 mmol/L in male or
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41 145 < 1.0 mmol/L in female, or treatment for this lipid abnormality. Subjects with the
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43 146 fasting glucose ≥ 7.0 mmol/L or currently undergoing drug treatment for blood glucose
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45 147 control were regarded as DM patients, and subjects with the systolic blood pressure
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47 148 ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or currently undertaking
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49 149 antihypertensive medication were regarded as HP patients. HU was defined as uric
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51 150 acid ≥ 416 $\mu\text{mol/L}$ for male and ≥ 360 $\mu\text{mol/L}$ for female or currently undergoing drug
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53 151 treatment for uric acid control.
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54 153 **Statistical analysis**

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56 154 The continuous data are expressed as mean (standard deviation), and the category data

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3 155 are expressed in percentage. Differences in continuous data were evaluated by
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5 156 one-way classification ANOVA (normally distributed data) or Kruskal-Wallis H test
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7 157 (non-normally distributed data), while differences in category data were assessed by
8
9 158 the χ^2 test. The serum Mg was classified into five categories based on the quintile
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11 159 distribution: ≤ 0.85 , 0.86-0.89, 0.90-0.92, 0.93-0.96 and ≥ 0.97 mmol/L. Logistic
12
13 160 regression was conducted in two models in order to calculate the adjusted odds ratios
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15 161 (ORs) with 95% confidence intervals (95%CI) for the associations of serum Mg with
16
17 162 MetS, DM, HP and HU. Three models were adjusted for the association. Model 1
18
19 163 were adjusted for age and sex. Then, model 2, a multivariable model was adopted.
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21 164 Covariates were chosen based on previous similar studies.^{27 33 50 51} Model 2 for the
22
23 165 association between serum Mg and MetS was adjusted for age (continuous data),
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25 166 gender (male, female), educational level (high school or above, lower than high
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27 167 school), smoking status (yes, no), activity level (continuous data) and alcohol drinking
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29 168 status (yes, no). Model 2 for the association between serum Mg and DM was adjusted
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31 169 for age (continuous data), BMI (≥ 25 kg/m², < 25 kg/m²), gender (male, female),
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33 170 educational level (high school or above, lower than high school), smoking status (yes,
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35 171 no), activity level (continuous data), alcohol drinking status (yes, no), HP (yes, no),
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37 172 and dyslipidemia (yes, no). Dyslipidemia was defined by triglycerides ≥ 1.7 mmol/L
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39 173 and/or HDL-cholesterol < 0.9 mmol/L in male or < 1.0 mmol/L in female, or treatment
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41 174 for this lipid abnormality. Model 2 for the association between serum Mg and HP was
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43 175 adjusted for age (continuous data), BMI (≥ 25 kg/m², < 25 kg/m²), gender (male,
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45 176 female), educational level (high school or above, lower than high school), smoking
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47 177 status (yes, no), activity level (continuous data), alcohol drinking status (yes, no), DM
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49 178 (yes, no), and dyslipidemia (yes, no). Model 2 for the association between serum Mg
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51 179 and HU was adjusted for age (continuous data), BMI (≥ 25 kg/m², < 25 kg/m²), gender
52
53 180 (male, female), educational level (high school or above, lower than high school),
54
55 181 smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes,
56
57 182 no), HP (yes, no), DM (yes, no) and dyslipidemia (yes, no). Model 3 for all
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59 183 associations were adjusted based on model 2, with additional factor of estimated
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184 glomerular filtration rate (eGFR). eGFR was calculated by serum creatinine (Scr), sex,

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3 and patients' age. The Modification of Diet in Renal Disease (MDRD) of eGFR
4 calculation formula was: $186 \times \text{Scr}^{-1.154} \times \text{age}^{-0.203} \times 1.210$ (if black) $\times 0.742$ (if
5 female).⁵² Tests for linear trends were conducted based on logistic regression using a
6
7 median variable of Mg concentration in each category. All data analyses were
8
9 performed using SPSS 17.0; $P \leq 0.05$ was considered to be statistically significant. All
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11 tests were two tailed.
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15 16 17 **Results**

18 A total of 962 subjects (377 females, accounting for 39.2%) were included in the
19 present cross-sectional study. The characteristics of the study population according to
20 quintiles of serum Mg were presented in Table 1. The mean age of the subjects was
21
22 54.9 \pm 7.6 years old. The overall prevalence of MetS, DM, HP and HU in OA patients
23
24 were 21.4%, 12.0%, 38.5% and 18.3% respectively. Significant differences were
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26 observed across the quintiles of serum Mg for fasting glucose, as well as the
27
28 prevalence of DM and HU.
29

30 The outcomes of multivariable adjusted associations between MetS and serum
31 Mg concentration were shown in Table 2. Compared with the lowest quintile, the
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33 age-sex adjusted ORs (Model 1) suggested significant decreased prevalence of MetS
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35 in the second (OR=0.61, 95%CI 0.38-0.97, P=0.038) and the highest (OR=0.59,
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37 95%CI 0.36-0.96, P=0.035) quintiles of serum Mg (P for trend =0.090); the
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39 multivariable adjusted ORs (Model 2) also suggested significant decreased prevalence
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41 of MetS in the second (OR=0.60, 95%CI 0.37-0.96, P=0.035) and the highest
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43 (OR=0.61, 95%CI 0.37-0.99, P=0.047) quintiles, and the P for trend was 0.120. The
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45 sensitivity analysis, by adding eGFR into model 2, also reached similar results -
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47 significant lower prevalence of MetS in the second (OR=0.58, 95%CI 0.36-0.94,
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49 P=0.026) and the highest quintiles (OR=0.56, 95%CI 0.34-0.93, P=0.024) compared
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51 with the reference quintile of serum Mg, and the P for trend was 0.066.
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53 Table 3 illustrated the multivariable adjusted relations between serum Mg and
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55 DM in OA patients. Both the age-sex adjusted OR values (Model 1) and the
56
57 multivariable adjusted OR values (Model 2) suggested a strong inverse association
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3 215 between serum Mg and DM. The age-sex adjusted ORs for the prevalence of DM
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5 216 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
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7 217 (95%CI 0.15-0.55, P<0.001), and 0.20 (95%CI 0.10-0.40, P<0.001) in the second,
8
9 218 third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was
10
11 219 <0.001. The multivariable adjusted ORs for the prevalence of DM were 0.38 (95%CI
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13 220 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,
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15 221 P<0.001), and 0.20 (95%CI 0.10-0.40, P<0.001) in the second, third, fourth and fifth
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17 222 quintiles of serum Mg respectively, and the P for trend was <0.001. The sensitivity
18
19 223 analysis, by adding eGFR into model 2, showed similar results - significant lower
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21 224 prevalence of DM in the second (OR=0.38, 95%CI 0.22-0.67, P=0.001), third
22
23 225 (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),
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25 226 and highest quintiles (OR=0.21, 95%CI 0.10-0.41, P<0.001) compared with the
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27 227 reference quintile of serum Mg, and the P for trend was <0.001.

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29 228 The multivariable-adjusted relations between serum Mg and HP in OA patients
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31 229 were illustrated in Table 4. According to both the age-sex adjusted ORs (Model 1) and
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33 230 the multivariable adjusted ORs (Model 2), there was no significant association
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35 231 between serum Mg and HP, and the P for trend were 0.929 and 0.423, respectively.
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37 232 The sensitivity analysis, by adding eGFR into model 2, reached the same results.

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39 233 The multivariable-adjusted relations between serum Mg and HU in OA patients
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41 234 were illustrated in Table 5. Both the age-sex adjusted OR values (Model 1) and the
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43 235 multivariable adjusted OR values (Model 2) suggested significant decreased
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45 236 prevalence of HU in the third quintile (age-sex adjusted OR=0.44, 95%CI 0.26-0.75,
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47 237 P=0.002; multivariable adjusted OR=0.42, 95%CI 0.24-0.73, P=0.002) and fifth
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49 238 quintile (age-sex adjusted OR=0.51, 95%CI 0.30-0.85, P=0.010; multivariable
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51 239 adjusted OR=0.50, 95%CI 0.29-0.86, P=0.012) compared with the lowest quintile of
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53 240 serum Mg, and the P for trend were 0.008 and 0.007, respectively. The sensitivity
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55 241 analysis, by adding eGFR into model 2, showed similar outcomes - significant lower
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57 242 prevalence of HU in the third (OR=0.36, 0.20-0.63, P<0.001), fourth (OR=0.54,
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59 243 95%CI 0.31-0.93, P=0.026), and highest quintiles (OR=0.39, 95%CI 0.22-0.68,
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244 P=0.001) compared with the reference quintile of serum Mg, and the P for trend was

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7 247 **Discussion**

8 248 The results of this study suggested that the serum Mg concentration was negatively
9 249 associated with the prevalence of MetS, DM and HU in subjects with radiographic
10 250 knee OA. In order to control potential confounders, several covariates including
11 251 characteristics, living habits and underlying diseases were selected, and even the
12 252 eGFR was added into the multivariable logistic regression models to eliminate the
13 253 influence of renal function on Mg excretion. The reverse associations mentioned
14 254 above remained significant after adjustments of these confounders. However, the
15 255 negative association between serum Mg and the prevalence of HP was not observed in
16 256 radiographic knee OA patients. Moreover, the linear associations were only observed
17 257 between serum Mg with DM and HU, but not between serum Mg and MetS.

18 258 Mg, the fourth most abundant cation in human body and the second most profuse
19 259 intracellular cation, is a metallic cofactor for over 300 enzymatic reactions. It appears
20 260 to play an important role in glucose metabolism and insulin homeostasis, which are
21 261 both highly correlated with metabolic diseases, especially MetS and DM. The
22 262 mechanisms involved in Mg deficiency in patients with MetS, DM and HU are
23 263 probably multifactorial. The most important factor may be insulin resistance, as Mg is
24 264 essential for insulin action and is a critical cofactor for several enzymes in
25 265 carbohydrate metabolism, which is important for the phosphorylation reactions of
26 266 tyrosine-kinase in the insulin receptor.^{31 53-57} Of course, it is necessary to highlight the
27 267 fact that insulin can also induce Mg excretion⁵⁸ and produce a significant decline of
28 268 plasma Mg through ion exchange.⁵⁹ Thus, there seems to be a vicious circle between
29 269 Mg deficiency and insulin resistance.

30 270 Other potential mechanisms include glucose transportation,⁵⁶ oxidative stress⁵⁶
31 271 and inflammatory cytokines,⁶⁰⁻⁶² and cellular calcium homeostasis.⁵⁴ Mg is an
32 272 essential cofactor of the high-energy phosphate-bound enzymatic pathways involved
33 273 in the modulation of glucose transport across cell membranes.⁵⁶ It also plays a role in
34 274 the mechanisms of cellular antioxidant defense.⁶³ The oxidative stress, defined as a

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3 275 persistent imbalance between the excessive production of reactive oxygen species
4 276 and/or defects in antioxidant defense, has been implicated in the pathogenesis of
5 277 diabetic complications.⁵⁶ Moreover, low serum Mg levels are strongly related to
6 278 elevated serum concentrations of both tumor necrosis factor alpha and C-reactive
7 279 protein (CRP),⁶⁴ suggesting that Mg deficiency may contribute to the development of
8 280 low-grade chronic inflammation syndrome and the development of glucose metabolic
9 281 disorders through the former pathway. In addition, lower Mg concentration can
10 282 enhance calcium-mediated vasoconstriction, blunt cardiac and smooth muscle
11 283 relaxation, and thus contribute to BP elevation.⁵⁴ However, the decreased serum
12 284 calcium concentration in radiographic knee OA patients may weaken the association
13 285 between Mg and HP.⁶⁵

14 286 MetS^{21 22} and DM^{4 23 24} were reported to be the risk factors of OA progression.
15 287 Moreover, serum Mg level has been proved to be significantly associated with the
16 288 CRP concentration,^{27 66-68} and higher CRP might serve as a prediction factor for OA
17 289 progression.^{69 70} Thus, OA progression may be delayed by elevating the serum Mg
18 290 level through reducing the prevalence of MetS and DM and decreasing the level of
19 291 CRP. Above all, the present study indicated that the elevation of serum Mg level has
20 292 the potential to reduce the prevalence of MetS, DM and HU in knee OA patients and
21 293 thereby may delay the progression of knee OA. However, the specific mechanism
22 294 needs to be further explored.

