ORIGINAL RESEARCH

Association of Serum Calcium and Insulin Resistance With Hypertension Risk: A Prospective Population-Based Study

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***Background*—**The temporal sequence between serum calcium and insulin resistance (IR) and their effects on hypertension are unclear. We studied the association between serum calcium and IR, with risk of hypertension events in a longitudinal cohort conducted in China.

***Methods and Results*—**Data from 8653 subjects aged 20 to 74 years with an average follow-up of 5.3 years were analyzed. Serum calcium, and fasting and 2-hour serum glucose and insulin were measured at baseline and follow-up. Cross-lagged panel and mediation analysis were used to examine the temporal relationship between serum calcium and IR and its impact on hypertension incidence. The conjoint effects of serum calcium and IR at baseline on hypertension at follow-up were observed (P=0.029 for HOMA_IR [hepatic IR] and P=0.009 for Gutt index [peripheral IR]). The cross-lagged path coefficient (β_2) from baseline serum calcium to follow-up peripheral IR were significantly greater than path coefficient (β_1) from baseline peripheral insulin resistance to follow-up serum calcium (β_2 =−0.354 versus β_1=−0.005; P=0.027). However, no directional relationships were observed in the serum calcium↔hepatic IR analysis. The mediation effect of peripheral IR on the association of serum calcium at baseline with hypertension at follow-up was estimated at 16.4% (P<0.001).

***Conclusions*—**Our findings demonstrate that higher serum calcium levels probably precede peripheral IR, and this 1-directional relation plays a role in the development of hypertension. **(*J Am Heart Assoc*. 2019;8:e009585. DOI: 10.1161/JAHA.118.009585.)**

Key Words: calcium • hypertension • insulin resistance

Prospective epidemiological studies have consistently shown that serum calcium (Ca) and insulin resistance (IR) are positively associated with hypertension.[1–7] It is generally considered that serum Ca and IR can influence each other based on pathophysiological and metabolic mechanisms.[8] IR and insulin secretion depend on Ca homeostasis.[9] Alteration of Ca concentrations may affect insulin secretion since initiation of insulin exocytosis from β-cells depends on Ca^{2+} influx through voltage-operated Ca^{2+} channels,[10,11] and on the contrary, insulin can affect Ca metabolism through blunting of Ca^{2+} influx.[12–14] As suggested by Levy, there exists a "vicious cycle" between Ca and glucose metabolism, by which abnormal Ca impairs glucose tolerance and poor glucose tolerance impairs Ca.[15] However, no data provide the evidence on which factor is the precursor in this "vicious cycle." Clarifying this issue would offer an early and effective target for preventing hypertension. In addition, although serum Ca and IR are extensively reported to be associated with hypertension, how their causal relation patterns influence hypertension and to what extent serum Ca is associated with hypertension through IR is largely unknown.

By using a longitudinal cohort data conducted in China, we aimed to assess the temporal relationship between serum Ca and IR, and then examine their impact on the development of hypertension.

Methods

The data, analytic methods, and study materials will not be made available to other researchers for purposes of

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Accompanying Tables S1, S2 and Figures S1 through S4 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.009585

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Clinical Perspective

What Is New?

- In the present large prospective cohort study, conjoint effects of serum calcium and insulin resistance on the development of hypertension were observed and evidence that increased serum calcium concentrations preceded peripheral insulin resistance was found.
- We demonstrated a significant mediation effect of peripheral insulin resistance on the serum calcium at baseline that was associated with the development of hypertension.

What Are the Clinical Implications?

- These findings indicate a potential role of targeting serum calcium concentration to reduce peripheral insulin resistance–related development of the hypertension.

reproducing the results or replicating the procedure by reason of ethical and data-protective legislation. However, the study group welcomes initiatives for cooperation, and data access may be granted upon application.

The Harbin Cohort Study on Diet, Nutrition and Chronic Noncommunicable Disease Study

The HDNNCDS study (Harbin Cohort Study on Diet, Nutrition and Chronic Noncommunicable Disease) was launched in 2010 by the national key discipline, department of nutrition and food hygiene at Harbin Medical University. It covered 7 urban administrative regions of Harbin. Each region was divided into 3 strata according to their financial situation, and a total of 42 communities were randomly selected from each stratum in each administrative region by performing a stratified multistage random cluster sampling design. Subjects were eligible to participate in the study if they (1) were between 20 and 74 years old; (2) had been living in Harbin for at least 2 years; and (3) were without cancer or type 1 diabetes mellitus. A total of 9734 subjects participated in the HDNNCDS, and the baseline survey was finished in 2012. The HDNNCDS methods have been previously described in detail.[16] During 2015 to 2016, 8913 subjects completed the first in-person follow-up survey with a response rate of 91.6%. The follow-up survey was conducted by an in-person interview by collecting information and health history much the same as those at the baseline survey. All data at baseline and the first follow-up were collected at the corresponding community service center to which the participant belonged. To dissect the natural temporal relationship between serum Ca and IR, 119 subjects who were taking insulin injections and 86 subjects who were taking vitamin D supplementation or calcium gluconate injection at either the baseline or the follow-up survey were excluded, because the medication could influence the serum levels of Ca and insulin, which probably further influence the temporal relationship between them. Fifty-five subjects who did not donate a blood sample at baseline were also excluded. Finally, 8653 subjects (2968 men and 5685 women) were included in this analysis, with an average follow-up of 5.3 years (range 3.1–6.8 years).

