

# Serum Calcium and the Risk of Incident Metabolic Syndrome: A 4.3-Year Retrospective Longitudinal Study

Jong Ha Baek<sup>1,2,\*</sup>, Sang-Man Jin<sup>1,\*</sup>, Ji Cheol Bae<sup>3</sup>, Jae Hwan Jee<sup>4</sup>, Tae Yang Yu<sup>1</sup>, Soo Kyoung Kim<sup>5</sup>, Kyu Yeon Hur<sup>1</sup>, Moon-Kyu Lee<sup>1</sup>, Jae Hyeon Kim<sup>1</sup>

<sup>1</sup>Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul,

<sup>2</sup>Division of Endocrinology and Metabolism, Department of Internal Medicine, Gyeongsang National University Changwon Hospital, Gyeongsang National University School of Medicine, Changwon,

<sup>3</sup>Division of Endocrinology and Metabolism, Department of Medicine, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon,

<sup>4</sup>Department of Health Promotion Center, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul,

<sup>5</sup>Department of Internal Medicine, Gyeongsang National University Hospital, Gyeongsang National University School of Medicine, Jinju, Korea

**Background:** An association between serum calcium level and risk of metabolic syndrome (MetS) has been suggested in cross-sectional studies. This study aimed to evaluate the association between baseline serum calcium level and risk of incident MetS in a longitudinal study.

**Methods:** We conducted a retrospective longitudinal study of 12,706 participants without MetS who participated in a health screening program, had normal range serum calcium level at baseline (mean age, 51 years), and were followed up for 4.3 years (18,925 person-years). The risk of developing MetS was analyzed according to the baseline serum calcium levels.

**Results:** A total of 3,448 incident cases (27.1%) of MetS developed during the follow-up period. The hazard ratio (HR) for incident MetS did not increase with increasing tertile of serum calcium level in an age- and sex-matched model ( $P$  for trend=0.915). The HRs (95% confidence interval [CI]) for incident MetS comparing the second and the third tertiles to the first tertile of baseline serum calcium level were 0.91 (95% CI, 0.84 to 0.99) and 0.85 (95% CI, 0.78 to 0.92) in a fully adjusted model, respectively ( $P$  for trend=0.001). A decreased risk of incident MetS in higher tertiles of serum calcium level was observed in subjects with central obesity and/or a metabolically unhealthy state at baseline.

**Conclusion:** There was no positive correlation between baseline serum calcium levels and incident risk of MetS in this longitudinal study. There was an association between higher serum calcium levels and decreased incident MetS in individuals with central obesity or two components of MetS at baseline.

**Keywords:** Calcium; Longitudinal studies; Metabolic syndrome; Obesity, abdominal

## INTRODUCTION

Metabolic syndrome (MetS) is characterized by a cluster of risk factors such as central obesity, dyslipidemia, elevated blood pressure, insulin resistance, and glucose intolerance, leading

MetS to be associated with a poor cardiovascular prognosis and increased mortality [1]. Although the prevalence of MetS varies according to age, sex, geographic location, and ethnicity [2-4], it has increased over the past decades [5,6] to become a global pandemic. Abdominal obesity is the most prevalent

Corresponding author: Jae Hyeon Kim  <http://orcid.org/0000-0001-5001-963X>  
Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea  
E-mail: jaehyeon@skku.edu

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\*Jong Ha Baek and Sang-Man Jin contributed equally to this study as first authors.

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manifestation of MetS [7], and obese patients with MetS are at higher risk of cardiovascular disease than non-obese patients with MetS [8]. Estimates from the National Health and Nutrition Examination Survey from 1999 to 2010 demonstrated that the prevalence of abdominal obesity has increased [4].

Intracellular calcium plays an important role in the regulation of lipid metabolism [9] and insulin sensitivity [10]. Serum calcium homeostasis is regulated within a narrow range, and is under tight hormonal control. Several epidemiological studies have demonstrated that a high level of serum calcium is associated with increased risk for type 2 diabetes mellitus (T2DM) [11], overweight or obesity [12], elevated blood pressure, hypercholesterolemia [13], as well as MetS [14]. Serum calcium concentrations were also positively correlated with increasing numbers of conventional MetS components [15]. Finally, an increased serum calcium concentration can predict increased all-cause and cardiovascular disease mortality in men [16].