23 295 The present study has several strengths. Firstly, this is the first study examining
24 296 the associations between serum Mg and the prevalence of MetS, DM, HP and HU in
25 297 radiographic knee OA patients. The results of this study will provide a new insight
26 298 into the treatment of knee OA. Secondly, the multivariable logistical regression
27 299 models were adjusted for a considerable number of potential confounding factors,
28 300 which greatly improved the reliability of the results. Thirdly, the kidney is the key
29 301 organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by
30 302 adding eGFR into multivariable logistic regression models which showed that the
31 303 reverse associations remained significant.

32 304 Limitations of the present study should also be admitted. The cross-sectional

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3 305 design precludes causal correlations, so further prospective studies and intervention
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5 306 trials should be undertaken to establish a causal association between serum Mg with
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7 307 the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. Since no
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9 308 previous research investigated such associations in knee OA patients, the value of this
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11 309 study should not be blotted out by the cross-sectional nature. Another limitation of
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13 310 this study lies in the relatively small sample size, and thus, extensive high-quality
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15 311 researches based on a larger sample are needed. Moreover, the dietary intake of Mg in
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17 312 relation to the prevalence of MetS, DM, HP and HU were not assessed in the present
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19 313 study. Last but not the least, it is important to highlight that Mg is an intracellular ion;
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21 314 therefore, the serum Mg concentration must be considered as a poor indicator of body
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23 315 Mg content,⁷¹ even though it has been used in many studies. However, blood Mg level
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25 316 is the second best indicator of body status.⁷²
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28 **Conclusions**

29 319 The present study concluded that the serum Mg concentration was inversely
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31 320 associated with the prevalence of MetS, DM and HU in radiographic knee OA
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33 321 patients.
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323 **Contributors**

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

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343 **Competing interests**

344 The authors declare that they have no conflict of interest.

346 **Ethics approval**

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

350 **Data sharing statement**

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

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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

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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

354 Data are mean (Standard Deviation), unless otherwise indicated; Mg, magnesium; OA, osteoarthritis; BMI, body mass index; HDL, high density lipoprotein; eGFR,
 355 estimated glomerular filtration rate; MetS, metabolic syndrome; DM, diabetes mellitus; HP, hypertension; HU, hyperuricemia.
 356 # P values are for test of difference across all quintiles of serum Mg.

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.026	0.818	0.109	0.024	-

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

360 *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
 361 (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
 362 based on model 2, with additional factor of eGFR (continuous data).

363

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.001	0.001	<0.001	<0.001	-

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

366 *Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (≥ 25 kg/m², < 25 kg/m²), gender
 367 (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status
 368 (yes, no), hypertension (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

369

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.218	0.629	0.577	0.978	-

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

372 * Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (≥ 25 kg/m², < 25 kg/m²), gender
 373 (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status
 374 (yes, no), diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

375

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.119	<0.001	0.026	0.001	-

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

378 * Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), BMI (≥ 25 kg/m², < 25 kg/m²), gender
 379 (male, female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status
 380 (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
 381 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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	-
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	-
		<i>Cross-sectional study</i> —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.

BMJ Open

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

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Manuscripts

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3 **1 Association between Serum Magnesium Concentration with Metabolic**
4 **2 Syndrome, Diabetes, Hypertension and Hyperuricemia in Knee Osteoarthritis**
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8 4 Yi-lun Wang¹, Jie Wei², Chao Zeng¹, Tuo Yang¹, Hui Li¹, Yang Cui³, Dong-xing Xie¹,
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1
2
3 **Abstract**

4
5 **Objectives:** To examine the associations between serum magnesium (Mg)
6
7 concentration with the prevalence of metabolic syndrome (MetS), diabetes mellitus
8
9 (DM), hypertension (HP) and hyperuricemia (HU) in radiographic knee osteoarthritis
10
11 (OA) patients.

12
13 **Methods:** The present study was conducted at the Health Management Center of
14
15 Xiangya Hospital. Radiographic OA was evaluated for patients aged over 40 years
16
17 with basic characteristics and blood biochemical assessment. Serum Mg concentration
18
19 was measured using the chemiluminescence method. MetS, DM, HP and HU were
20
21 diagnosed based on standard protocols. The associations between serum Mg
22
23 concentration with MetS, DM, HP and HU were evaluated by conducting
24
25 multivariable adjusted logistic regression.

26
27 **Results:** A total of 962 radiographic knee OA patients were included. Compared with
28
29 the lowest quintile, the multivariable-adjusted odds ratios (ORs) and related 95%
30
31 confidence intervals (95%CI) of DM were 0.40 (95%CI 0.23-0.70, P=0.001), 0.33
32
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
34
35 0.11-0.44, P<0.001) in the second, third, fourth and highest quintiles of serum Mg,
36
37 respectively (P for trend <0.001); the multivariable-adjusted ORs of HU were 0.33
38
39 (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
40
41 0.22-0.70, P=0.001) in the third, fourth and highest quintiles of serum Mg
42
43 respectively (P for trend <0.001); and the multivariable-adjusted ORs of MetS were
44
45 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)
46
47 in the highest quintiles of serum Mg (P for trend =0.067). There was no significant
48
49 association between serum Mg and HP in OA patients.

50
51 **Conclusions:** The serum Mg concentration was inversely associated with the
52
53 prevalence of MetS, DM and HU in radiographic knee OA patients.

54
55 **Level of Evidence:** Level III, cross-sectional study.

56
57 **Key words:** osteoarthritis, magnesium, metabolic syndrome, diabetes, hypertension,
58
59 hyperuricemia

60

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3 48 **Strengths and limitations of this study**
4

- 5 49 1. This is the first study examining the associations between serum magnesium (Mg)
6 and the prevalence of metabolic syndrome, diabetes mellitus, hypertension and
7 50 and the prevalence of metabolic syndrome, diabetes mellitus, hypertension and
8 hyperuricemia in radiographic knee osteoarthritis patients.
9 51
10 52 2. The multivariable logistical regression models in this study were adjusted for a
11 considerable number of potential confounding factors, which greatly improved the
12 53 reliability of the results.
13 54
14 55 3. The kidney is the key organ in maintaining Mg homeostasis. This study conducted
15 a sensitivity analysis by adding estimated glomerular filtration rate into the
16 56 multivariable logistic regression models, and the reverse associations remained
17 significant.
18 57
19 58
20 59 4. This study adopted cross-sectional design which precluded causal correlations.
21 60
22 61 5. Serum Mg concentration was adopted as the indicator of body Mg content in this
23 study which may not be the best indicator of body status.
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63 Introduction

64 The association between osteoarthritis (OA) and metabolic diseases, especially
65 metabolic syndrome (MetS)^{1 2} and diabetes mellitus (DM),³⁻⁵ has drawn increasing
66 attention in the past few years. OA includes three specific phenotypes: metabolic OA,
67 age-related OA and injury-related OA.⁶ A large number of studies have indicated that
68 the prevalence of MetS,⁷⁻⁹ DM¹⁰⁻¹⁸ and hypertension (HP)^{7 9-13 19 20} is either higher in
69 OA patients or associated with OA. In addition, some other studies reported that
70 MetS,^{21 22} DM^{23 24} and HP^{21 22} are risk factors of OA progression. Thus, it appears
71 necessary to pay more attention and adopt appropriate measures to reduce the high
72 prevalence of metabolic diseases in OA patients, which also seems to be beneficial in
73 delaying OA progression.

74 Serum magnesium (Mg), one of the most important micronutrients for human
75 health, has been reported to be negatively associated with MetS,²⁵⁻²⁹ DM³⁰⁻³⁸ and HP³⁰
76 ³⁹⁻⁴¹ by lots of studies. Meanwhile, our previous study showed an inverse association
77 between serum Mg and hyperuricemia (HU).⁴² However, to the best knowledge of the
78 authors, there is not yet a study examining the association between the serum Mg
79 concentration and the aforementioned metabolic diseases (MetS, DM, HP and HU) in
80 OA patients. On the other hand, we have previously shown that the serum Mg
81 concentration may be inversely associated with radiographic knee OA.⁴³ Therefore,
82 we speculate that the prevalence of MetS, DM, HP and HU in OA patients may be
83 reduced by elevating the level of serum Mg, which can in turn delay OA progression.
84 Thus, the objective of the present study was to examine the associations between the
85 serum Mg concentration with the prevalence of MetS, DM, HP and HU in
86 radiographic knee OA patients. It was hypothesized that serum Mg concentration was
87 inversely associated with these diseases.

88

89 Methods

90 Study population

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

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3 93 published previously.⁴²⁻⁴⁶ The protocol has been reviewed and approved by the Ethics
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5 94 Committee of Xiangya Hospital, Central South University (reference numbers:
6
7 95 201312459), and the methods were developed in “accordance” with the approved
8
9 96 guidelines. Informed consent has been obtained from all participants. Registered
10
11 97 nurses were engaged to interview all participants during the examination using a
12
13 98 standard questionnaire, with the purpose to collect information on demographic
14
15 99 characteristics and health-related habits. Participants were selected based on the
16
17 100 following inclusion criteria: 1) 40 years old or above; 2) undergoing weight-bearing
18
19 101 bilateral anteroposterior radiography of the knee, and diagnosed with knee OA
20
21 102 according to the Kellgren-Lawrence (K-L) radiographic atlas (knee joint was graded
22
23 103 K-L 2 or above); 3) availability of all basic characteristics, including age, gender,
24
25 104 body mass index (BMI) and blood pressure; 4) availability of biochemical test results,
26
27 105 including serum Mg concentration; 5) availability of information related to the living
28
29 106 habits, including education background, activity level, smoking, drinking and
30
31 107 medication status. Initially, the present cross-sectional study retrieved 1820
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33 108 radiographic knee OA patients aged over 40 years who exhibited sound basic
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35 109 characteristics and required blood biochemical assessment (including serum Mg
36
37 110 concentration). Among them, 962 patients offered demographic characteristics and
38
39 111 health-related habits and were finally included in this study.