The study protocol of the HDNNCDS was approved by the Ethics Committee of Harbin Medical University, and written informed consents were provided by all subjects.

Questionnaire Survey and Anthropometric Measurements

Detailed in-person interviews were administered by trained personnel using a structured questionnaire to collect information on demographic characteristics, dietary habits, lifestyles, physical condition, and anthropometric characteristics at baseline. Current drinkers were defined as those who consumed ≥1 alcoholic drink each month in the 12 months before the survey. Current smokers were defined as those who smoked at least 100 cigarettes in a lifetime or smoked every day or currently smoked some days. Regular exercise was defined as any kind of recreational or sport physical activity other than walking for work or life performed at least 30 minutes for 3 or more days per week.

Weight and height were measured with participants standing without shoes and wearing light clothing at baseline recruitment. BMI (kg/m^2) was calculated as weight (kg) divided by the square of the height in meters (m^2).

Outcome Measures

Blood pressures were measured 3 times by using an electronic blood pressure monitor (OMRON HEM-7112) on the right arm of each subject after a 10-minute rest in a sitting position, and the mean values were used for analysis. Each participant was measured twice: the first was at baseline and the second was in the first follow-up survey. Hypertension was defined as systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg, or self-reports of a history of hypertension diagnosis with diagnostic record, or receiving treatment for hypertension with names of prescriptions for hypertension. The number of new incident hypertension cases was 1319.

Biochemical Analyses

Blood samples, including fasting and postprandial (2 hours after drinking 75 g of water containing glucose), were collected at baseline and at the first follow-up survey. After

collection, plasma samples were kept in a portable, insulated bag with ice packs (at ≈0–4°C) and were processed within 6 hours for long-term storage at −80°C. Serum Ca concentration was measured by a TOSOH automated enzyme immunoassay analyzer (AIA2000ST). An oral glucose tolerance test was carried out according to the World Health Organization guidelines.[17] Diabetes mellitus was identified by self-reports of a history of diabetes mellitus diagnosis, and/or fasting blood glucose ≥7.0 mmol/L, and/or 2-hour glucose ≥11.1 mmol/L, and/or receiving treatment for diabetes mellitus. Fasting and 2-hour serum insulin was measured by the immunofluorescence method (TOSOH automated enzyme immunoassay analyzer AIA2000ST). Gutt index was calculated as an indicator of peripheral IR, which is based on glucose uptake rates, metabolic clearance rates, and mean serum insulin by the following equation: [75 000+(fasting glucose−2-h glucose)×0.19×body weight]/(120×log [(fasting insulin+2-h insulin)/2]×[(fasting glucose+2-h glucose)/2]).[18] HOMA models were used to estimate hepatic insulin resistance (HOMA-IR) according to the formula: fasting glucose (mmol/L)×fasting insulin (mIU/L)/22.5.[19]

Taking into account both statistical power considerations and budget constraints, we randomly selected 100 subjects among all the study participants to measure the biochemical albumin levels. The 100 participants were similar to the parent cohort regarding age, body mass index, current smoking, current drinking, and caloric intake, suggesting that the biomarker study participants are representative of the whole cohort (Table S1). After analysis, we found that the serum Ca levels were quite similar to those of serum Ca levels adjusted for albumin levels (Table S1), which was comparable to the results in other reported results.[20] In addition, all participants in the present study were without cancer or type 1 diabetes mellitus and thus, we did not measure albumin levels for all participants.

Statistical Analysis

Serum Ca, HOMA-IR, and Gutt index were log-transformed to improve the normality of the distribution. Serum Ca, HOMA-IR, and Gutt index were categorized by quartile distribution with the lowest quartile serving as the reference, respectively. Odds ratios and 95% CIs were calculated to estimate the association of serum Ca concentration or IR indices with hypertension by using logistic regression models after adjustment for covariates. In addition, the multiplicative interaction term of continuous serum Ca with HOMA-IR or Gutt index was added to the main effects model to evaluate their conjoint effects on incident hypertension, respectively. Rothman's synergy index (S) was used to evaluate the interaction effect of serum calcium and HOMA_IR/Gutt index on hypertension.[21]