A longitudinal study that evaluated the effect of serum calcium on the development of T2DM among the non-diabetes mellitus general population ( $n=863$ ) demonstrated that higher serum calcium levels are associated with an increased risk of developing T2DM [11]. On the other hand, Becerra-Tomas et al. [17] reported that an increased risk of T2DM was associated with change in serum calcium concentration, but not with baseline serum calcium levels among individuals at high cardiovascular risk ( $n=707$ ). As shown in previous studies, the association between serum calcium and incident metabolic diseases, including T2DM and MetS, is affected by several factors, including study design, patient population, and baseline metabolic health status. To our knowledge, there have been no longitudinal studies to date investigating the association between normal range of serum calcium level and risk of incident MetS. Therefore, the aim of this study was to investigate the association between serum calcium within the normal range and risk of incident MetS in a longitudinal study.

## METHODS

### Subjects

We conducted a retrospective longitudinal study in subjects who visited a health care center at the Samsung Medical Center for an annual or biannual comprehensive medical check-up. Of the 24,178 people who underwent at least four medical check-ups within 7 years (from January 2006 to December 2012), 12,706 individuals were included for analysis. Medical

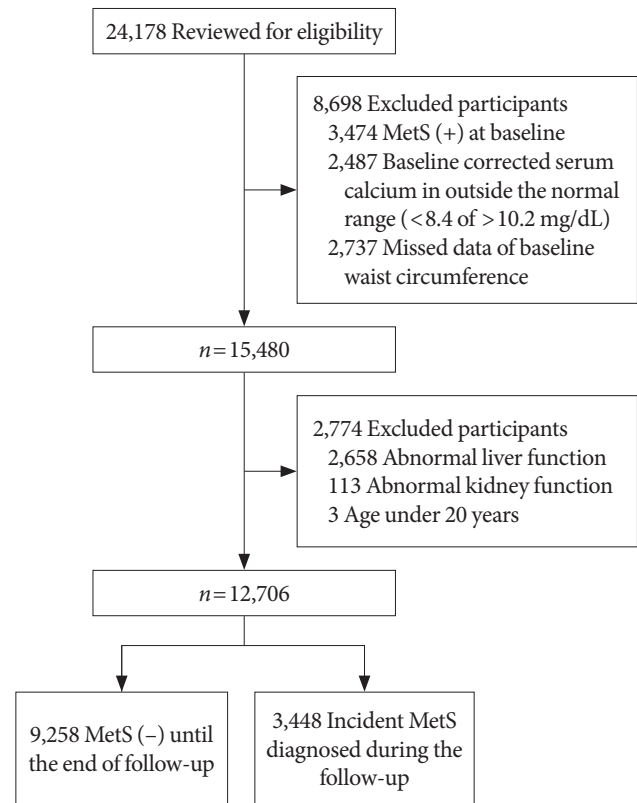


Fig. 1. Subject inclusion. MetS, metabolic syndrome.

check-up records from the first visit served as baseline data. We excluded 11,472 people based on the following criteria: initial visit records showed that they had already met the criteria for MetS ( $n=3,474$ ); baseline albumin-corrected serum calcium levels were outside the normal range (8.4 to 10.2 mg/dL,  $n=2,487$ ); baseline waist circumference (WC) data were unavailable ( $n=2,737$ ); total bilirubin or liver enzymes were elevated to greater than twice the upper limit of normal ( $n=2,658$ ); estimated glomerular filtration rate calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula was  $<60$  mL/min/1.73 m<sup>2</sup> ( $n=113$ ); or they were under 20 years of age ( $n=3$ ) (Fig. 1). The Institutional Review Board of Samsung Medical Center approved this study, and waived the informed consent requirement for this study, as the researcher accessed the database solely for analytic purposes, and did not access any personal information.

### Definition of MetS

MetS was defined according to the modified criteria proposed by the Adult Treatment Program III of the National Cholesterol Education Program (NCEP-ATP III) [18]. People with three

or more of the following criteria were classified as having MetS: (1) WC  $\geq 90$  cm in men or  $\geq 80$  cm in women; (2) triglycerides (TG)  $\geq 150$  mg/dL; (3) high density lipoprotein cholesterol (HDL-C)  $< 40$  mg/dL in men or  $< 50$  mg/dL in women; (4) elevated blood pressure (systolic blood pressure [SBP]  $\geq 130$  mm Hg and/or diastolic blood pressure [DBP]  $\geq 85$  mm Hg) or being on anti-hypertensive medications; or (5) fasting serum glucose (FPG)  $\geq 100$  mg/dL or being treated for diabetes [19].