112

113 **Blood biochemistry**

114 All blood samples were drawn after a 12-hour overnight fast and were kept at 4°C
115 until analysis. Blood tests were undertaken using the Beckman Coulter AU 5800
116 (Beckman Coulter Inc., Brea, CA, USA). The inter- and intra-assay coefficients of
117 variation were tested at both low concentrations (2.5 mmol/L for glucose, 118 µmol/L
118 for uric acid and 0.60 mmol/L for serum Mg) and high concentrations (6.7 mmol/L for
119 glucose, 472 µmol/L for uric acid and 1.00 mmol/L for serum Mg) of standard human
120 samples. The intra-assay coefficients of variation were 0.98% (2.5 mmol/L) and 1.72%
121 (6.7 mmol/L) for glucose, 1.39% (118 µmol/L) and 0.41% (472 µmol/L) for uric acid,
122 and 1.86% (0.60 mmol/L) and 1.65% (1.00 mmol/L) for serum Mg respectively. The

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3 123 inter-assay coefficients of variation were 2.45% (2.5 mmol/L) and 1.46% (6.7 mmol/L)
4 for glucose, 1.40% (118 $\mu\text{mol/L}$) and 1.23% (472 $\mu\text{mol/L}$) for uric acid, and 1.87%
5 (0.60 mmol/L) and 1.70% (1.00 mmol/L) for serum Mg respectively.
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127 **Assessment of other exposures**

128 Blood pressure was measured by an electronic sphygmomanometer. The weight and
129 height of each subjects was measured respectively to calculate the BMI. Information
130 on the average frequency of physical activity (never, one to two times per week, three
131 to four times per week, five times and above per week) and average duration of
132 physical activity (less than half an hour, half an hour to one hour, one to two hours,
133 more than two hours) was collected through survey questionnaire. The smoking,
134 alcohol drinking and medication status were collected during the face-to-face
135 interview.
136

137 **Assessment of MetS, DM, HP and HU**

138 MetS was diagnosed based on the Chinese Diabetes Society (CDS) criteria,⁴⁷⁻⁴⁹ which
139 requires meeting at least 3 of the following 4 items: (1) BMI ≥ 25 kg/m²; (2) Fasting
140 plasma glucose (FPG) ≥ 6.1 mmol/L, or diagnosed DM; (3) Systolic blood pressure
141 (BP) ≥ 140 mmHg or diastolic BP ≥ 90 mmHg, or treatment of previously diagnosed
142 HP; (4) Triglycerides ≥ 1.7 mmol/L and/or HDL-cholesterol < 0.9 mmol/L in male or
143 < 1.0 mmol/L in female, or treatment for this lipid abnormality. Subjects with the
144 fasting glucose ≥ 7.0 mmol/L or currently undergoing drug treatment for blood glucose
145 control were regarded as DM patients, and subjects with the systolic blood pressure
146 ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or currently undertaking
147 antihypertensive medication were regarded as HP patients. HU was defined as uric
148 acid ≥ 416 $\mu\text{mol/L}$ for male and ≥ 360 $\mu\text{mol/L}$ for female or currently undergoing drug
149 treatment for uric acid control.
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151 **Statistical analysis**

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

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3 153 data are expressed in percentage. Differences in continuous data were evaluated by
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5 154 one-way classification ANOVA (normally distributed data) or Kruskal-Wallis H test
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7 155 (non-normally distributed data), while differences in category data were assessed by
8
9 156 the χ^2 test. The serum Mg was classified into five categories based on the quintile
10
11 157 distribution: ≤ 0.85 , 0.86-0.89, 0.90-0.92, 0.93-0.96 and ≥ 0.97 mmol/L. The
12
13 158 prevalence of MetS, DM, HP and HU in each quintile of serum Mg in OA patients
14
15 159 were assessed by scatter plots.

16
17 160 Logistic regression was conducted to calculate the odds ratios (ORs) with 95%
18
19 161 confidence intervals (95%CI) for the associations between serum Mg and MetS, DM,
20
21 162 HP and HU. Specifically, model 1 was adjusted by covariates of age (continuous data)
22
23 163 and gender (male, female). Then, model 2 was adjusted by additional covariates of
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25 164 BMI (continuous data), educational level (high school or above, lower than high
26
27 165 school), smoking status (yes, no), activity level (continuous data), alcohol drinking
28
29 166 status (yes, no), HP (yes, no), DM (yes, no), and dyslipidemia (yes, no) on the basis of
30
31 167 model 1. Dyslipidemia was defined as triglycerides ≥ 1.7 mmol/L and/or
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33 168 HDL-cholesterol < 0.9 mmol/L in male or < 1.0 mmol/L in female, or treatment for this
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35 169 lipid abnormality. Notably, the selection of covariates in model 2 varied slightly for
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37 170 examining different associations (between serum Mg and MetS, DM, HP or HU). For
38
39 171 example, BMI, HP and dyslipidemia were adjusted for the association between serum
40
41 172 Mg and DM, but not for the association between serum Mg and MetS, simply because
42
43 173 MetS was diagnosed based on BMI, HP and dyslipidemia status. Model 3 was
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45 174 established based on model 2, with adjustment of an additional covariate, estimated
46
47 175 glomerular filtration rate (eGFR). eGFR (continuous data) was calculated from the
48
49 176 Chronic Kidney Disease Epidemiology Collaboration equation.⁵⁰ All covariates in the
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51 177 present study were chosen referring to some of the previous similar studies.^{27 33 51 52}
52
53 178 Tests for linear trends were conducted based on logistic regression using a median
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55 179 variable of Mg concentration in each category.
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57 180 Scatter plots were plotted using R 3.4.4.⁵³ Other data analyses were performed using
58
59 181 SPSS 17.0; $P \leq 0.05$ was considered to be statistically significant. All tests were two
60
182 tailed.

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5 184 **Patient and public involvement**6
7 185 No patients were involved in setting the research question or the outcome measures,
8
9 186 nor were they involved in the design or implementation of the study. There are no
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11 187 plans to disseminate the results of the research to study participants

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13
14 189 **Results**15
16 190 A total of 962 subjects (377 females, accounting for 39.2%) were included in the
17
18 191 present cross-sectional study. The characteristics of the study population according to
19
20 192 quintiles of serum Mg were presented in Table 1. The mean age of the subjects was
21
22 193 54.9±7.6 years old. The overall prevalence of MetS, DM, HP and HU in OA patients
23
24 194 were 21.4%, 12.0%, 38.5% and 18.3% respectively. Significant differences were
25
26 195 observed across the quintiles of serum Mg for fasting glucose, as well as the
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28 196 prevalence of DM and HU.29
30 197 The prevalence of MetS in each quintile of serum Mg in OA patients was shown
31
32 198 in Figure 1 (A). The outcomes of multivariable adjusted associations between MetS
33
34 199 and serum Mg concentration were shown in Table 2. Compared with the lowest
35
36 200 quintile, the age-gender adjusted ORs (Model 1) suggested significant decreased
37
38 201 prevalence of MetS in the second (OR=0.61, 95%CI 0.38-0.97, P=0.038) and the
39
40 202 highest (OR=0.59, 95%CI 0.36-0.96, P=0.035) quintiles of serum Mg (P for trend
41
42 203 =0.090); the multivariable adjusted ORs (Model 2) also suggested significant
43
44 204 decreased prevalence of MetS in the second (OR=0.60, 95%CI 0.37-0.96, P=0.035)
45
46 205 and the highest (OR=0.61, 95%CI 0.37-0.99, P=0.047) quintiles, and the P for trend
47
48 206 was 0.120. The sensitivity analysis, by adding eGFR into model 2, also reached
49
50 207 similar results - significant lower prevalence of MetS in the second (OR=0.59, 95%CI
51
52 208 0.36-0.94, P=0.027) and the highest quintiles (OR=0.56, 95%CI 0.34-0.93, P=0.024)
53
54 209 compared with the reference quintile of serum Mg, and the P for trend was 0.067.55
56 210 Figure 1 (B) showed the prevalence of DM in each category of serum Mg in OA
57
58 211 patients. Table 3 illustrated the multivariable adjusted relations between serum Mg
59
60 212 and DM in OA patients. Both the age-gender adjusted OR values (Model 1) and the

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3 213 multivariable adjusted OR values (Model 2) suggested a strong inverse association
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5 214 between serum Mg and DM. The age-gender adjusted ORs for the prevalence of DM
6
7 215 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
8
9 216 (95%CI 0.15-0.55, P<0.001), and 0.20 (95%CI 0.10-0.40, P<0.001) in the second,
10
11 217 third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was
12
13 218 <0.001. The multivariable adjusted ORs for the prevalence of DM were 0.40 (95%CI
14
15 219 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,
16
17 220 P<0.001), and 0.21 (95%CI 0.11-0.42, P<0.001) in the second, third, fourth and fifth
18
19 221 quintiles of serum Mg respectively, and the P for trend was <0.001. The sensitivity
20
21 222 analysis, by adding eGFR into model 2, showed similar results - significant lower
22
23 223 prevalence of DM in the second (OR=0.40, 95%CI 0.23-0.70, P=0.001), third
24
25 224 (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),
26
27 225 and highest quintiles (OR=0.22, 95%CI 0.11-0.44, P<0.001) compared with the
28
29 226 reference quintile of serum Mg, and the P for trend was <0.001.

30
31 227 The prevalence of HP in each quintile of serum Mg in OA patients was depicted
32
33 228 in Figure 1 (C). The multivariable-adjusted relations between serum Mg and HP in
34
35 229 OA patients were illustrated in Table 4. According to both the age-gender adjusted
36
37 230 ORs (Model 1) and the multivariable adjusted ORs (Model 2), there was no
38
39 231 significant association between serum Mg and HP, and the P for trend were 0.929 and
40
41 232 0.377, respectively. The sensitivity analysis, by adding eGFR into model 2, reached
42
43 233 the same results.

44
45 234 The prevalence of HU in each category of serum Mg in OA patients was shown
46
47 235 in Figure 1 (D). The multivariable-adjusted relations between serum Mg and HU in
48
49 236 OA patients were illustrated in Table 5. Both the age-gender adjusted OR values
50
51 237 (Model 1) and the multivariable adjusted OR values (Model 2) suggested significant
52
53 238 decreased prevalence of HU in the third quintile (age-gender adjusted OR=0.44,
54
55 239 95%CI 0.26-0.75, P=0.002; multivariable adjusted OR=0.38, 95%CI 0.22-0.67,
56
57 240 P=0.001) and fifth quintile (age-gender adjusted OR=0.51, 95%CI 0.30-0.85, P=0.010;
58
59 241 multivariable adjusted OR=0.50, 95%CI 0.29-0.87, P=0.013) compared with the
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242 lowest quintile of serum Mg, and the P for trend were 0.008 and 0.006, respectively.

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3 243 The sensitivity analysis, by adding eGFR into model 2, showed similar outcomes -
4
5 244 significant lower prevalence of HU in the third (OR=0.33, 0.19-0.59, P<0.001), fourth
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7 245 (OR=0.52, 95%CI 0.30-0.91, P=0.022), and highest quintiles (OR=0.39, 95%CI
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9 246 0.22-0.70, P=0.001) compared with the reference quintile of serum Mg, and the P for
10
11 247 trend was <0.001.

12 248

13 249 **Discussion**

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16 250 The results of this study suggested that the serum Mg concentration was negatively
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18 251 associated with the prevalence of MetS, DM and HU in subjects with radiographic
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20 252 knee OA. In order to control potential confounders, several covariates including
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22 253 characteristics, living habits and underlying diseases were selected, and even the
23
24 254 eGFR was added into the multivariable logistic regression models to eliminate the
25
26 255 influence of renal function on Mg excretion. The reverse associations mentioned
27
28 256 above remained significant after adjustments of these confounders. However, the
29
30 257 negative association between serum Mg and the prevalence of HP was not observed in
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32 258 radiographic knee OA patients. Moreover, the linear associations were only observed
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34 259 between serum Mg with DM and HU, but not between serum Mg and MetS.