Longitudinal changes in serum Ca, HOMA-IR, and Gutt index measured at 2 time points can be modeled using a cross-lagged panel design. In this modeling approach, each variable in the model is regressed on all of the variables that precede it in time. A simplified, conceptual version of the model used in this analysis is presented in Figure 1. The path coefficient with β_1 describes the effect of baseline HOMA-IR or Gutt index on the subsequent serum Ca, and the path coefficient with β_2 describes the effect of the baseline serum Ca on the subsequent HOMA-IR or Gutt index. Before the cross-lagged path analysis, the baseline and follow-up serum Ca, HOMA-IR, and Gutt index were adjusted for age, sex, current smoking, current drinking, regular exercise, caloric intake, and follow-up years using a regression residual analysis and then were standardized by Z-transformation (mean=0, SD, 1). Pearson correlation coefficients of the Z-transformed quantitative variables of biochemical indices at baseline and follow-up were calculated. The cross-lagged path coefficients (β_1 and β_2) were estimated simultaneously based on the correlation matrix. The percentile CI of cross-lagged path coefficient was estimated using bootstrap simulation for the cross-lagged model. The validity of model fitting was indicated by the root mean square residual and comparative fitness index. Root mean square residual <0.05 and comparative fitness index >0.90 indicate relatively well fit to the observed data. The difference between β_1 and β_2 derived from the standardized variables was tested using Fisher's Z-test. Although the significance of individual β_1 or β_2 suggests a directional relationship, a significant difference between β_1 and β_2 provides stronger evidence for a temporal relationship in the model.

Once the temporal relationships of these biochemical indices had been established, a causal mediation model was constructed to examine whether the association of serum Ca with hypertension was mediated by IR after adjusting for age, sex, current smoking, current drinking, regular exercise, caloric intake, and follow-up years.

All statistical analyses were performed using R 2.15.3 (http://www.r-project.org/). The statistical test was 2-sided and a $P<0.05$ was considered statistically significant.

Results

Characteristics of the Study Population

Mean levels of study variables at baseline and follow-up by sex are summarized in Table 1.

Association of Serum Ca, HOMA-IR, and Gutt Index With Hypertension

The associations of baseline serum Ca, HOMA-IR, and Gutt index with incident hypertension are shown in Table 2. With increasing baseline serum Ca concentration, values of odds

