ORIGINAL RESEARCH

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Nutritional Epidemiology and Public Health

Serum Calcium Concentrations, Chronic Inflammation and Glucose Metabolism: A Cross-Sectional Analysis in the Andhra Pradesh Children and Parents Study (APCaPS)

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ABSTRACT

Background: Evidence suggests a role for elevated serum calcium in dysregulated glucose metabolism, linked through low-level chronic inflammation.

Objectives: We investigated the association of elevated serum calcium concentrations (corrected for albumin) with markers of dysregulated glucose metabolism and type II diabetes and tested if these associations were accounted for by chronic inflammation in a rural Indian population.

Methods: A cross-sectional analysis of participants aged 40–84 y from the Andhra Pradesh Children and Parents Study (APCaPS; $n = 2699$, 52.2% women) was conducted. Comprehensive information on household, sociodemographic, and lifestyle factors; medical and family history; physical measurements; blood measurements including fasting plasma glucose (FPG), fasting insulin (FI), serum calcium, albumin, phosphorous, vitamin D (in a subset), and creatinine were analyzed. Additionally, in a random sample of healthy participants ($n = 1000$), inflammatory biomarkers (interleukins 6 and 18, soluble intercellular adhesion molecule 1, adiponectin, and high-sensitivity C-reactive protein) were measured and an inflammatory score (IScore) calculated.

Results: After adjustments for sociodemographics, lifestyle factors, and anthropometry the highest calcium quartile (Q4 compared with Q1) was associated with FI ($\beta = 1.4$ μU/ml; 95% CI: 1.2, 1.5 μU/ml; P-trend < 0.001), the homeostasis model assessment for insulin resistance (HOMA-IR) ($\beta = 1.4$; 95% CI: 1.2, 1.5; P-trend < 0.001), and was modestly associated with FPG ($\beta = 2.1$ mg/dL; 95% CI: −0.9, 5.2 mg/dL; P-trend $= 0.058$) and prevalent type II diabetes (OR = 1.6; 95% CI: 1.0, 2.6; P-trend= 0.020). In the healthy subgroup, the association of the highest calcium quartile was similar for FI and HOMA-IR. Additional adjustment with IScore did not alter the associations. Further, in a subset, all these associations were independent of endogenous regulators of calcium metabolism (serum vitamin D, phosphorus, and creatinine). Independently, after accounting for potential confounders, the highest IScore quartile (Q4 compared with Q1) was positively associated with FPG, FI, HOMA-IR, and prevalent prediabetes, and also with serum calcium concentrations in men.

Conclusions: Elevated serum calcium was positively associated with markers of dysregulated glucose metabolism and prevalent type II diabetes in a rural Indian population. Chronic inflammation did not mediate this association but was independently associated with markers of dysregulated glucose metabolism. Inflammation might be responsible for elevated serum calcium concentrations in men. *Curr Dev Nutr* 2019;3:nzy085.

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Keywords: calcium, glucose metabolism, insulin resistance, type II diabetes, prediabetes, chronic inflammation, India, APCaPS

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Supplemental Material, Supplemental Figures 1-2 and Supplemental Tables 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.

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Abbreviations used: APCaPS, Andhra Pradesh Children and Parents Study; COPD, chronic obstructive respiratory disease; CVD, cardiovascular disease; DALY, disability-adjusted life-year; FPG, fasting plasma glucose; FI, fasting insulin; hsCRP, high-sensitivity C-reactive protein; IScore, inflammatory score; PAL, physical activity level; PTH, parathyroid hormone; sICAM-1, soluble intercellular adhesion molecule 1; SLI, Standard of Living Index.

Introduction

Type II diabetes is one of the leading causes of morbidity and mortality due to a noncommunicable disease globally (1–3). Over the past decade, although major noncommunicable diseases including cardiovascular disease (CVD) and chronic obstructive respiratory disease (COPD) have either increased modestly or notably declined in some geographic regions, diabetes has increased globally [relative increase from 2006 to 2016: 24.4%; 95% CI: 22.7%, 26.2%) in disability-adjusted life-years (DALYs) and 31.1% (95% CI: 28.9%, 33.4%) in absolute deaths] (1–3). The estimated 1.4 million deaths and over 570 million DALYs due to diabetes in 2016 are also likely underestimates (1, 2). Asia, with the most populous countries of the world (i.e., China and India), is at the epicenter of this burden, although the pattern of disease differs from that found in the West (3), and novel etiopathologic explorations are therefore imperative for planning effective preventive and therapeutic strategies (3, 4).