35 260 Mg, the fourth most abundant cation in human body and the second most profuse
36
37 261 intracellular cation, is a metallic cofactor for over 300 enzymatic reactions. It appears
38
39 262 to play an important role in glucose metabolism and insulin homeostasis, which are
40
41 263 both highly correlated with metabolic diseases, especially MetS and DM. The
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43 264 mechanisms involved in Mg deficiency in patients with MetS, DM and HU are
44
45 265 probably multifactorial. The most important factor may be insulin resistance, as Mg is
46
47 266 essential for insulin action and is a critical cofactor for several enzymes in
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49 267 carbohydrate metabolism, which is important for the phosphorylation reactions of
50
51 268 tyrosine-kinase in the insulin receptor.^{31 54-58} Of course, it is necessary to highlight the
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53 269 fact that insulin can also induce Mg excretion⁵⁹ and produce a significant decline of
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55 270 plasma Mg through ion exchange.⁶⁰ Thus, there seems to be a vicious circle between
56
57 271 Mg deficiency and insulin resistance.

58
59 272 Other potential mechanisms include glucose transportation,⁵⁷ oxidative stress⁵⁷

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2
3 273 and inflammatory cytokines,⁶¹⁻⁶³ and cellular calcium homeostasis.⁵⁵ Mg is an
4 274 essential cofactor of the high-energy phosphate-bound enzymatic pathways involved
5 275 in the modulation of glucose transport across cell membranes.⁵⁷ It also plays a role in
6
7 276 the mechanisms of cellular antioxidant defense.⁶⁴ The oxidative stress, defined as a
8
9 277 persistent imbalance between the excessive production of reactive oxygen species
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11 278 and/or defects in antioxidant defense, has been implicated in the pathogenesis of
12
13 279 diabetic complications.⁵⁷ Moreover, low serum Mg levels are strongly related to
14
15 280 elevated serum concentrations of both tumor necrosis factor alpha and C-reactive
16
17 281 protein (CRP),⁶⁵ suggesting that Mg deficiency may contribute to the development of
18
19 282 low-grade chronic inflammation syndrome and the development of glucose metabolic
20
21 283 disorders through the former pathway. In addition, lower Mg concentration can
22
23 284 enhance calcium-mediated vasoconstriction, blunt cardiac and smooth muscle
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25 285 relaxation, and thus contribute to BP elevation.⁵⁵ However, the decreased serum
26
27 286 calcium concentration in radiographic knee OA patients may weaken the association
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29 287 between Mg and HP.⁶⁶

30 288 MetS^{21 22} and DM^{4 23 24} were reported to be the risk factors of OA progression.
31
32 289 Moreover, serum Mg level has been proved to be significantly associated with the
33
34 290 CRP concentration,^{27 67-69} and higher CRP might serve as a prediction factor for OA
35
36 291 progression.^{70 71} Thus, OA progression may be delayed by elevating the serum Mg
37
38 292 level through reducing the prevalence of MetS and DM and decreasing the level of
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40 293 CRP. Above all, the present study indicated that the elevation of serum Mg level has
41
42 294 the potential to reduce the prevalence of MetS, DM and HU in knee OA patients and
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44 295 thereby may delay the progression of knee OA. However, the specific mechanism
45
46 296 needs to be further explored.

47 297 The present study has several strengths. Firstly, this is the first study examining
48
49 298 the associations between serum Mg and the prevalence of MetS, DM, HP and HU in
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51 299 radiographic knee OA patients. The results of this study will provide a new insight
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53 300 into the treatment of knee OA. Secondly, the multivariable logistical regression
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55 301 models were adjusted for a considerable number of potential confounding factors,
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57 302 which greatly improved the reliability of the results. Thirdly, the kidney is the key

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2
3 303 organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by
4 304 adding eGFR into multivariable logistic regression models which showed that the
5
6 305 reverse associations remained significant.
7

8 306 Limitations of the present study should also be admitted. The cross-sectional
9
10 307 design precludes causal correlations, so further prospective studies and intervention
11
12 308 trials should be undertaken to establish a causal association between serum Mg with
13
14 309 the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. Since no
15
16 310 previous research investigated such associations in knee OA patients, the value of this
17
18 311 study should not be blotted out by the cross-sectional nature. Another limitation of
19
20 312 this study lies in the relatively small sample size, and thus, extensive high-quality
21
22 313 researches based on a larger sample are needed. Moreover, the dietary intake of Mg in
23
24 314 relation to the prevalence of MetS, DM, HP and HU were not assessed in the present
25
26 315 study. Last but not the least, it is important to highlight that Mg is an intracellular ion;
27
28 316 therefore, the serum Mg concentration must be considered as a poor indicator of body
29
30 317 Mg content,⁷² even though it has been used in many studies. However, blood Mg level
31
32 318 is the second best indicator of body status.⁷³
33

34

35 320 **Conclusions**

36 321 The present study concluded that the serum Mg concentration was inversely
37
38 322 associated with the prevalence of MetS, DM and HU in radiographic knee OA
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40 323 patients.
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42 324

325 **Contributors**

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

334

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344

345 **Competing interests**

346 The authors declare that they have no conflict of interest.

347

348 **Ethics approval**

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

351

352 **Data sharing statement**

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

355

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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

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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

569 Data are mean (Standard Deviation), unless otherwise indicated; Mg, magnesium; OA, osteoarthritis; BMI, body mass index; HDL, high density lipoprotein; eGFR,
570 estimated glomerular filtration rate; MetS, metabolic syndrome; DM, diabetes mellitus; HP, hypertension; HU, hyperuricemia.
571 # P values are for test of difference across all quintiles of serum Mg.

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6 573 Figure 1 The prevalence of MetS (A), DM (B), HP (C) and HU (D) in each quintile of serum Mg in OA patients
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575 Table 2 Multivariable-adjusted relations of serum Mg and MetS in OA patients (n = 962)

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.027	0.830	0.114	0.024	-

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

577 *Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), gender (male, female), educational
 578 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
 579 adjusted based on model 2, with additional factor of eGFR (continuous data).

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.001	<0.001	<0.001	<0.001	-

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

583 *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,
584 female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no),
585 hypertension (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.235	0.574	0.727	0.818	-

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

589 * 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,
 590 female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no),
 591 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					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> 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)

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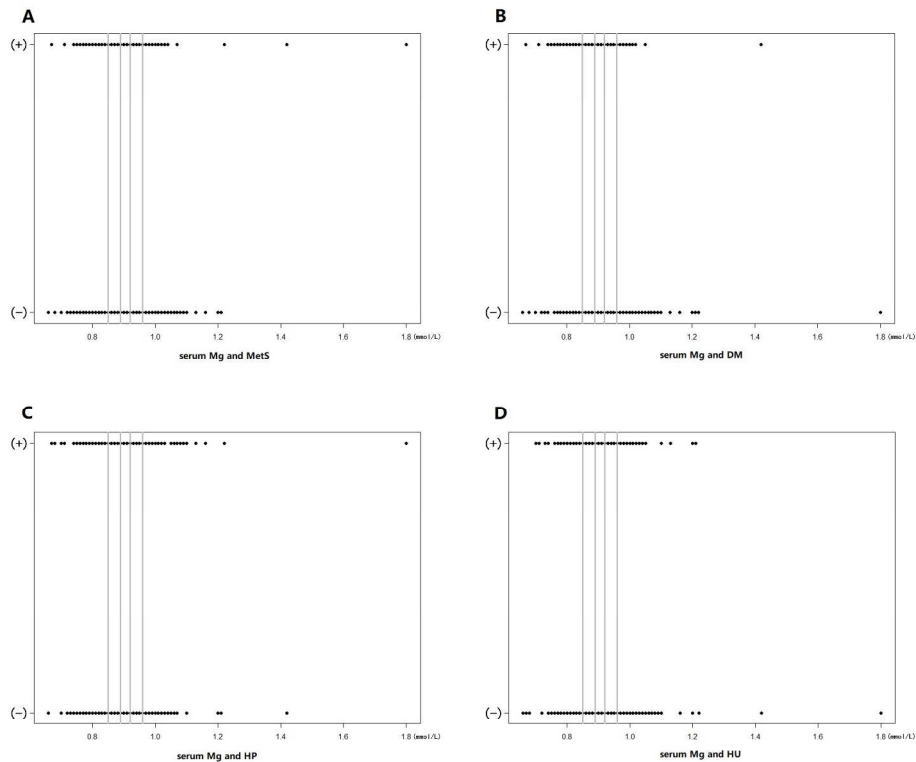


Figure 1 The prevalence of MetS (A), DM (B), HP (C) and HU (D) in each quintile of serum Mg in OA patients.

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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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4-5
		<i>Case-control study</i> —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	
		<i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed	-
		<i>Case-control study</i> —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- 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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed	4- 5
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	
		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(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
		(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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	-
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	-
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	8-10 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	8-10 8-9
		(b) Report category boundaries when continuous variables were categorized	8-10 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	8-10 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 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.

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

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		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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
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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
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		(c) Explain how missing data were addressed	-
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —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
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Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	-
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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
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BMJ Open

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

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Primary Subject Heading:	Rheumatology
Secondary Subject Heading:	Rheumatology, Public health, Epidemiology
Keywords:	osteoarthritis, magnesium, metabolic syndrome, diabetes, Hypertension < CARDIOLOGY, hyperuricemia

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Manuscripts

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3 **1 Association between Serum Magnesium Concentration with Metabolic**
4 **2 Syndrome, Diabetes, Hypertension and Hyperuricemia in Knee Osteoarthritis**
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3 **Abstract**

4
5 **Objectives:** To examine the associations between serum magnesium (Mg)
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7 concentration with the prevalence of metabolic syndrome (MetS), diabetes mellitus
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9 (DM), hypertension (HP) and hyperuricemia (HU) in radiographic knee osteoarthritis
10
11 (OA) patients.

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13 **Methods:** The present study was conducted at the Health Management Center of
14
15 Xiangya Hospital. Radiographic OA was evaluated for patients aged over 40 years
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17 with basic characteristics and blood biochemical assessment. Serum Mg concentration
18
19 was measured using the chemiluminescence method. MetS, DM, HP and HU were
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21 diagnosed based on standard protocols. The associations between serum Mg
22
23 concentration with MetS, DM, HP and HU were evaluated by conducting
24
25 multivariable adjusted logistic regression.

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27 **Results:** A total of 962 radiographic knee OA patients were included. Compared with
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29 the lowest quintile, the multivariable-adjusted odds ratios (ORs) and related 95%
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31 confidence intervals (95%CI) of DM were 0.40 (95%CI 0.23-0.70, P=0.001), 0.33
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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
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35 0.11-0.44, P<0.001) in the second, third, fourth and highest quintiles of serum Mg,
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37 respectively (P for trend <0.001); the multivariable-adjusted ORs of HU were 0.33
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39 (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
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41 0.22-0.70, P=0.001) in the third, fourth and highest quintiles of serum Mg
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43 respectively (P for trend <0.001); and the multivariable-adjusted ORs of MetS were
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45 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)
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47 in the highest quintiles of serum Mg. However, the inverse association between serum
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49 Mg and the prevalence of MetS was nonlinear (P for trend =0.067). There was no
50
51 significant association between serum Mg and HP in OA patients.

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53 **Conclusions:** The serum Mg concentration was inversely associated with the
54
55 prevalence of MetS, DM and HU in radiographic knee OA patients.