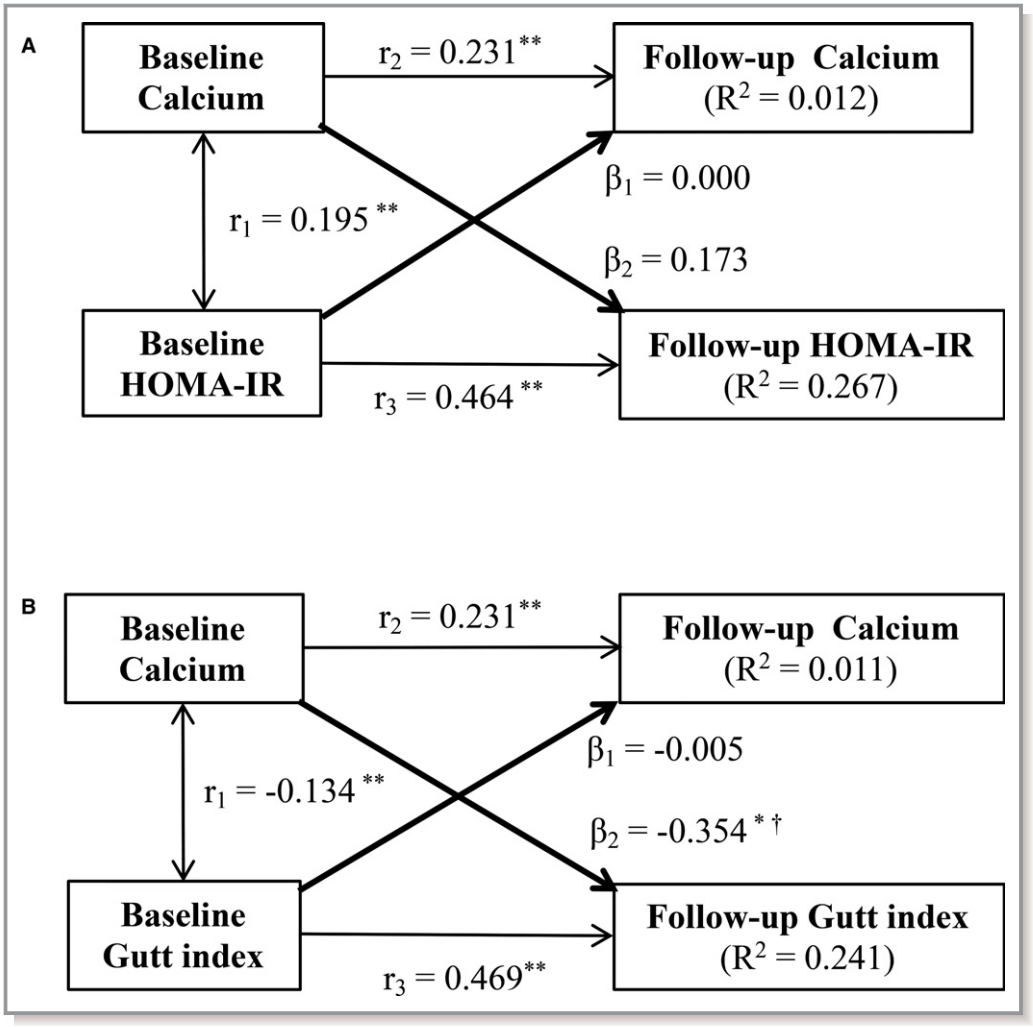

Figure 1. The detailed parameter information on cross-lagged path analysis models. Results were adjusted for age, sex, current smoking, current drinking, regular exercise, caloric intake, and follow-up years. **A** and **B**, β_1, cross-lagged path coefficients from baseline HOMA-IR/Gutt index to follow-up serum calcium concentration; β_2, cross-lagged path coefficients from baseline serum calcium concentration to follow-up HOMA-IR/Gutt index; r1 represents synchronous correlations; r2 and r3 represents tracking correlations; R^2: variance explained. $^{**}P<0.05$, $^{*}P<0.0001$ for coefficients being different from 0; †Difference between β_1 and β_2 for being different from 0; Gutt index=[75 000+(fasting glucose−2-h glucose)×0.19×body weight]/(120×log [(fasting insulin+2-h insulin)/2]×[(fasting glucose+2-h glucose)/2]). HOMA-IR indicates higher glucose, insulin, and insulin resistance.

ratios for incident hypertension had a significant increasing trend (*P*-trend<0.0001). Statistically significant interaction was observed between serum Ca and HOMA_IR (*P*-interaction=0.029)/Gutt index (*P*-interaction=0.009). The values of S were 3.83 and 4.37, respectively. The above results did not change when the data were analyzed by sex groups (Table 3).

Temporal Relationship Between Serum Ca and IR

Cross-lagged analysis coefficients are shown in Figure 1, adjusted for age, sex, current smoking, current drinking, regular exercise, caloric intake, and follow-up years among all participants were adjusted. Serum Ca was significantly correlated with hepatic IR (r=0.195, P<0.0001) and peripheral IR (r=−0.134, P<0.0001). The path coefficients (β_1=−0.354) from the baseline serum Ca to the follow-up Gutt index were significantly greater than the path coefficients (β_2=−0.005) from the baseline Gutt index to the follow-up serum Ca (P=0.027 for the difference between β_1 and β_2). The values of root mean square residual and comparative fitness index were 0.05 and 0.915, respectively. No directional relationships were observed in the serum Ca↔HOMA-IR-models analysis. The above associations did not change when the data were analyzed by sex groups (Figures S1 and S2).

Table 1. Characteristics Regarding the Study Variables at Baseline and Follow-Up by Sex Groups in the HDNNCDS Study, 2010–2016

Characteristic	Men (n=2968)	Women (n=5685)	*P* Value
Baseline			
Age, y	48.15 (10.04)	47.63 (9.22)	0.199
BMI, kg/m^2	25.14 (3.43)	23.79 (3.23)	<0.0001
Exercised regularly, %	48.57	41.71	<0.0001
Current smoking, %	37.41	3.85	<0.0001
Current drinking, %	58.97	21.80	<0.0001
Caloric intake, kcal/d	2644.99 (959.78)	2241.92 (842.59)	<0.0001
Serum calcium concentration, mmol/L	2.26 (0.10)	2.25 (0.11)	0.033
Fasting glucose, mmol/L	4.57 (0.91)	4.49 (0.79)	<0.0001
2-h glucose, mmol/L	5.90 (2.22)	5.87 (1.90)	0.520
Fasting insulin, U/mL	8.29 (6.50)	8.24 (6.48)	0.806
2-h insulin, U/mL	41.85 (39.32)	43.63 (36.16)	0.141
Hemoglobin A_{1c}	4.82 (0.6)	4.75 (0.47)	0.0002
Gutt index	43.68 (26.82)	39.52 (15.95)	<0.0001
HOMA-IR	1.90 (1.34)	1.93 (1.49)	0.0003
Systolic blood pressure, mm Hg	137.43 (17.75)	132.39 (18.46)	<0.0001
Diastolic blood pressure, mm Hg	83.92 (10.09)	79.98 (8.28)	<0.0001
Follow-up			
Serum calcium concentration, mmol/L	2.27 (0.09)	2.28 (0.11)	<0.0001
Fasting glucose, mmol/L	4.70 (1.22)	6.60 (3.08)	<0.0001
2-h glucose, mmol/L	6.60 (3.08)	6.34 (2.41)	0.0007
Fasting insulin, U/mL	9.66 (9.23)	9.20 (8.46)	0.111
2-h insulin, U/mL	54.84 (47.78)	58.23 (45.48)	0.032
Hemoglobin A_{1c}	5.54 (1.30)	5.50 (2.40)	0.542
Gutt index	40.43 (21.7)	38.53 (15.72)	0.014
HOMA-IR	2.11 (2.67)	1.93 (1.74)	<0.0001
Systolic blood pressure, mm Hg	139.28 (19.61)	130.22 (20.19)	<0.0001
Diastolic blood pressure, mm Hg	85.18 (24.83)	77.26 (11.70)	<0.0001