### Clinical evaluation

During the clinical visits, we collected lifestyle behavior information and medical histories through a standardized self-reported questionnaire. We also assessed cigarette smoking and previously or newly diagnosed disease (hypercholesterolemia, diabetes, and hypertension).

Blood pressure was measured in the seated position after 5 minutes of rest, and was taken by trained nurses using a mercury sphygmomanometer in accordance with the Hypertension Detection and Follow-up Program protocol [20]. We measured patient height and body weight while wearing light clothing and no shoes. WC was measured at the narrowest point between the costal margin and the iliac crest.

### Laboratory methods

We obtained blood samples by venipuncture after an overnight fast, and samples were analyzed at the same certified laboratory at the Samsung Medical Center. Serum calcium concentrations (normal range, 8.4 to 10.2 mg/dL) were determined by the *o*-cresolphthalein complexone method (intra-assay coefficient of variation  $< 2\%$ ). Serum albumin levels (normal range, 3.8 to 5.3 g/dL) were measured by bromocresol green using a Roche modular DP analyzer (Roche Diagnostics, Basel, Switzerland). Albumin-corrected serum calcium (mg/dL) was calculated as  $[\text{serum calcium} + 0.8 \times (4 - \text{albumin})]$ . Serum TG and HDL-C levels were determined by an enzymatic colorimetric method using the Roche modular DP analyzer. Serum glucose levels were determined by the hexokinase method using a GLU kit (Roche Diagnostics) on the Roche modular DP analyzer. Glycosylated hemoglobin (HbA1c) measurements were taken by HPLC using the G8 Elution buffer kits (Tosoh, Yokkaichi, Japan) on HLC-723G8 (Tosoh). The HbA1c results were standardized to the reference method suggested by the Diabetes Control and Complication Trial, and met the National Glycohemoglobin Standardization Program standards. The homeostasis model assessment 2 of insulin resistance (HOMA2-IR)

calculation was based on model-derived estimates (rather than linear approximations) using the HOMA2 calculator version 2.2.3 (Diabetes Trials Unit, University of Oxford, Oxford, UK; <http://www.dtu.ox.ac.uk/homacalculator/download.php>) [21].

### Statistical analysis

Baseline characteristics were presented as the mean  $\pm$  standard deviation for continuous variables and the number and percentage for categorical variables. One-way analysis of covariance was used for continuous variables, and chi-square tests were used for categorical variables in order to assess the baseline characteristics according to the albumin-corrected serum calcium tertile. Pearson correlation coefficients were used to examine the strength of the relationship between serum calcium and the MetS conditions, as well as the HOMA2-IR.

We used multivariate Cox proportional regression models to evaluate the relative risk of incident MetS according to the albumin-corrected serum calcium tertiles, and as a continuous variable per 1 mg/dL increase of albumin-corrected serum calcium at baseline. Collinearity tests for variables used in multivariate Cox proportional regression analysis were performed using linear modeling of the outcome variables with calculation of the variance inflation factor (VIF) of the independent predictors. A VIF  $< 5$  was considered optimal for stability. Two different Cox regression models were used to adjust for potential confounding factors. The first model (model 1) was adjusted for sex and age, and the second model (model 2) was adjusted for age, sex, smoking status (current, ex-smoker, non-smoker), WC, SBP, DBP, TG level, HDL-C, FPG, and treatment for medical conditions, including hypertension, diabetes, and hypercholesterolemia.

The analysis was further stratified by presence of central obesity and presence of two components of MetS at baseline. Tests of linearity were conducted by identifying the mean value of the albumin-corrected serum calcium in each tertile as a continuous variable. A separate subgroup analysis for incident MetS in the metabolic risk group, such as the presence of central obesity or presence of two components of MetS at baseline, was also performed. Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and  $P \leq 0.05$  (two-tailed) were considered statistically significant. Meanwhile,  $P \leq 0.025$  (two-tailed) were considered statistically significant after correcting for multiple comparisons in the subgroup multivariate Cox regression analysis, according to central obesity and presence of two components of MetS.