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

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59 **Key words:** osteoarthritis, magnesium, metabolic syndrome, diabetes, hypertension,
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61 hyperuricemia

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3 48 **Strengths and limitations of this study**
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- 5 49 1. This is the first study examining the associations between serum magnesium (Mg)
6 and the prevalence of metabolic syndrome, diabetes mellitus, hypertension and
7 50 and the prevalence of metabolic syndrome, diabetes mellitus, hypertension and
8 hyperuricemia in radiographic knee osteoarthritis patients.
9 51
10 52 2. The multivariable logistical regression models in this study were adjusted for a
11 considerable number of potential confounding factors, which greatly improved the
12 53 reliability of the results.
13 54
14 55 3. The kidney is the key organ in maintaining Mg homeostasis. This study conducted
15 a sensitivity analysis by adding estimated glomerular filtration rate into the
16 56 multivariable logistic regression models, and the reverse associations remained
17 significant.
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19 58
20 59 4. This study adopted cross-sectional design which precluded causal correlations.
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22 61 5. Serum Mg concentration was adopted as the indicator of body Mg content in this
23 study which may not be the best indicator of body status.
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63 Introduction

64 The association between osteoarthritis (OA) and metabolic diseases, especially
65 metabolic syndrome (MetS)^{1 2} and diabetes mellitus (DM),³⁻⁵ has drawn increasing
66 attention in the past few years. OA includes three specific phenotypes: metabolic OA,
67 age-related OA and injury-related OA.⁶ A large number of studies have indicated that
68 the prevalence of MetS,⁷⁻⁹ DM¹⁰⁻¹⁸ and hypertension (HP)^{7 9-13 19 20} is either higher in
69 OA patients or associated with OA. In addition, some other studies reported that
70 MetS,^{21 22} DM^{23 24} and HP^{21 22} are risk factors of OA progression. Thus, it appears
71 necessary to pay more attention and adopt appropriate measures to reduce the high
72 prevalence of metabolic diseases in OA patients, which also seems to be beneficial in
73 delaying OA progression.

74 Serum magnesium (Mg), one of the most important micronutrients for human
75 health, has been reported to be negatively associated with MetS,²⁵⁻²⁹ DM³⁰⁻³⁸ and HP³⁰
76 ³⁹⁻⁴¹ by lots of studies. Meanwhile, our previous study showed an inverse association
77 between serum Mg and hyperuricemia (HU).⁴² However, to the best knowledge of the
78 authors, there is not yet a study examining the association between the serum Mg
79 concentration and the aforementioned metabolic diseases (MetS, DM, HP and HU) in
80 OA patients. On the other hand, we have previously shown that the serum Mg
81 concentration may be inversely associated with radiographic knee OA.⁴³ Therefore,
82 we speculate that the prevalence of MetS, DM, HP and HU in OA patients may be
83 reduced by elevating the level of serum Mg, which can in turn delay OA progression.
84 Thus, the objective of the present study was to examine the associations between the
85 serum Mg concentration with the prevalence of MetS, DM, HP and HU in
86 radiographic knee OA patients. It was hypothesized that serum Mg concentration was
87 inversely associated with these diseases.

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89 Methods

90 Study population

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

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3 93 published previously.⁴²⁻⁴⁶ The protocol has been reviewed and approved by the Ethics
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5 94 Committee of Xiangya Hospital, Central South University (reference numbers:
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7 95 201312459), and the methods were developed in “accordance” with the approved
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9 96 guidelines. Informed consent has been obtained from all participants. Registered
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11 97 nurses were engaged to interview all participants during the examination using a
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13 98 standard questionnaire, with the purpose to collect information on demographic
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15 99 characteristics and health-related habits. Participants were selected based on the
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17 100 following inclusion criteria: 1) 40 years old or above; 2) undergoing weight-bearing
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19 101 bilateral anteroposterior radiography of the knee, and diagnosed with knee OA
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21 102 according to the Kellgren-Lawrence (K-L) radiographic atlas (knee joint was graded
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23 103 K-L 2 or above); 3) availability of all basic characteristics, including age, gender,
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25 104 body mass index (BMI) and blood pressure; 4) availability of biochemical test results,
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27 105 including serum Mg concentration; 5) availability of information related to the living
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29 106 habits, including education background, activity level, smoking, drinking and
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31 107 medication status. Initially, the present cross-sectional study retrieved 1820
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33 108 radiographic knee OA patients aged over 40 years who exhibited sound basic
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35 109 characteristics and required blood biochemical assessment (including serum Mg
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37 110 concentration). Among them, 962 patients offered demographic characteristics and
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39 111 health-related habits and were finally included in this study.

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113 **Blood biochemistry**

114 All blood samples were drawn after a 12-hour overnight fast and were kept at 4°C
115 until analysis. Blood tests were undertaken using the Beckman Coulter AU 5800
116 (Beckman Coulter Inc., Brea, CA, USA). The inter- and intra-assay coefficients of
117 variation were tested at both low concentrations (2.5 mmol/L for glucose, 118 µmol/L
118 for uric acid and 0.60 mmol/L for serum Mg) and high concentrations (6.7 mmol/L for
119 glucose, 472 µmol/L for uric acid and 1.00 mmol/L for serum Mg) of standard human
120 samples. The intra-assay coefficients of variation were 0.98% (2.5 mmol/L) and 1.72%
121 (6.7 mmol/L) for glucose, 1.39% (118 µmol/L) and 0.41% (472 µmol/L) for uric acid,
122 and 1.86% (0.60 mmol/L) and 1.65% (1.00 mmol/L) for serum Mg respectively. The

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3 123 inter-assay coefficients of variation were 2.45% (2.5 mmol/L) and 1.46% (6.7 mmol/L)
4 for glucose, 1.40% (118 $\mu\text{mol/L}$) and 1.23% (472 $\mu\text{mol/L}$) for uric acid, and 1.87%
5 (0.60 mmol/L) and 1.70% (1.00 mmol/L) for serum Mg respectively.
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127 **Assessment of other exposures**

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

138 MetS was diagnosed based on the Chinese Diabetes Society (CDS) criteria,⁴⁷⁻⁴⁹ which
139 requires meeting at least 3 of the following 4 items: (1) BMI ≥ 25 kg/m²; (2) Fasting
140 plasma glucose (FPG) ≥ 6.1 mmol/L, or diagnosed DM; (3) Systolic blood pressure
141 (BP) ≥ 140 mmHg or diastolic BP ≥ 90 mmHg, or treatment of previously diagnosed
142 HP; (4) Triglycerides ≥ 1.7 mmol/L and/or HDL-cholesterol < 0.9 mmol/L in male or
143 < 1.0 mmol/L in female, or treatment for this lipid abnormality. Subjects with the
144 fasting glucose ≥ 7.0 mmol/L or currently undergoing drug treatment for blood glucose
145 control were regarded as DM patients, and subjects with the systolic blood pressure
146 ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or currently undertaking
147 antihypertensive medication were regarded as HP patients. HU was defined as uric
148 acid ≥ 416 $\mu\text{mol/L}$ for male and ≥ 360 $\mu\text{mol/L}$ for female or currently undergoing drug
149 treatment for uric acid control.
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151 **Statistical analysis**

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

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3 153 category data were expressed in percentage. Differences in continuous data were
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5 154 evaluated by one-way classification ANOVA (normally distributed data) or
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7 155 Kruskal-Wallis H test (non-normally distributed data), while differences in category
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9 156 data were assessed by the χ^2 test. The serum Mg was classified into five categories
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11 157 based on the quintile distribution: ≤ 0.85 , 0.86-0.89, 0.90-0.92, 0.93-0.96 and ≥ 0.97
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13 158 mmol/L. The prevalence of MetS, DM, HP and HU in each quintile of serum Mg in
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15 159 OA patients were assessed by scatter plots.

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17 160 Logistic regression was conducted to calculate the odds ratios (ORs) with 95%
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19 161 confidence intervals (95%CI) for the associations between serum Mg and MetS, DM,
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21 162 HP and HU. Specifically, model 1 was adjusted by covariates of age (continuous data)
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23 163 and gender (male, female). Then, model 2 was adjusted by additional covariates of
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25 164 BMI (continuous data), educational level (high school or above, lower than high
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27 165 school), smoking status (yes, no), activity level (continuous data), alcohol drinking
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29 166 status (yes, no), HP (yes, no), DM (yes, no), and dyslipidemia (yes, no) on the basis of
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31 167 model 1. Dyslipidemia was defined as triglycerides ≥ 1.7 mmol/L and/or
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33 168 HDL-cholesterol < 0.9 mmol/L in male or < 1.0 mmol/L in female, or treatment for this
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35 169 lipid abnormality. Notably, the selection of covariates in model 2 varied slightly for
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37 170 examining different associations (between serum Mg and MetS, DM, HP or HU). For
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39 171 example, BMI, HP and dyslipidemia were adjusted for the association between serum
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41 172 Mg and DM, but not for the association between serum Mg and MetS, simply because
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43 173 MetS was diagnosed based on BMI, HP and dyslipidemia status. Model 3 was
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45 174 established based on model 2, with adjustment of an additional covariate, estimated
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47 175 glomerular filtration rate (eGFR). eGFR (continuous data) was calculated from the
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49 176 Chronic Kidney Disease Epidemiology Collaboration equation.⁵⁰ All covariates in the
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51 177 present study were chosen referring to some of the previous similar studies.^{27 33 51 52}
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53 178 Tests for linear trends were conducted based on logistic regression using a median
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55 179 variable of Mg concentration in each category.

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57 180 Scatter plots were plotted using R 3.4.4.⁵³ Other data analyses were performed
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59 181 using SPSS 17.0; $P \leq 0.05$ was considered to be statistically significant. All tests were
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182 two tailed.

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184 Patient and public involvement

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

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189 Results

190 A total of 962 subjects (377 females, accounting for 39.2%) were included in the
191 present cross-sectional study. The characteristics of the study population according to
192 quintiles of serum Mg were presented in Table 1. The mean age of the subjects was
193 54.9 ± 7.6 years old. The overall prevalence of MetS, DM, HP and HU in OA patients
194 were 21.4%, 12.0%, 38.5% and 18.3% respectively. Significant differences were
195 observed across the quintiles of serum Mg for fasting glucose, as well as the
196 prevalence of DM and HU.

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

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

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3 213 and DM in OA patients. Both the age-gender adjusted OR values (Model 1) and the
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5 214 multivariable adjusted OR values (Model 2) suggested a strong inverse association
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7 215 between serum Mg and DM. The age-gender adjusted ORs for the prevalence of DM
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9 216 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
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11 217 (95%CI 0.15-0.55, P<0.001), and 0.20 (95%CI 0.10-0.40, P<0.001) in the second,
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13 218 third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was
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15 219 <0.001. The multivariable adjusted ORs for the prevalence of DM were 0.40 (95%CI
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17 220 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,
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19 221 P<0.001), and 0.21 (95%CI 0.11-0.42, P<0.001) in the second, third, fourth and fifth
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21 222 quintiles of serum Mg respectively, and the P for trend was <0.001. The sensitivity
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23 223 analysis, by adding eGFR into model 2, showed similar results - significant lower
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25 224 prevalence of DM in the second (OR=0.40, 95%CI 0.23-0.70, P=0.001), third
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27 225 (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),
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29 226 and highest quintiles (OR=0.22, 95%CI 0.11-0.44, P<0.001) compared with the
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31 227 reference quintile of serum Mg, and the P for trend was <0.001.