Continuous variables are presented as the means (SD). BMI indicates body mass index; HDNNCDS, Harbin Cohort Study on Diet, Nutrition and Chronic Noncommunicable Disease; HOMA-IR, higher glucose, insulin, and insulin resistance.

Cross-lagged path analysis models of serum Ca with HOMA-IR and Gutt index in normotensives and hypertensives are presented in Figure 2, adjusted for age, sex, current smoking, current drinking, regular exercise, caloric intake, and follow-up years. The unidirectional relationship from serum Ca to Gutt index did not change in normotensives or hypertensives.

Mediation Role of IR in the Association Between Serum Ca and Hypertension

The mediation effect of Gutt index on the serum Ca at baseline-hypertension at follow-up is presented in Figure 3 with adjustment for age, sex, current smoking, current drinking, regular exercise, caloric intake, and follow-up years. The total effect of serum Ca on hypertension measured as standardized regression coefficient (β_{Tot}=2.692; P<0.0001) was estimated without Gutt index in the model. Indirect effect 1 (β_1=−0.783) was significantly greater than indirect effect 2 (β_2=−0.493), with the overall indirect effect being 0.386 (−0.783×−0.493). The percentage of the total effect mediated by Gutt index was estimated at 16.4% (P<0.0001). In addition, similar results were found among male and female participants (Figure 3 [male] and Figure 3 [female]), and the percentages of the total effect mediated by Gutt index were estimated at 13.6% (P<0.0001) for males and 18.8% (P<0.0001) for females, respectively.

Table 2. Association of Serum Calcium Concentrations or Insulin Resistance Indices at Baseline With Incident Hypertension in the HDNNCDS Study, 2010–2016

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* Trend
Serum calcium concentrations					
Means, SD	2.13 (0.06)	2.23 (0.02)	2.30 (0.02)	2.42 (0.08)	
No. of cases	227	319	318	464	
Age and sex-adjusted OR	1.00	0.76 (0.61, 0.93)	0.73 (0.59, 0.90)	0.47 (0.39, 0.58)	<0.0001
Multivariate OR*	1.00	1.37 (1.10, 1.70)	1.45 (1.17, 1.81)	2.18 (1.77, 2.68)	<0.0001
Interaction with HOMA-IR					
Rothman's synergy index	3.83				
Interaction with Gutt index					
Rothman's synergy index	4.37				

HDNNCDS indicates Harbin Cohort Study on Diet, Nutrition and Chronic Noncommunicable Diseases; HOMA-IR, higher glucose, insulin, and insulin resistance; OR, odds ratio.
*Adjusted for age, sex, current smoking, current drinking, regular exercise, caloric intake, and follow-up years.

Analysis Based on the New American College of Cardiology–American Heart Association Guidelines (130/80 mm Hg) for Hypertension

For the association of serum Ca and Gutt index with hypertension, the results (Table S2, Figures S3 and S4) were comparable to the above results in which hypertension was defined by the American College of Cardiology–American Heart Association guidelines (140/90 mm Hg). For the association of serum Ca and HOMA-IR with hypertension, although the unidirectional relationship from serum Ca to HOMA-IR was found in normotensives or hypertensives (Figure S3), the results of no directional relationships in the serum Ca↔HOMA-IR-models analysis were independent of

Table 3. Association of Serum Calcium Concentrations or Insulin Resistance Indices at Baseline With Incident Hypertension by Sex in the HDNNCDS Study, 2010–2016