Calcium modulates enzymatic functions in the liver that are responsible for the production of glucose and the breakdown of glycogen. Calcium concentrations in blood and tissues are vital for secretion of insulin by the β cells of the pancreas (4). Thus, glucose production and β cell function are calcium dependent, and calcium homeostasis in blood and tissues is important for the control of glucose metabolism (4). Elevated serum calcium concentrations have been associated with an increased risk of type II diabetes in longitudinal studies (5–8) independent of potential confounders including adiposity and endogenous regulators of calcium metabolism such as vitamin D, phosphorus, parathyroid hormone (PTH), or renal function (9). Further, cross-sectional studies (10–13) have shown positive associations of elevated serum calcium concentrations with markers of dysregulated glucose metabolism, such as increased levels of fasting glucose, glucose intolerance, insulin resistance, and defective β cell function, as well as type II diabetes.

Chronic low levels of inflammation triggered by energy excess constitute an important biological mechanism which may be associated with elevated serum calcium concentrations (4) and mediate the association of calcium with dysregulated glucose metabolism (4, 14). However, given the complexity of endogenous (vitamin D, renal function, hormones) and exogenous regulators (dietary intake, supplements, medications such as diuretics) of calcium metabolism (15), testing these associations in different settings and populations (9) and understanding the potential biological mechanism underlying the associations are vital (15). Data from rural Indian adults, with a different pattern of type II diabetes (3, 16) and distinctive risk exposures such as tobacco chewing, regional dietary patterns, possible infectious history, and genetics, provide the opportunity to assess the relationship between calcium, inflammation, and glucose metabolism within a different structure of confounders compared with the populations more often studied. The Andhra Pradesh Children and Parents Study (APCaPS) is a prospective birth cohort study with data collected from rural Indian adolescents and adults at 3 time points (2003–05, 2009–10, 2011–12) (17). We evaluated the association of serum calcium concentrations with levels of fasting plasma glucose (FPG), fasting insulin (FI), HOMA-IR and the prevalence of prediabetes (FPG 100–125 mg/dL) and type II diabetes (doctor-diagnosed plus FPG $\geq$126 mg/dL) in rural Indian adults aged $\geq$40 y based on the data from the 2011–12 collection round. We included additional inflammatory data in a subset of healthy participants to test if these associations were partly or fully accounted for by chronic inflammation after excluding participants with self-reported history of any chronic disease including hypertension, diabetes, CVD, COPD, and cancer.

Methods

The analyses in this study used data from the third follow-up of APCaPS (2010–12), a prospective cohort study established through long-term follow-up of the Hyderabad Nutrition Trial (1987–90). Brief details of the study design are included as **Supplemental Material** along with a participant flow chart and venn diagram (**Supplemental Figure 1-2**). A total of 6944 out of a potential 10,213 individuals participated during this phase. This included adults born between 1987 and 1990, their parents, and household members. Our analysis included men and women aged $\geq$40 y (n = 2699) from the third follow-up phase. Comprehensive information on household, sociodemographic, socioeconomic, lifestyle factors, medical and family history, as well as physical measurements (anthropometrics and blood pressure) were collected. Fasting blood samples (8 h) were collected, and biochemical concentrations of FPG and FI, and serum concentrations of nutrients including calcium, phosphorus as well as vitamin D in a subset (n = 1713), along with full blood count, and kidney function tests (e.g., serum creatinine, albumin) were measured. Vitamin D data were available for adults born between 1987 and 1990 and their parents (n = 1713/2699), but were not available for other members of the household or community. Inflammatory biomarkers IL-6, IL-18, soluble intercellular adhesion molecule 1 (sICAM-1), adiponectin, and high-sensitivity C-reactive protein (hsCRP) and were measured in a subset of randomly selected healthy participants aged $\geq$40 y (n = 1000) excluding participants with self-reported history of any chronic disease including hypertension, diabetes, CVD, COPD, and cancer.

Data collection

Questionnaire data.

Structured interviewer-administered questionnaires were used to collect data from all participants between January 2010 and December 2012. Comprehensive data on sociodemographics, socioeconomics, and lifestyle factors were collected. The study participants lived in rural locations with gradual improvements in existing facilities and infrastructure over the course of the study period (2003–2012). This degree of urbanization was calculated through the use of a composite urbanization score, and the participants were classified into tertiles. A subset of questions (14/29) from the National Health Family Survey-2 (18) was used to estimate socioeconomic position based on information about the the quality of housing, toilet facilities, source of lighting, drinking water, household articles, and agricultural land. A Standard of Living Index (SLI) was derived based on these estimates.

Clinical assessments.