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33 228 The prevalence of HP in each quintile of serum Mg in OA patients was depicted
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35 229 in Figure 1 (C). The multivariable-adjusted relations between serum Mg and HP in
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37 230 OA patients were illustrated in Table 4. According to both the age-gender adjusted
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39 231 ORs (Model 1) and the multivariable adjusted ORs (Model 2), there was no
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41 232 significant association between serum Mg and HP, and the P for trend were 0.929 and
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43 233 0.377, respectively. The sensitivity analysis, by adding eGFR into model 2, reached
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45 234 the same results.

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47 235 The prevalence of HU in each category of serum Mg in OA patients was shown
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49 236 in Figure 1 (D). The multivariable-adjusted relations between serum Mg and HU in
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51 237 OA patients were illustrated in Table 5. Both the age-gender adjusted OR values
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53 238 (Model 1) and the multivariable adjusted OR values (Model 2) suggested significant
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55 239 decreased prevalence of HU in the third quintile (age-gender adjusted OR=0.44,
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57 240 95%CI 0.26-0.75, P=0.002; multivariable adjusted OR=0.38, 95%CI 0.22-0.67,
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59 241 P=0.001) and fifth quintile (age-gender adjusted OR=0.51, 95%CI 0.30-0.85, P=0.010;
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242 multivariable adjusted OR=0.50, 95%CI 0.29-0.87, P=0.013) compared with the

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3 243 lowest quintile of serum Mg, and the P for trend were 0.008 and 0.006, respectively.
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5 244 The sensitivity analysis, by adding eGFR into model 2, showed similar outcomes -
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7 245 significant lower prevalence of HU in the third (OR=0.33, 0.19-0.59, P<0.001), fourth
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9 246 (OR=0.52, 95%CI 0.30-0.91, P=0.022), and highest quintiles (OR=0.39, 95%CI
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11 247 0.22-0.70, P=0.001) compared with the reference quintile of serum Mg, and the P for
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13 248 trend was <0.001.

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16 250 **Discussion**

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18 251 The results of this study suggested that the serum Mg concentration was negatively
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20 252 associated with the prevalence of MetS, DM and HU in subjects with radiographic
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22 253 knee OA. To control potential confounders, several covariates including
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24 254 characteristics, living habits and underlying diseases were selected, and even the
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26 255 eGFR was added into the multivariable logistic regression models to eliminate the
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28 256 influence of renal function on Mg excretion. The reverse associations mentioned
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30 257 above remained significant after adjustments of these confounders. However, the
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32 258 association between serum Mg and the prevalence of MetS was nonlinear, with no
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34 259 clear trend in the third and fourth quintiles of serum Mg. Moreover, the negative
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36 260 association between serum Mg and the prevalence of HP was not observed in
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38 261 radiographic knee OA patients.

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40 262 Mg, the fourth most abundant cation in human body and the second most profuse
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42 263 intracellular cation, is a metallic cofactor for over 300 enzymatic reactions. It appears
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44 264 to play an important role in glucose metabolism and insulin homeostasis, which are
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46 265 both highly correlated with metabolic diseases, especially MetS and DM. The
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48 266 mechanisms involved in Mg deficiency in patients with MetS, DM and HU are
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50 267 probably multifactorial. The most important factor may be insulin resistance, as Mg is
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52 268 essential for insulin action and is a critical cofactor for several enzymes in
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54 269 carbohydrate metabolism, which is important for the phosphorylation reactions of
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56 270 tyrosine-kinase in the insulin receptor.^{31 54-58} Of course, it is necessary to highlight the
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58 271 fact that insulin can also induce Mg excretion⁵⁹ and produce a significant decline of
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60 272 plasma Mg through ion exchange.⁶⁰ Thus, there seems to be a vicious circle between

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3 273 Mg deficiency and insulin resistance.

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5 274 Other potential mechanisms include glucose transportation,⁵⁷ oxidative stress⁵⁷
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7 275 and inflammatory cytokines,⁶¹⁻⁶³ and cellular calcium homeostasis.⁵⁵ Mg is an
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9 276 essential cofactor of the high-energy phosphate-bound enzymatic pathways involved
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11 277 in the modulation of glucose transport across cell membranes.⁵⁷ It also plays a role in
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13 278 the mechanisms of cellular antioxidant defense.⁶⁴ The oxidative stress, defined as a
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15 279 persistent imbalance between the excessive production of reactive oxygen species
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17 280 and/or defects in antioxidant defense, has been implicated in the pathogenesis of
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19 281 diabetic complications.⁵⁷ Moreover, low serum Mg levels are strongly related to
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21 282 elevated serum concentrations of both tumor necrosis factor alpha and C-reactive
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23 283 protein (CRP),⁶⁵ suggesting that Mg deficiency may contribute to the development of
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25 284 low-grade chronic inflammation syndrome and the development of glucose metabolic
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27 285 disorders through the former pathway. In addition, lower Mg concentration can
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29 286 enhance calcium-mediated vasoconstriction, blunt cardiac and smooth muscle
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31 287 relaxation, and thus contribute to BP elevation.⁵⁵ However, the decreased serum
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33 288 calcium concentration in radiographic knee OA patients may weaken the association
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35 289 between Mg and HP.⁶⁶

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37 290 MetS^{21 22} and DM^{4 23 24} were reported to be the risk factors of OA progression.
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39 291 Moreover, serum Mg level has been proved to be significantly associated with the
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41 292 CRP concentration,^{27 67-69} and higher CRP might serve as a prediction factor for OA
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43 293 progression.^{70 71} Thus, OA progression may be delayed by elevating the serum Mg
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45 294 level through reducing the prevalence of MetS and DM and decreasing the level of
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47 295 CRP. Above all, the present study indicated that the elevation of serum Mg level has
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49 296 the potential to reduce the prevalence of MetS, DM and HU in knee OA patients and
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51 297 thereby may delay the progression of knee OA. However, the specific mechanism
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53 298 needs to be further explored.

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55 299 The present study has several strengths. Firstly, this is the first study examining
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57 300 the associations between serum Mg and the prevalence of MetS, DM, HP and HU in
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59 301 radiographic knee OA patients. The results of this study will provide a new insight
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302 into the treatment of knee OA. Secondly, the multivariable logistical regression

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3 303 models were adjusted for a considerable number of potential confounding factors,
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5 304 which greatly improved the reliability of the results. Thirdly, the kidney is the key
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7 305 organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by
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9 306 adding eGFR into multivariable logistic regression models which showed that the
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11 307 reverse associations remained significant.

12 308 Limitations of the present study should also be admitted. The cross-sectional
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14 309 design precludes causal correlations, so further prospective studies and intervention
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16 310 trials should be undertaken to establish a causal association between serum Mg with
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18 311 the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. Since no
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20 312 previous research investigated such associations in knee OA patients, the value of this
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22 313 study should not be blotted out by the cross-sectional nature. Another limitation of
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24 314 this study lies in the relatively small sample size, and thus, extensive high-quality
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26 315 researches based on a larger sample are needed. Moreover, the dietary intake of Mg in
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28 316 relation to the prevalence of MetS, DM, HP and HU were not assessed in the present
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30 317 study. Last but not the least, it is important to highlight that Mg is an intracellular ion;
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32 318 therefore, the serum Mg concentration must be considered as a poor indicator of body
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34 319 Mg content,⁷² even though it has been used in many studies. However, blood Mg level
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36 320 is the second best indicator of body status.⁷³

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39 322 **Conclusions**

40 323 The present study concluded that the serum Mg concentration was inversely
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42 324 associated with the prevalence of MetS, DM and HU in radiographic knee OA
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44 325 patients.

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327 **Contributors**

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

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347 **Competing interests**

348 The authors declare that they have no conflict of interest.

350 **Ethics approval**

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

354 **Data sharing statement**

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

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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

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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

571 Data are mean (Standard Deviation), unless otherwise indicated; Mg, magnesium; OA, osteoarthritis; BMI, body mass index; HDL, high density lipoprotein; eGFR,
572 estimated glomerular filtration rate; MetS, metabolic syndrome; DM, diabetes mellitus; HP, hypertension; HU, hyperuricemia.
573 # P values are for test of difference across all quintiles of serum Mg.

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7 575 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

8 576 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

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10 577 horizontal axis denotes the serum Mg level, and the vertical axis indicates whether a subject is diagnosed with the specific disease: (+) - disease; (-) - no disease.

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12 578 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

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14 579 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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581 Table 2 Multivariable-adjusted relations of serum Mg and MetS in OA patients (n = 962)

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.027	0.830	0.114	0.024	-

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

583 *Model 1 was adjusted for age (continuous data) and gender (male, female); Model 2 was adjusted for age (continuous data), gender (male, female), educational
 584 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
 585 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					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.001	<0.001	<0.001	<0.001	-

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

589 *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,
590 female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no),
591 hypertension (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.235	0.574	0.727	0.818	-

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

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 diabetes (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.142	<0.001	0.022	0.001	-

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

601 * 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,
 602 female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no),
 603 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)

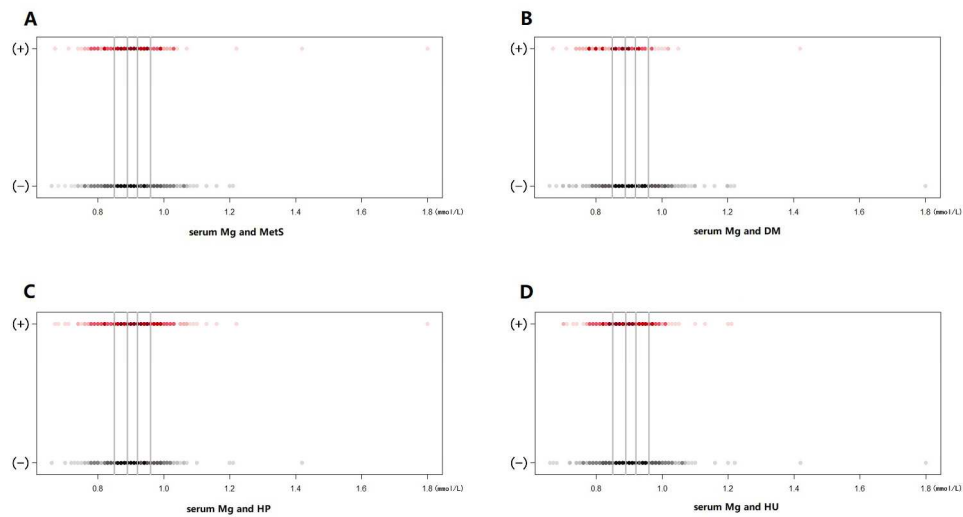


Figure 1 The prevalence of MetS (A), DM (B), HP (C) and HU (D) in each quintile of serum Mg in radiographic knee OA patients

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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	-
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	-
		<i>Cross-sectional study</i> —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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Keywords:	osteoarthritis, magnesium, metabolic syndrome, diabetes, Hypertension < CARDIOLOGY, hyperuricemia

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Manuscripts

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3 **1 Association between serum magnesium concentration and metabolic syndrome,**
4 **2 diabetes, hypertension and hyperuricemia in knee osteoarthritis: a**
5 **3 cross-sectional study in Hunan Province, China**
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2
3 **Abstract**

4
5 **Objectives:** To examine the associations between serum magnesium (Mg)
6
7 concentration with the prevalence of metabolic syndrome (MetS), diabetes mellitus
8
9 (DM), hypertension (HP) and hyperuricemia (HU) in radiographic knee osteoarthritis
10
11 (OA) patients.