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* Trend
Males					
Serum calcium concentrations					
Means, SD	2.13 (0.05)	2.23 (0.02)	2.30 (0.02)	2.41 (0.07)	
Age and sex-adjusted OR	1.00	1.23 (0.96, 1.57)	1.49 (1.16, 1.90)	1.84 (1.44, 2.34)	<0.0001
Multivariate OR*	1.00	1.40 (1.08, 1.82)	1.53 (1.19, 1.99)	1.96 (1.52, 2.53)	<0.0001
Interaction with HOMA-IR					
Rothman's synergy index	1.71				
Interaction with Gutt index					
Rothman's synergy index	5.92				
Females					
Serum calcium concentrations					
Means, SD	2.13 (0.07)	2.23 (0.02)	2.30 (0.02)	2.42 (0.09)	
Age- and sex-adjusted OR	1.00	0.91 (0.77, 1.09)	1.32 (1.11, 1.57)	1.60 (1.35, 1.90)	<0.0001
Multivariate OR*	1.00	0.92 (0.76, 1.10)	1.37 (1.14, 1.65)	1.65 (1.38, 1.98)	<0.0001
Interaction with HOMA-IR					
Rothman's synergy index	4.37				
Interaction with Gutt index					
Rothman's synergy index	3.02				

HDNNCDS indicates Harbin Cohort Study on Diet, Nutrition and Chronic Noncommunicable Diseases; HOMA-IR, higher glucose, insulin, and insulin resistance; OR, odds ratio.
*Adjusted for age, current smoking, current drinking, regular exercise, caloric intake, and follow-up years.

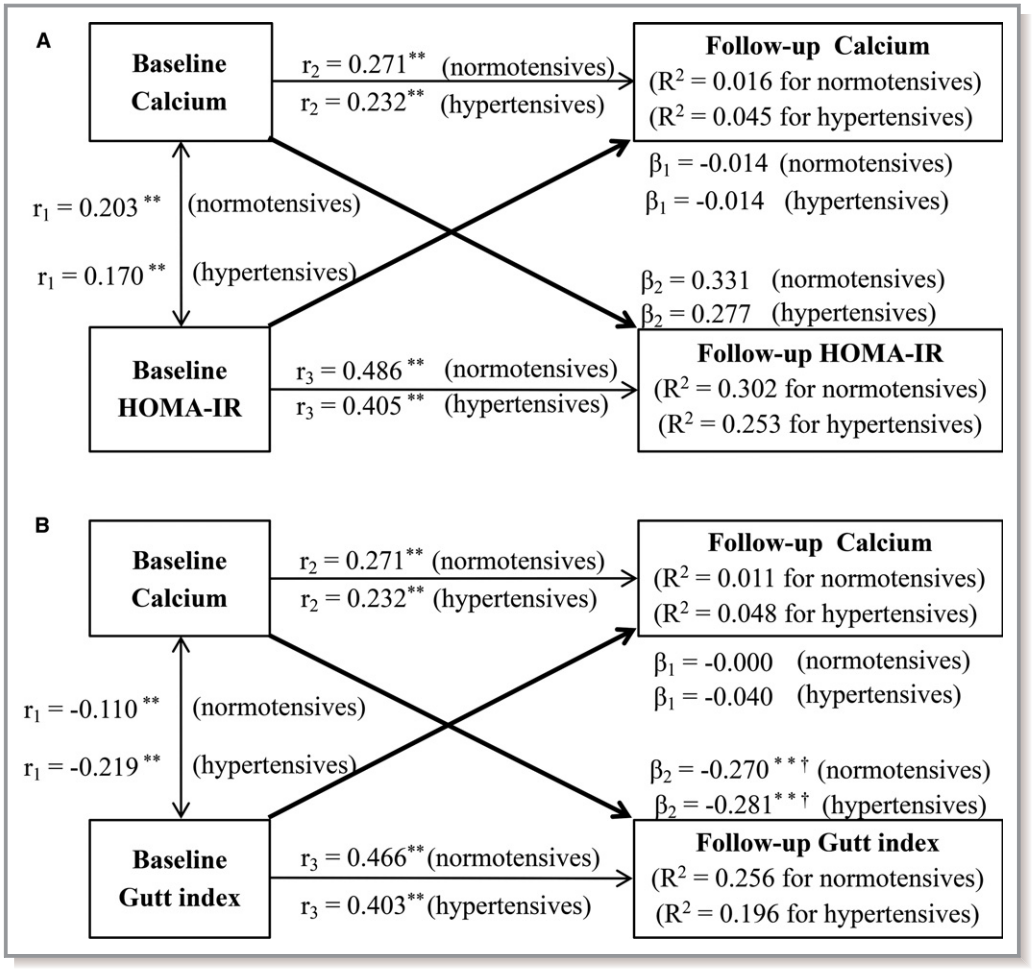

Figure 2. The detailed parameter information on cross-lagged path analysis models in normotensives and hypertensives, adjusted for age, sex, current smoking, current drinking, regular exercise, caloric intake, and follow-up years. **A** and **B**, β_1, cross-lagged path coefficients from baseline HOMA-IR/Gutt index to follow-up serum calcium concentration; β_2, cross-lagged path coefficients from baseline serum calcium concentration to follow-up HOMA-IR/Gutt index; r1 represents synchronous correlations; r2 and r3 represent tracking correlations; R^2: variance explained. $^{**}P<0.05$ for coefficients being different from 0; †Difference between β_1 and β_2 for being different from 0; Gutt index=[75 000+(fasting glucose−2-h glucose)×0.19×body weight]/(120×log [(fasting insulin+2-h insulin)/2]×[(fasting glucose+2-h glucose)/2]). HOMA-IR indicates higher glucose, insulin, and insulin resistance.

the definition for hypertension and thus, no mediation effect of HOMA-IR between the association of serum Ca and hypertension (defined by new American College of Cardiology–American Heart Association guidelines) was observed.