## RESULTS

**Albumin-corrected serum calcium levels and metabolic conditions at baseline**

Of all subjects ( $n=12,706$ ), the mean age was 51 years and 6,873 (54.1%) were men. Baseline characteristics of the anthropometric and biochemical parameters according to the albumin-corrected serum calcium at baseline are shown in Table 1. With increasing serum calcium tertiles, there was a significant increase in age, SBP, DBP, FPG, HbA1c, and TG levels (all  $P<0.001$ ). In our simple linear regression analyses, there were also positive correlations between baseline albumin-corrected

serum calcium levels and age, SBP, DBP, HDL-C, FPG, and HbA1c. Meanwhile, HOMA2-IR was inversely correlated with albumin-corrected serum calcium levels ( $r=-0.026$ ,  $P=0.021$ ) (Table 2).

After a mean follow-up period of 4.3 years, a total of 3,448 incident cases (27.1%) of MetS developed. In crude and age- and sex-matched models, the hazard ratio (HR) for incident MetS comparing the second and third quartiles to the first quartile of change in serum calcium level did not increase significantly. However, the HR for incident MetS comparing the second and third quartiles to the first quartile of change in serum calcium level significantly decreased in the fully adjusted

**Table 1.** Baseline characteristics according to albumin-corrected serum calcium tertile

Characteristic	Corrected serum calcium tertile, mg/dL			Total	P value <sup>a</sup>
	Tertile 1 ( $<8.70$ )	Tertile 2 ( $8.70-8.94$ )	Tertile 3 ( $>8.94$ )		
Number	4,174	4,334	4,198	12,706	
Incident case	1,263 (28.0)	1,095 (27.4)	1,090 (26.0)	3,448 (27.1)	0.089
Male sex	2,319 (55.6)	2,395 (55.3)	2,159 (51.4)	6,873 (54.1)	$<0.001^b$
Smoker					0.045 <sup>b</sup>
Never	2,352 (56.3)	2,431 (56.1)	2,474 (58.9)	7,257 (57.1)	
Current	697 (16.7)	734 (16.9)	689 (16.4)	2,120 (16.7)	
Past	1,125 (27.0)	1,169 (27.0)	1,035 (24.7)	3,329 (26.2)	
Age, yr	50.5 $\pm$ 8.1	51.0 $\pm$ 8.3	51.3 $\pm$ 8.2	51.0 $\pm$ 8.2	$<0.001$
WC, cm	81.1 $\pm$ 8.7	81.4 $\pm$ 8.7	80.9 $\pm$ 8.6	81.1 $\pm$ 8.7	0.029
BMI, kg/m <sup>2</sup>	23.3 $\pm$ 2.6	23.4 $\pm$ 2.6	23.3 $\pm$ 2.6	23.3 $\pm$ 2.6	0.170
SBP, mm Hg	110 $\pm$ 14	112 $\pm$ 14	113 $\pm$ 15	112 $\pm$ 14	$<0.001$
DBP, mm Hg	68 $\pm$ 10	69 $\pm$ 10	70 $\pm$ 10	69 $\pm$ 10	$<0.001$
FPG, mg/dL	88 $\pm$ 13	89 $\pm$ 13	91 $\pm$ 15	89 $\pm$ 14	$<0.001$
HbA1c, %	5.4 $\pm$ 0.5	5.4 $\pm$ 0.5	5.5 $\pm$ 0.6	5.4 $\pm$ 0.6	$<0.001$
Triglyceride, mg/dL	111 $\pm$ 58	115 $\pm$ 61	117 $\pm$ 68	114 $\pm$ 62	$<0.001$
HDL-C, mg/dL	59 $\pm$ 14	59 $\pm$ 13	60 $\pm$ 14	59 $\pm$ 14	0.001
Corrected calcium, mg/dL	8.56 $\pm$ 0.08	8.82 $\pm$ 0.07	9.17 $\pm$ 0.18	8.85 $\pm$ 0.28	-
Diabetes	170 (4.1)	174 (4.0)	201 (4.8)	545 (4.3)	0.148 <sup>b</sup>
Hypertension	404 (9.7)	495 (11.4)	512 (12.2)	1,411 (11.1)	0.001 <sup>b</sup>
Hypercholesterolemia	78 (1.9)	119 (2.7)	133 (3.2)	330 (2.6)	0.001 <sup>b</sup>
Presence of two components of MetS	938 (22.5)	1,107 (25.5)	1,123 (26.8)	3,168 (24.9)	$<0.001^b$
HOMA2-IR ( $n=7,945$ )	1.16 (0.49)	1.16 (0.48)	1.13 (0.45)	1.15 (0.47)	0.066

Values are presented as number (%) or mean  $\pm$  standard deviation.