12
13 **Methods:** The present study was conducted at the Health Management Center of
14
15 Xiangya Hospital. Radiographic OA was evaluated for patients aged over 40 years
16
17 with basic characteristics and blood biochemical assessment. Serum Mg concentration
18
19 was measured using the chemiluminescence method. MetS, DM, HP and HU were
20
21 diagnosed based on standard protocols. The associations between serum Mg
22
23 concentration with MetS, DM, HP and HU were evaluated by conducting
24
25 multivariable adjusted logistic regression.

26
27 **Results:** A total of 962 radiographic knee OA patients were included. Compared with
28
29 the lowest quintile, the multivariable-adjusted odds ratios (ORs) and related 95%
30
31 confidence intervals (95%CI) of DM were 0.40 (95%CI 0.23-0.70, P=0.001), 0.33
32
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
34
35 0.11-0.44, P<0.001) in the second, third, fourth and highest quintiles of serum Mg,
36
37 respectively (P for trend <0.001); the multivariable-adjusted ORs of HU were 0.33
38
39 (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
40
41 0.22-0.70, P=0.001) in the third, fourth and highest quintiles of serum Mg
42
43 respectively (P for trend <0.001); and the multivariable-adjusted ORs of MetS were
44
45 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)
46
47 in the highest quintiles of serum Mg. However, the inverse association between serum
48
49 Mg and the prevalence of MetS was nonlinear (P for trend =0.067). There was no
50
51 significant association between serum Mg and HP in OA patients.

52
53 **Conclusions:** The serum Mg concentration was inversely associated with the
54
55 prevalence of MetS, DM and HU in radiographic knee OA patients.

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

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59 **Key words:** osteoarthritis, magnesium, metabolic syndrome, diabetes, hypertension,
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61 hyperuricemia

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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.

64 Introduction

65 The association between osteoarthritis (OA) and metabolic diseases, especially
66 metabolic syndrome (MetS)^{1 2} and diabetes mellitus (DM),³⁻⁵ has drawn increasing
67 attention in the past few years. OA includes three specific phenotypes: metabolic OA,
68 age-related OA and injury-related OA.⁶ A large number of studies have indicated that
69 the prevalence of MetS,⁷⁻⁹ DM¹⁰⁻¹⁸ and hypertension (HP)^{7 9-13 19 20} is either higher in
70 OA patients or associated with OA. In addition, some other studies reported that
71 MetS,^{21 22} DM^{23 24} and HP^{21 22} are risk factors of OA progression. Thus, it appears
72 necessary to pay more attention and adopt appropriate measures to reduce the high
73 prevalence of metabolic diseases in OA patients, which also seems to be beneficial in
74 delaying OA progression.

75 Serum magnesium (Mg), one of the most important micronutrients for human
76 health, has been reported to be negatively associated with MetS,²⁵⁻²⁹ DM³⁰⁻³⁸ and HP³⁰
77 ³⁹⁻⁴¹ by lots of studies. Meanwhile, our previous study showed an inverse association
78 between serum Mg and hyperuricemia (HU).⁴² However, to the best knowledge of the
79 authors, there is not yet a study examining the association between the serum Mg
80 concentration and the aforementioned metabolic diseases (MetS, DM, HP and HU) in
81 OA patients. On the other hand, we have previously shown that the serum Mg
82 concentration may be inversely associated with radiographic knee OA.⁴³ Therefore,
83 we speculate that the prevalence of MetS, DM, HP and HU in OA patients may be
84 reduced by elevating the level of serum Mg, which can in turn delay OA progression.
85 Thus, the objective of the present study was to examine the associations between the
86 serum Mg concentration with the prevalence of MetS, DM, HP and HU in
87 radiographic knee OA patients. It was hypothesized that serum Mg concentration was
88 inversely associated with these diseases.

90 Methods

91 Study population

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

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3 94 published previously.⁴²⁻⁴⁶ The protocol has been reviewed and approved by the Ethics
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5 95 Committee of Xiangya Hospital, Central South University (reference numbers:
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7 96 201312459), and the methods were developed in “accordance” with the approved
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9 97 guidelines. Informed consent has been obtained from all participants. Registered
10
11 98 nurses were engaged to interview all participants during the examination using a
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13 99 standard questionnaire, with the purpose to collect information on demographic
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15 100 characteristics and health-related habits. Participants were selected based on the
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17 101 following inclusion criteria: 1) 40 years old or above; 2) undergoing weight-bearing
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19 102 bilateral anteroposterior radiography of the knee, and diagnosed with knee OA
20
21 103 according to the Kellgren-Lawrence (K-L) radiographic atlas (knee joint was graded
22
23 104 K-L 2 or above); 3) availability of all basic characteristics, including age, gender,
24
25 105 body mass index (BMI) and blood pressure; 4) availability of biochemical test results,
26
27 106 including serum Mg concentration; 5) availability of information related to the living
28
29 107 habits, including education background, activity level, smoking, drinking and
30
31 108 medication status. Initially, the present cross-sectional study retrieved 1820
32
33 109 radiographic knee OA patients aged over 40 years who exhibited sound basic
34
35 110 characteristics and required blood biochemical assessment (including serum Mg
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37 111 concentration). Among them, 962 patients offered demographic characteristics and
38
39 112 health-related habits and were finally included in this study.

113

114 **Blood biochemistry**

115 All blood samples were drawn after a 12-hour overnight fast and were kept at 4°C
116 until analysis. Blood tests were undertaken using the Beckman Coulter AU 5800
117 (Beckman Coulter Inc., Brea, CA, USA). The inter- and intra-assay coefficients of
118 variation were tested at both low concentrations (2.5 mmol/L for glucose, 118 µmol/L
119 for uric acid and 0.60 mmol/L for serum Mg) and high concentrations (6.7 mmol/L for
120 glucose, 472 µmol/L for uric acid and 1.00 mmol/L for serum Mg) of standard human
121 samples. The intra-assay coefficients of variation were 0.98% (2.5 mmol/L) and 1.72%
122 (6.7 mmol/L) for glucose, 1.39% (118 µmol/L) and 0.41% (472 µmol/L) for uric acid,
123 and 1.86% (0.60 mmol/L) and 1.65% (1.00 mmol/L) for serum Mg respectively. The

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3 124 inter-assay coefficients of variation were 2.45% (2.5 mmol/L) and 1.46% (6.7 mmol/L)
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5 125 for glucose, 1.40% (118 $\mu\text{mol/L}$) and 1.23% (472 $\mu\text{mol/L}$) for uric acid, and 1.87%
6
7 126 (0.60 mmol/L) and 1.70% (1.00 mmol/L) for serum Mg respectively.
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11 128 **Assessment of other exposures**

12 129 Blood pressure was measured by an electronic sphygmomanometer. The weight and
13
14 130 height of each subject were measured respectively to calculate the BMI. Information
15
16 131 on the average frequency of physical activity (never, one to two times per week, three
17
18 132 to four times per week, five times and above per week) and average duration of
19
20 133 physical activity (less than half an hour, half an hour to one hour, one to two hours,
21
22 134 more than two hours) were collected through survey questionnaire. The smoking,
23
24 135 alcohol drinking and medication status were collected during the face-to-face
25
26 136 interview.
27

28 137

29 138 **Assessment of MetS, DM, HP and HU**

30 139 MetS was diagnosed based on the Chinese Diabetes Society (CDS) criteria,⁴⁷⁻⁴⁹ which
31
32 140 requires meeting at least 3 of the following 4 items: (1) BMI ≥ 25 kg/m²; (2) Fasting
33
34 141 plasma glucose (FPG) ≥ 6.1 mmol/L, or diagnosed DM; (3) Systolic blood pressure
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36 142 (BP) ≥ 140 mmHg or diastolic BP ≥ 90 mmHg, or treatment of previously diagnosed
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38 143 HP; (4) Triglycerides ≥ 1.7 mmol/L and/or HDL-cholesterol < 0.9 mmol/L in male or
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40 144 < 1.0 mmol/L in female, or treatment for this lipid abnormality. Subjects with the
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42 145 fasting glucose ≥ 7.0 mmol/L or currently undergoing drug treatment for blood glucose
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44 146 control were regarded as DM patients, and subjects with the systolic blood pressure
45
46 147 ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or currently undertaking
47
48 148 antihypertensive medication were regarded as HP patients. HU was defined as uric
49
50 149 acid ≥ 416 $\mu\text{mol/L}$ for male and ≥ 360 $\mu\text{mol/L}$ for female or currently undergoing drug
51
52 150 treatment for uric acid control.
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54 151

55 152 **Statistical analysis**

56 153 The continuous data were expressed as mean with standard deviation, and the

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3 154 category data were expressed in percentage. Differences in continuous data were
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5 155 evaluated by one-way classification ANOVA (normally distributed data) or
6
7 156 Kruskal-Wallis H test (non-normally distributed data), while differences in category
8
9 157 data were assessed by the χ^2 test. The serum Mg was classified into five categories
10
11 158 based on the quintile distribution: ≤ 0.85 , 0.86-0.89, 0.90-0.92, 0.93-0.96 and ≥ 0.97
12
13 159 mmol/L. The prevalence of MetS, DM, HP and HU in each quintile of serum Mg in
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15 160 OA patients were assessed by scatter plots.

16
17 161 Logistic regression was conducted to calculate the odds ratios (ORs) with 95%
18
19 162 confidence intervals (95%CI) for the associations between serum Mg and MetS, DM,
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21 163 HP and HU. Specifically, model 1 was adjusted by covariates of age (continuous data)
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23 164 and gender (male, female). Then, model 2 was adjusted by additional covariates of
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25 165 BMI (continuous data), educational level (high school or above, lower than high
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27 166 school), smoking status (yes, no), activity level (continuous data), alcohol drinking
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29 167 status (yes, no), HP (yes, no), DM (yes, no), and dyslipidemia (yes, no) on the basis of
30
31 168 model 1. Dyslipidemia was defined as triglycerides ≥ 1.7 mmol/L and/or
32
33 169 HDL-cholesterol < 0.9 mmol/L in male or < 1.0 mmol/L in female, or treatment for this
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35 170 lipid abnormality. Notably, the selection of covariates in model 2 varied slightly for
36
37 171 examining different associations (between serum Mg and MetS, DM, HP or HU). For
38
39 172 example, BMI, HP and dyslipidemia were adjusted for the association between serum
40
41 173 Mg and DM, but not for the association between serum Mg and MetS, simply because
42
43 174 MetS was diagnosed based on BMI, HP and dyslipidemia status. Model 3 was
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45 175 established based on model 2, with adjustment of an additional covariate, estimated
46
47 176 glomerular filtration rate (eGFR). eGFR (continuous data) was calculated from the
48
49 177 Chronic Kidney Disease Epidemiology Collaboration equation.⁵⁰ All covariates in the
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51 178 present study were chosen referring to some of the previous similar studies.^{27 33 51 52}
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53 179 Tests for linear trends were conducted based on logistic regression using a median
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55 180 variable of Mg concentration in each category.

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57 181 Scatter plots were plotted using R 3.4.4.⁵³ Other data analyses were performed
58
59 182 using SPSS 17.0; $P \leq 0.05$ was considered to be statistically significant. All tests were
60
183 two tailed.

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185 Patient and public involvement

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

189

190 Results

191 A total of 962 subjects (377 females, accounting for 39.2%) were included in the
192 present cross-sectional study. The characteristics of the study population according to
193 quintiles of serum Mg were presented in Table 1. The mean age of the subjects was
194 54.9 ± 7.6 years old. The overall prevalence of MetS, DM, HP and HU in OA patients
195 were 21.4%, 12.0%, 38.5% and 18.3% respectively. Significant differences were
196 observed across the quintiles of serum Mg for fasting glucose, as well as the
197 prevalence of DM and HU.