Discussion

In the present study, we observed conjoint effects of serum Ca and IR on the development of hypertension. We have clarified for the first time that there is a unidirectional relationship from serum Ca to peripheral IR, and we found that peripheral IR mediated a considerable amount of the total effect of serum Ca on the development of hypertension. To the best of our knowledge, this study is the first to directly provide quantifiable mechanistic evidence linking serum Ca to hypertension.

Our results on the significant association between serum Ca and IR were consistent with previous findings that alteration of serum Ca is significantly correlated with the abnormality of IR.[8,14] However, existing data are limited about whether an increase in serum Ca measures antedate increases in IR or vice versa, or whether the relationship is bidirectional. The present study investigated the temporal relationship between serum Ca and IR in a longitudinal cohort by using a cross-lagged path analysis model, a powerful statistical approach to dissecting a causal relationship between intercorrelated variables.[22] The results showed that there was a 1-directional relationship from serum Ca to

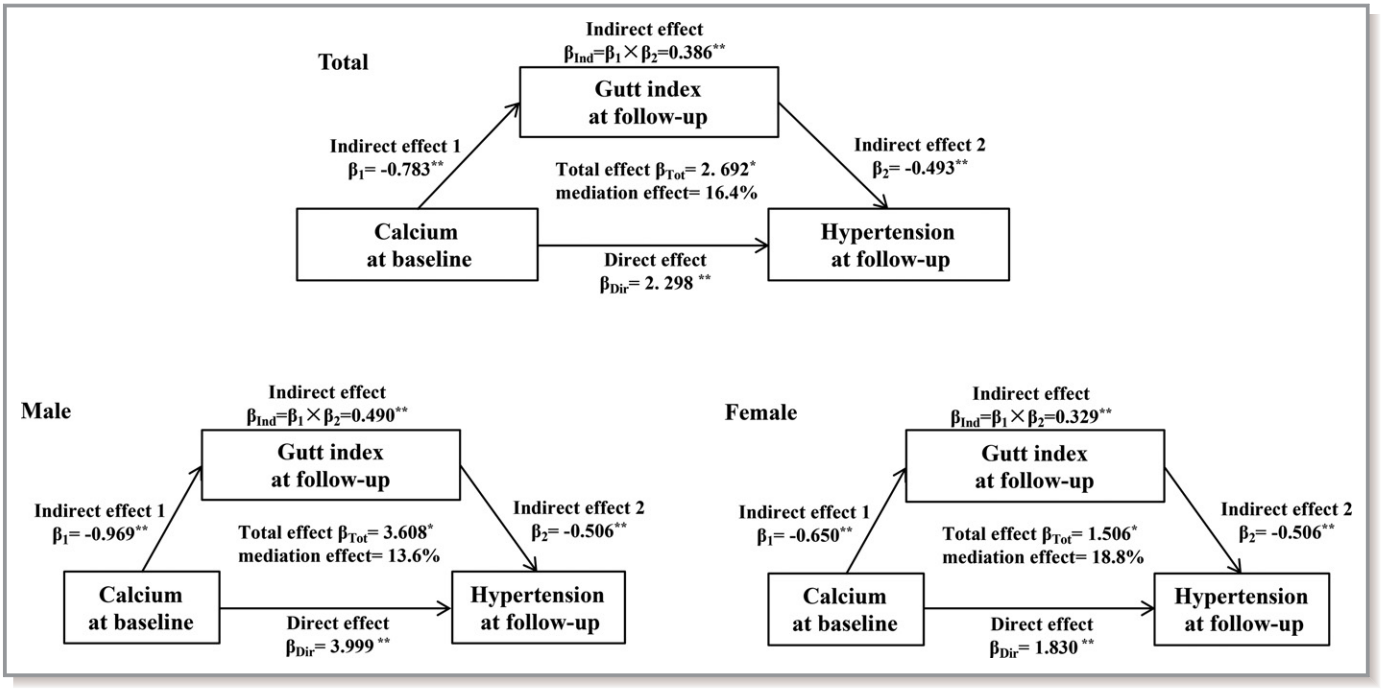

Figure 3. Mediation effect of Gutt index at follow-up on the serum calcium concentration–hypertension association for total participants, male participants, and female participants, respectively. $^{**}P<0.0001$, $^{*}P<0.01$ for coefficients being different from 0.

peripheral IR, which provided evidence that serum Ca may probably be a causal factor of IR. In terms of hepatic IR, we found no directional relationships between them, which was different from that with peripheral IR. Although hepatic IR is frequently associated with peripheral IR, the severity of IR may differ among the various tissues in different individuals.[23] This indicates that treatment for IR should be organ-specific.