WC, waist circumference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; HDL-C, high density lipoprotein cholesterol; MetS, metabolic syndrome; HOMA2-IR, homeostasis model assessment index 2 for insulin resistance.

<sup>a</sup>One-way analysis of variance for continuous variables, <sup>b</sup>Chi-square test.

model ( $P$  for trend=0.001) (Table 3).

**Albumin-corrected serum calcium and the risk of incident MetS stratified by presence of central obesity at baseline**

When the analysis was stratified by presence of central obesity, incident cases of MetS developed more frequently in those with central obesity (48.6% vs. 19.9%,  $P<0.001$ ). With regard to baseline central obesity, the association between the albu-

min-corrected serum calcium and the risk for incident MetS showed different trends between the central obesity group and the non-central obesity group (Table 4). In the subjects with central obesity ( $n=3,223$ ), the HR of incident MetS comparing the second and third quartiles to the first quartile of change in serum calcium level significantly decreased in the crude ( $P$  for trend=0.030), age- and sex-matched ( $P$  for trend=0.090), and fully adjusted models ( $P$  for trend=0.009). However, the decrease in HR was not significant in subjects without central obesity (Table 4).

**Table 2.** Simple correlation coefficient for corrected serum calcium and metabolic syndrome condition at baseline ( $n=12,704$ )

Variable	B	P value
Age, yr	0.041	<0.001
BMI, kg/m <sup>2</sup>	0.282	<0.001
SBP, mm Hg	0.081	<0.001
DBP, mm Hg	0.091	<0.001
TG, mg/dL	0.042	<0.001
HDL-C, mg/dL	0.033	<0.001
FPG, mg/dL	0.095	<0.001
HbA1c, %	0.100	<0.001
HOMA2-IR ( $n=7,945$ )	-0.026	0.021

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglyceride; HDL-C, high density lipoprotein; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; HOMA2-IR, homeostasis model assessment index 2 for insulin resistance.

**Albumin-corrected serum calcium and the risk of incident MetS stratified by presence of two components of MetS**

When the analysis was stratified by the presence of two components of MetS based on the NCEP-ATP III criteria [19], incident cases of MetS developed more frequently in those with two components of MetS at baseline than those with fewer than two components (57.7% vs. 17.0%,  $P<0.001$ ). In the subjects having two components of MetS at baseline ( $n=3,168$ ), the HR for incident MetS comparing the second and the third quartiles to the first quartile of change in serum calcium levels significantly decreased in the crude ( $P$  for trend=0.016), age- and sex-matched ( $P$  for trend=0.024), and fully adjusted model ( $P$  for trend=0.002). However, the decrease in HR was not significant in subjects with fewer than two components of MetS at baseline (Table 5).

**Table 3.** Risk of developing MetS based on corrected serum calcium tertile at baseline

Variable	Corrected serum calcium tertile, mg/dL			P for trend <sup>a</sup>	Continuous variables HR (95% CI)
	Tertile 1 (<8.70)	Tertile 2 (8.70-8.94)	Tertile 3 (>8.94)		
Number	4,174	4,334	4,198	0.482 <sup>b</sup>	
Incident cases, $n$ (%)	1,164 (27.9)	1,194 (27.5)	1,090 (26.0)		
Person-years of follow-up	18,925	18,974	17,320		
Incident cases of MetS/1,000 person-year	61.5	62.9	62.9		
Adjusted HR (95% CI) <sup>a</sup>					
Crude model	1 (reference)	1.02 (0.94-1.11)	1.02 (0.94-1.11)	0.849	1.05 (0.93-1.19)
Model 1	1 (reference)	1.01 (0.93-1.10)	1.02 (0.94-1.11)	0.915	1.06 (0.94-1.20)
Model 2	1 (reference)	0.91 (0.84-0.99)	0.85 (0.78-0.92)	0.001	0.80 (0.71-0.91)

Model 1: adjusted for age and sex; Model 2: adjusted for model 1+systolic blood pressure, diastolic blood pressure, triglyceride, high density lipoprotein, fasting plasma glucose, smoking status, and conditions under medical treatment, including hypertension, diabetes, and hypercholesterolemia.