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

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

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3 214 and DM in OA patients. Both the age-gender adjusted OR values (Model 1) and the
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5 215 multivariable adjusted OR values (Model 2) suggested a strong inverse association
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7 216 between serum Mg and DM. The age-gender adjusted ORs for the prevalence of DM
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9 217 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
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11 218 (95%CI 0.15-0.55, P<0.001), and 0.20 (95%CI 0.10-0.40, P<0.001) in the second,
12
13 219 third, fourth and fifth quintiles of serum Mg respectively, and the P for trend was
14
15 220 <0.001. The multivariable adjusted ORs for the prevalence of DM were 0.40 (95%CI
16
17 221 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,
18
19 222 P<0.001), and 0.21 (95%CI 0.11-0.42, P<0.001) in the second, third, fourth and fifth
20
21 223 quintiles of serum Mg respectively, and the P for trend was <0.001. The sensitivity
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23 224 analysis, by adding eGFR into model 2, showed similar results - significant lower
24
25 225 prevalence of DM in the second (OR=0.40, 95%CI 0.23-0.70, P=0.001), third
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27 226 (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),
28
29 227 and highest quintiles (OR=0.22, 95%CI 0.11-0.44, P<0.001) compared with the
30
31 228 reference quintile of serum Mg, and the P for trend was <0.001.

32
33 229 The prevalence of HP in each quintile of serum Mg in OA patients was depicted
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35 230 in Figure 1 (C). The multivariable-adjusted relations between serum Mg and HP in
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37 231 OA patients were illustrated in Table 4. According to both the age-gender adjusted
38
39 232 ORs (Model 1) and the multivariable adjusted ORs (Model 2), there was no
40
41 233 significant association between serum Mg and HP, and the P for trend were 0.929 and
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43 234 0.377, respectively. The sensitivity analysis, by adding eGFR into model 2, reached
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45 235 the same results.

46
47 236 The prevalence of HU in each category of serum Mg in OA patients was shown
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49 237 in Figure 1 (D). The multivariable-adjusted relations between serum Mg and HU in
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51 238 OA patients were illustrated in Table 5. Both the age-gender adjusted OR values
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53 239 (Model 1) and the multivariable adjusted OR values (Model 2) suggested significant
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55 240 decreased prevalence of HU in the third quintile (age-gender adjusted OR=0.44,
56
57 241 95%CI 0.26-0.75, P=0.002; multivariable adjusted OR=0.38, 95%CI 0.22-0.67,
58
59 242 P=0.001) and fifth quintile (age-gender adjusted OR=0.51, 95%CI 0.30-0.85, P=0.010;
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243 multivariable adjusted OR=0.50, 95%CI 0.29-0.87, P=0.013) compared with the

244 lowest quintile of serum Mg, and the P for trend were 0.008 and 0.006, respectively.
245 The sensitivity analysis, by adding eGFR into model 2, showed similar outcomes -
246 significant lower prevalence of HU in the third (OR=0.33, 0.19-0.59, P<0.001), fourth
247 (OR=0.52, 95%CI 0.30-0.91, P=0.022), and highest quintiles (OR=0.39, 95%CI
248 0.22-0.70, P=0.001) compared with the reference quintile of serum Mg, and the P for
249 trend was <0.001.

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251 Discussion

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

263 Mg, the fourth most abundant cation in human body and the second most profuse
264 intracellular cation, is a metallic cofactor for over 300 enzymatic reactions. It appears
265 to play an important role in glucose metabolism and insulin homeostasis, which are
266 both highly correlated with metabolic diseases, especially MetS and DM. The
267 mechanisms involved in Mg deficiency in patients with MetS, DM and HU are
268 probably multifactorial. The most important factor may be insulin resistance, as Mg is
269 essential for insulin action and is a critical cofactor for several enzymes in
270 carbohydrate metabolism, which is important for the phosphorylation reactions of
271 tyrosine-kinase in the insulin receptor.^{31 54-58} Of course, it is necessary to highlight the
272 fact that insulin can also induce Mg excretion⁵⁹ and produce a significant decline of
273 plasma Mg through ion exchange.⁶⁰ Thus, there seems to be a vicious circle between

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3 274 Mg deficiency and insulin resistance.

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5 275 Other potential mechanisms include glucose transportation,⁵⁷ oxidative stress⁵⁷
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7 276 and inflammatory cytokines,⁶¹⁻⁶³ and cellular calcium homeostasis.⁵⁵ Mg is an
8
9 277 essential cofactor of the high-energy phosphate-bound enzymatic pathways involved
10
11 278 in the modulation of glucose transport across cell membranes.⁵⁷ It also plays a role in
12
13 279 the mechanisms of cellular antioxidant defense.⁶⁴ The oxidative stress, defined as a
14
15 280 persistent imbalance between the excessive production of reactive oxygen species
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17 281 and/or defects in antioxidant defense, has been implicated in the pathogenesis of
18
19 282 diabetic complications.⁵⁷ Moreover, low serum Mg levels are strongly related to
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21 283 elevated serum concentrations of both tumor necrosis factor alpha and C-reactive
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23 284 protein (CRP),⁶⁵ suggesting that Mg deficiency may contribute to the development of
24
25 285 low-grade chronic inflammation syndrome and the development of glucose metabolic
26
27 286 disorders through the former pathway. In addition, lower Mg concentration can
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29 287 enhance calcium-mediated vasoconstriction, blunt cardiac and smooth muscle
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31 288 relaxation, and thus contribute to BP elevation.⁵⁵ However, the decreased serum
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33 289 calcium concentration in radiographic knee OA patients may weaken the association
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35 290 between Mg and HP.⁶⁶

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37 291 MetS^{21 22} and DM^{4 23 24} were reported to be the risk factors of OA progression.
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39 292 Moreover, serum Mg level has been proved to be significantly associated with the
40
41 293 CRP concentration,^{27 67-69} and higher CRP might serve as a prediction factor for OA
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43 294 progression.^{70 71} Thus, OA progression may be delayed by elevating the serum Mg
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45 295 level through reducing the prevalence of MetS and DM and decreasing the level of
46
47 296 CRP. Above all, the present study indicated that the elevation of serum Mg level has
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49 297 the potential to reduce the prevalence of MetS, DM and HU in knee OA patients and
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51 298 thereby may delay the progression of knee OA. However, the specific mechanism
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53 299 needs to be further explored.

54
55 300 The present study has several strengths. Firstly, this is the first study examining
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57 301 the associations between serum Mg and the prevalence of MetS, DM, HP and HU in
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59 302 radiographic knee OA patients. The results of this study will provide a new insight
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303 into the treatment of knee OA. Secondly, the multivariable logistical regression

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3 304 models were adjusted for a considerable number of potential confounding factors,
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5 305 which greatly improved the reliability of the results. Thirdly, the kidney is the key
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7 306 organ in maintaining Mg homeostasis. This study conducted a sensitivity analysis by
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9 307 adding eGFR into multivariable logistic regression models which showed that the
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11 308 reverse associations remained significant.

12 309 Limitations of the present study should also be admitted. The cross-sectional
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14 310 design precludes causal correlations, so further prospective studies and intervention
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16 311 trials should be undertaken to establish a causal association between serum Mg with
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18 312 the prevalence of MetS, DM, HP and HU in radiographic knee OA patients. Since no
19
20 313 previous research investigated such associations in knee OA patients, the value of this
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22 314 study should not be blotted out by the cross-sectional nature. Another limitation of
23
24 315 this study lies in the relatively small sample size, and thus, extensive high-quality
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26 316 researches based on a larger sample are needed. Moreover, the dietary intake of Mg in
27
28 317 relation to the prevalence of MetS, DM, HP and HU were not assessed in the present
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30 318 study. Last but not the least, it is important to highlight that Mg is an intracellular ion;
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32 319 therefore, the serum Mg concentration must be considered as a poor indicator of body
33
34 320 Mg content,⁷² even though it has been used in many studies. However, blood Mg level
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36 321 is the second best indicator of body status.⁷³

37 322

38 323 **Conclusions**

39
40 324 The present study concluded that the serum Mg concentration was inversely
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42 325 associated with the prevalence of MetS, DM and HU in radiographic knee OA
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44 326 patients.

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328 **Contributors**

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

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348 **Competing interests**

349 The authors declare that they have no conflict of interest.

351 **Ethics approval**

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

355 **Data sharing statement**

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

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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

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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.
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7 576 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

8 577 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

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10 578 horizontal axis denotes the serum Mg level, and the vertical axis indicates whether a subject is diagnosed with the specific disease: (+) - disease; (-) - no disease.

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12 579 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

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14 580 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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582 Table 2 Multivariable-adjusted relations of serum Mg and MetS in OA patients (n = 962)

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> 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).

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.001	<0.001	<0.001	<0.001	-

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

590 *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,
591 female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no),
592 hypertension (yes, no), and dyslipidemia (yes, no); Model 3 was adjusted based on model 2, with additional factor of eGFR (continuous data).

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.235	0.574	0.727	0.818	-

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

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

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

	Quintiles of serum Mg					<i>P</i> for trend
	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	-
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
<i>P</i> 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
<i>P</i> 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
<i>P</i> value	-	0.142	<0.001	0.022	0.001	-

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

602 * 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,
 603 female), educational level (high school or above, lower than high school), smoking status (yes, no), activity level (continuous data), alcohol drinking status (yes, no),
 604 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)

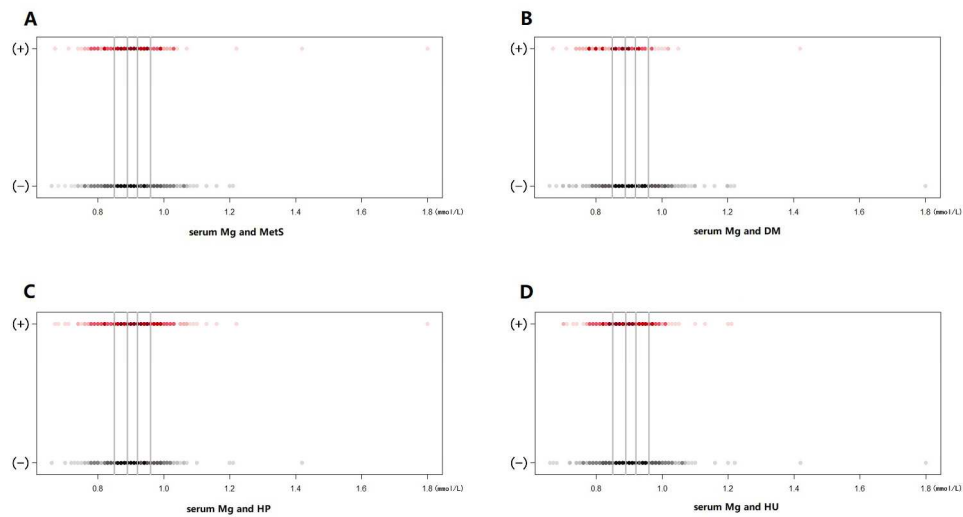


Figure 1 The prevalence of MetS (A), DM (B), HP (C) and HU (D) in each quintile of serum Mg in radiographic knee OA patients

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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4-5
		<i>Case-control study</i> —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	
		<i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed	-
		<i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed	4-5
		<i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed	
		<i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	-
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	-
		<i>Cross-sectional study</i> —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.