Higher serum Ca was reported to be associated with hypertension risk in some epidemiological studies,[1,24] while other studies observed that lower serum Ca was related to hypertension risk.[25] Our results support the association that higher serum Ca is a risk factor for hypertension. In line with results from previous studies,[6,7] positive associations of IR and hypertension were found in the present study. In addition, the conjoint effect of elevated serum Ca and IR on the incident hypertension was first evaluated in the present study and was found to be statistically significant. The possible mechanisms underlying the association of serum Ca and IR with hypertension may include (1) a direct effect on vasculature by enhanced vascular resistance; (2) renal vasoconstriction causing kidney dysfunction; (3) hyperactivity of the renin–angiotensin system from hyperparathyroidism; (4) direct effects of insulin to stimulate renal sodium reabsorption; and (5) sympathetic stimulation of the heart, blood vessels, and kidney.[26–30] The present study provided evidence that there was a 1-directional relationship from serum Ca to peripheral IR and demonstrated that altered serum Ca preceded IR. IR can be considered an important link between elevated serum Ca and hypertension based on the above-described mechanisms. However, to date, data are lacking about the mediation effect of IR on the elevated serum Ca–hypertension association. In the present study, the serum Ca-to-IR directionality was first established in the cross-lagged analysis models, and then the temporal patterns were linked to incident hypertension. The findings of the present mediation analysis showed that the association of serum Ca at baseline and hypertension at follow-up was partially mediated by peripheral IR (16.4%); however, it is smaller than the direct effect of serum Ca at baseline on hypertension at follow-up (34.1%). Some other mechanisms linking serum calcium with hypertension might exist and need to be estimated in further studies. In addition, the evidence provided by the present study suggests that the underlying pathophysiological and metabolic mechanisms for the serum Ca–hypertension relationship might be more important than mechanisms for the effect of IR on hypertension. No data in this regard are available for comparison; further studies are needed to validate the findings from the present study.

The potential origins of high serum Ca were possibly because of hyperparathyroidism, malignancy, vitamin D intoxication, and so on. They were characterized by different mechanisms. Hyperparathyroidism increases serum Ca, possibly via decreasing Ca excretion in urine by an increase in the renal tubular reabsorption of calcium, or stimulation of osteoclast-mediated resorption of bone, which releases calcium into serum.[31] The most discussed mechanism for the pathophysiology of cancer-

associated high serum Ca is the production of parathyroid hormone–related peptide with bone and kidney actions along with increasing Ca levels.[32,33] Furthermore, ingestion of excessive amounts of vitamin D3 (or vitamin D2) results in high serum Ca, possibly because of the formation of supra-physiological amounts of 25-hydroxyvitamin D [25(OH)D] that bind to the vitamin D receptor, albeit with lower affinity than the active form of the vitamin, 1,25(OH)2D, and the formation of 5,6-*trans* 25(OH)D, which binds to the vitamin D receptor more tightly than 25(OH)D. These possible origins of high serum Ca need to be further evaluated in long-term cohort studies to prevent the occurrence of hypertension.[34]

The strengths of our study include the following. This study examined the temporal relationships of serum Ca with IR using a novel theoretical model; hepatic and peripheral IR were analyzed, which provided more information for these temporal relationships; mediation analysis was used to explore the mediation effect of IR in the association of serum Ca with hypertension. However, our work has some limitations. First, although we adjusted for confounders, we cannot exclude the possibility of residual confounding. Second, the hepatic IR and peripheral IR were calculated based on glucose and insulin concentrations. The results of this study need to be validated in other studies by more sophisticated methods, such as glucose clamp technique and intravenous glucose tolerance test. Third, the present study was designed to be 1 measurement per subject in each survey including baseline and the first follow-up, so the results should be further validated in cohort studies with more than 1 measurement.

The present study observed conjoint effects of serum Ca and IR on the development of hypertension and demonstrates that increased serum Ca concentrations preceded peripheral IR in a longitudinal assessment of the directionality analysis between serum Ca and insulin using a cross-lagged path analysis model. There was a significant mediation effect of peripheral IR on the serum Ca at baseline; an association with incident hypertension was demonstrated. These findings of the causal inference analysis of serum Ca and IR in relation to incident hypertension would improve our understanding of the pathobiology, mechanisms, and natural history of human essential hypertension and facilitate selection of novel therapeutic and intervention strategies by targeting the causal factors to prevent subsequent hypertension.

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Disclosures

None.

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