Weight was measured to the nearest 0.1 kg with a digital balance (SECA 899), and standing height measured to the nearest 1 mm with a plastic stadiometer (Leicester height measure; Chasmors). Measurements were taken twice and the average of 2 values was used in the analysis. BMI was calculated as weight (kg)/height (m^2). Systolic and diastolic blood

pressure was measured with a validated oscillometric device (Omron M5-I; Matsusaka Co.) in the supine position, with appropriate cuff sizes. Each measure was taken 3 times and the average used. Physical activity level (PAL) was assessed based on metabolic equivalent tasks (19) (see Supplementary Material for details). Hypertension included doctor-diagnosed disease, a systolic blood pressure ≥140 mm Hg or a diastolic blood pressure ≥90 mm Hg at the time of the interview. Diabetes included doctor-diagnosed disease or an FPG >126 mg/dL at the time of the interview. Prediabetes included an FPG 100–125 mg/dL at the time of the interview.

Biochemical measurements.

Participants attended morning clinics and were asked to fast overnight. Venous blood samples (20 mL) were drawn in plain and fluoride vacutainers (Becton & Dickinson) by a trained phlebotomist, the samples were centrifuged at 800 × *g* for 15 min at 22-25°C, and transferred to the laboratory on ice. All assays, except for FPG and vitamin D (measured at the National Institute of Nutrition, Hyderabad, India), were performed in the Genetics and Biochemistry Laboratory of the South Asia Network for Chronic Disease, Public Health Foundation of India, New Delhi. FPG was analyzed by an enzymatic method based on the use of glucose oxidase/peroxidase-4- aminophenazone-phenol on the same day, and the remaining serum samples were stored at −80°C for further analysis; FI was determined by electrochemi-luminescence immunoassay based on the sandwich principle with the use of a Roche Diagnostics Cobas 411 autoanalyzer and Roche reagents; albumin was measure by the bromocresol green method and creatinine by Jaffe's method, rate blanked and compensated. Serum calcium was estimated with *o*-cresolphthalein complex 1 under alkaline conditions. Total serum calcium was corrected for albumin through the use of the formula: (serum calcium mg/dL) + [0.8 × (4 – serum albumin g/dL)]. Inorganic phosphate was measured through formation of the ammonium phosphomolybdenum complex; vitamin D was measured by quantitative HPLC and detected at 265 nm with a UV detector (CV 7%). hsCRP was measured by a particle-enhanced immunoturbidimetry method, IL-6, IL-18, and sICAM-1 were assayed by ELISA, (Krishgen BioSystems), and adiponectin was assayed by a sandwich assay that used kits from Mediagnost, Diagnostika GmbH. The intra-assay and interassay CV were at <3% and <5%, respectively.

HOMA-IR was calculated from the formula [(fasting glucose mg/dL × fasting insulin μU/ml)/405] if fasting insulin was not equal to −1 μU/ml (20). Chronic inflammation was assessed through calculation of a standardized composite inflammatory score (IScore) by summing the *z* scores of markers as follows: [(*z* score IL-6) + (*z* score hsCRP) + (*z* score IL-18) + (*z* score sICAM-1)] – (*z* score adiponectin) as previously described in Tabung et al. (21) with the exception of TNF-α (a proinflammatory marker) which was not available. Participants were categorized as anti- or proinflammatory based on negative and positive IScores, respectively, as described by Tabung et al. (21).

Ethical approval

The study received approvals from the ethics committees of the National Institute of Nutrition, the Indian Council of Medical Research, Hyderabad, India (latest approval 03/CR/2011/IV, dated 23 December 2011) and the London School of Hygiene and Tropical Medicine, London, UK (latest approval LSHTM ethics ref: 6471, dated 29 July 2013). Approval was also sought from the village heads and their committees in each of the 29 villages. The participants provided written informed consent, or a witnessed thumbprint if illiterate, before their inclusion in the study. This analysis received ethical approval from the London School of Hygiene and Tropical Medicine, London, UK (LSHTM MSc ethics ref: 12242, dated 16 June 2017) and Public Health Foundation of India, Gurugram, Haryana, India (TRC-IEC-349/17, dated 10 July 2017).