MetS, metabolic syndrome; HR, hazard ratio; CI, confidence interval.

<sup>a</sup>Cox proportional hazard model, <sup>b</sup>Chi-square test for linear by linear association.

**Table 4.** Risk of developing metabolic syndrome by baseline albumin-corrected serum calcium tertile, stratified by the presence of central obesity

Variable	Corrected calcium tertile, mg/dL			P for trend <sup>a</sup>	Continuous variables HR (95% CI)
	Tertile 1 (<8.70)	Tertile 2 (8.70–8.94)	Tertile 3 (>8.94)		
Subjects with central obesity ( <i>n</i> =3,223)	1,032	1,121	1,070		
No. of subjects incident cases, <i>n</i> (%)	547 (53.0)	545 (48.6)	473 (44.2)	<0.001 <sup>b</sup>	
Adjusted HR (95% CI) <sup>a</sup>					
Crude model	1 (reference)	0.92 (0.81–1.03)	0.85 (0.75–0.96)	0.030	0.84 (0.70–1.00)
Model 1	1 (reference)	0.92 (0.82–1.04)	0.87 (0.77–0.99)	0.090	0.88 (0.73–1.05)
Model 2	1 (reference)	0.87 (0.77–0.98)	0.83 (0.74–0.94)	0.009	0.82 (0.68–0.98)
Subjects without central obesity ( <i>n</i> =9,483)	3,142	3,213	3,128		
No. of subjects incident cases, <i>n</i> (%)	617 (19.6)	649 (20.2)	617 (19.7)	0.833 <sup>b</sup>	
Adjusted HR (95% CI) <sup>a</sup>					
Crude model	1 (reference)	1.08 (0.97–1.20)	1.13 (1.01–1.27)	0.087	1.19 (1.01–1.40)
Model 1	1 (reference)	1.07 (0.95–1.19)	1.13 (1.01–1.26)	0.117	1.20 (1.02–1.41)
Model 2	1 (reference)	0.99 (0.88–1.10)	0.91 (0.81–1.02)	0.183	0.83 (0.70–0.98)

Model 1: adjusted for age and sex; Model 2: adjusted for model 1+systolic blood pressure, diastolic blood pressure, triglyceride, high density lipoprotein, fasting plasma glucose, smoking status, and conditions under medical treatment, including hypertension, diabetes, and hypercholesterolemia.

HR, hazard ratio; CI, confidence interval;

<sup>a</sup>Cox proportional hazard model ( $P < 0.025$  considered statistically significant after correction for multiple comparisons), <sup>b</sup>Chi-square test for linear by linear association.

**Table 5.** Risk of developing MetS by baseline albumin-corrected serum calcium tertile, stratified by the presence of two components of MetS

Variable	Corrected calcium tertile, mg/dL			P for trend <sup>a</sup>	Continuous variables HR (95% CI)
	Tertile 1 (<8.70)	Tertile 2 (8.70–8.94)	Tertile 3 (>8.94)		
Subjects with two components of MetS ( <i>n</i> =3,168)	1,027	1,101	1,040		
No. of subjects incident cases, <i>n</i> (%)	643 (62.6)	630 (57.2)	554 (53.3)	<0.001 <sup>b</sup>	
Adjusted HR (95% CI) <sup>a</sup>					
Crude model	1 (reference)	0.89 (0.80–1.00)	0.85 (0.76–0.95)	0.016	0.76 (0.64–0.89)
Model 1	1 (reference)	0.89 (0.80–1.00)	0.86 (0.77–0.96)	0.024	0.77 (0.65–0.90)
Model 2	1 (reference)	0.87 (0.78–0.97)	0.82 (0.73–0.92)	0.002	0.71 (0.60–0.84)
Subjects with fewer than two components of MetS ( <i>n</i> =9,538)	3,236	3,227	3,075		
No. of subjects incident cases, <i>n</i> (%)	566 (17.5)	565 (17.5)	490 (15.9)	0.103 <sup>b</sup>	
Adjusted HR (95% CI) <sup>a</sup>					
Crude model	1 (reference)	1.05 (0.93–1.18)	1.03 (0.91–1.16)	0.724	1.08 (0.90–1.29)
Model 1	1 (reference)	1.05 (0.93–1.17)	1.03 (0.91–1.17)	0.751	1.10 (0.92–1.32)
Model 2	1 (reference)	0.94 (0.83–1.05)	0.89 (0.79–1.01)	0.173	0.89 (0.74–1.08)

Model 1: adjusted for age, sex; Model 2: adjusted for model 1+waist circumference, systolic blood pressure, diastolic blood pressure, triglyceride, high density lipoprotein, fasting plasma glucose, smoking status, and conditions under medical treatment, including hypertension, diabetes, and hypercholesterolemia.

MetS, metabolic syndrome; HR, hazard ratio; CI, confidence interval.

<sup>a</sup>Cox proportional hazard model ( $P < 0.025$  considered statistically significant after correction for multiple comparisons), <sup>b</sup>Chi-square test for linear by linear association.

## DISCUSSION

In this retrospective longitudinal cohort study, the positive correlation between serum calcium and MetS observed in previous cross-sectional studies was not found. Unexpectedly, risk of MetS was inversely associated with serum calcium level in individuals with central obesity or two components of MetS.

The association between serum calcium level and risk factors for MetS has been well documented in the cross-sectional study design [14,15,22-24]. Previous studies demonstrated that serum calcium concentrations were associated with MetS components [14] and with increased MetS risk scores [23], as well as non-conventional cardio-metabolic risk factors (markers of oxidative stress, including uric acid, homocysteine, and  $\gamma$ -glutamyltransferase) [15]. We confirmed positive correlations between serum calcium and MetS components at baseline, including blood pressure, BMI, TGs, HDL-C, FPG, and HbA1c. Impaired insulin secretion [22] and insulin sensitivity [25], or increased insulin resistance [26], may explain the association between increased baseline serum calcium levels and the risk of T2DM or MetS. Furthermore, increased serum calcium levels have been shown to cause calcium influx into arterial smooth muscle, which induces muscle contraction, resulting in blood pressure elevation and increased peripheral vascular resistance [27].

To the best of our knowledge, the present study is the first to evaluate the association between baseline serum calcium and the development of MetS in a longitudinal study. In the present study, the positive correlation between serum calcium and MetS observed in previous cross-sectional studies [14,15,22-24] was not found. Moreover, the fully adjusted model showed an unexpectedly decreased risk of incident MetS during the follow-up period, in contrast with the results in the crude and age- and sex-matched models. For this reason, we stratified the analysis by presence of central obesity and two components of MetS at baseline. The results of the stratified analysis consistently showed decreased risk of MetS associated with increasing serum calcium level in subjects with central obesity and/or two components of MetS at baseline. Although it is difficult to explain the mechanisms, these findings suggest that it is important to accurately adjust for metabolic risk factors and to optimize subject selection to those bearing similar metabolic risk factors in assessing the modulating effect of calcium on the risk of incident MetS, because serum calcium level is closely associated with each component of MetS. Alternatively, an

increase in serum calcium level might possibly be a defense mechanism for increased insulin resistance. If a cause-and-effect relationship of the findings in this study is confirmed, further studies are warranted to understand the mechanisms involved between alterations in serum calcium levels and insulin resistance.

There are several limitations to our study. First, we did not collect serum levels of parathyroid hormone (PTH) or 25-hydroxy vitamin D, and did not gather information on dietary and supplemental calcium or vitamin D intake, although serum PTH is associated with several factors involved in MetS [28,29]. The strength of our study lies in its longitudinal design with a large sample size, which allowed us to evaluate the association between basal serum calcium and the incidence of MetS after adjusting for multiple metabolic risk factors and/or stratification.

In contrast to the results of the cross-sectional analysis in the previous studies, there was no positive correlation between serum calcium levels and incident risk of MetS in this retrospective longitudinal cohort study. The unexpected association between higher serum calcium levels and decreased incident MetS, which was shown in individuals with central obesity or two components of MetS, is hypothesis-generating. Further prospective studies designed to specifically address the hypothesis could explain the discrepancy.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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