Statistical methods

All data are presented as mean ± SD, median (IQR), or as numbers (%). The levels of outcome variables (FPG, FI, HOMA-IR, and IScore) were continuous. Corrected serum calcium concentrations were grouped in quartiles. Bivariate comparisons for differences in means, proportions or medians of sociodemographics, lifestyle factors, anthropometrics and biochemical parameters based on gender (men compared with women), and different quartiles of calcium concentrations (Q1–Q4) were made with the use of appropriate tests of significance (*t* test, ANOVA, chi-square test, Wilcoxon rank-sum test, and Kruskal-Wallis ANOVA as per data type). As the data were correlated at household level, multilevel random-effects regression models (linear or logistic) accounting for household clustering were conducted to investigate the association of calcium quartiles with FPG, FI, HOMA-IR, prevalent prediabetes, and type II diabetes after adjusting for sociodemographics [age (years), gender (men/women), degree of urbanization (tertiles)], SLI tertiles, lifestyle factors [tobacco (never/ever), alcohol (never, daily-weekly-monthly, or on special occasions), physical activity (categories based on PALs as extremely inactive, sedentary, moderately active, and vigorously active)], anthropometrics [BMI (kg/m^2), waist circumference (cm)] as well as endogenous regulators of serum calcium concentrations such as serum phosphorus (mg/dL), serum creatinine (mg/dL), and vitamin D (ng/ml) in a subset. Evidence of statistical interactions was assessed by the log-likelihood ratio test of models with and without the interaction terms. We also conducted sensitivity analysis by excluding participants who used any antihypertensive medications. Missing data for different variables were <5% and were excluded from the analysis. To assess if observed effects might be mediated through chronic inflammation, we used the approach of Baron and Kenny (22, 23) This was done in a random sample of a healthy subgroup of participants. Multilevel random-effects models for the associations of corrected serum calcium concentrations with FPG, FI, HOMA-IR, and prevalent prediabetes were conducted with and without adjustments for IScore. Further, independent associations of IScore with corrected serum calcium concentrations, FPG, FI, HOMA-IR, and prevalent prediabetes were also tested. All analyses were performed with STATA Software, version 10.1 (Stata Corp.), and the multilevel sampling scheme was reflected in all of the analyses.

Results

Associations of serum calcium concentrations with markers of dysregulated glucose metabolism and prevalent type II diabetes

The analysis included 2699 APCaPS participants aged 50.3 ± 7.0 y (range: 40–84 y); 52.2% were women. For the whole population, 86.1% were village dwellers, most with no formal education (85.7%).

One-fourth of the participants were current tobacco users (24.9%); 21.5% consumed alcohol regularly; 98.4% ate a nonvegetarian diet; and 50.9% reported a sedentary lifestyle. Median corrected serum calcium was 9.3 mg/dL (IQR: 8.9, 9.7 mg/dL), mean phosphorus was 4.1 ± 0.8 mg/dL and mean vitamin-D was 20.0 ± 9.1 ng/mL (data available for a subset of participants; $n = 1713$). These characteristics stratified by sex are shown in **Table 1**. Although serum calcium concentrations were not different between men and women, there were significant differences in serum concentrations of phosphorus, vitamin D, and creatinine, as well as fasting insulin. Baseline characteristics stratified by quartiles of corrected serum calcium concentrations are presented in **Table 2**. Overall, participants in the highest quartile tended to be living in locations with a higher urbanization score and had higher serum phosphorus, creatinine, fasting glucose, fasting insulin, and insulin resistance, as well as prevalent type II diabetes.

Multivariable adjusted results for the association of corrected serum calcium quartiles with markers of glucose metabolism, prevalent prediabetes and type II diabetes are presented in **Table 3**. After adjustments for demographic, lifestyle, and anthropometric characteristics, participants in the highest calcium quartile were more likely to have higher levels of FI and HOMA-IR with a modest increase in FPG and prevalence of type II diabetes than those in the lowest quartile (Q4 compared with Q1) (FI: $\beta = 1.4$, 95% CI: 1.2, 1.5 μU/ml, P-trend < 0.001; HOMA-IR: $\beta = 1.4$, 95% CI: 1.2, 1.5, P-trend < 0.001; FPG $\beta = 2.1$, 95% CI: −0.9, 5.2, P-trend $= 0.058$ and prevalent type II diabetes OR: 1.6, 95% CI: 1.0, 2.6, P-trend $= 0.020$). There was no association of serum calcium with prevalent prediabetes, and there were no significant interactions in associations between serum calcium and the outcomes by sex. We conducted 2 sensitivity analyses. In the first of these analyses, which used the subset with the vitamin D data ($n = 1713$), a similar pattern as whole population was found, but with slightly stronger associations, including when adjusting for serum concentrations of vitamin D, phosphorus, and creatinine **(Supplemental Table 1).** The vitamin D subset comprised younger men and women with lower SLI than the participants for whom vitamin D data was not available (data not shown). In the second sensitivity analyses we excluded participants who regularly used any hypertensive medication ($n = 188$). Again, the results were similar, although slightly attenuated (Supplemental Table 1).

Mediation of chronic inflammation in associations between serum calcium and markers of dysregulated glucose metabolism in a healthy subset of participants

The characteristics of the healthy subset of participants ($n = 1000$) with inflammatory markers assessed were similar to the whole population and are shown stratified by sex in **Supplementary Table 2**. Men had a higher inflammatory score (IScore) than women (mean IScore: 0.2 ± 2.4 compared with −0.3 ± 2.2 in women). Men had higher amounts of IL-18 but lower IL-6 and adiponectin than women ($P < 0.001$). Although there were no differences in serum calcium concentrations between men and women, the concentrations of serum phosphorus, vitamin D, and fasting insulin, as well as the insulin resistance differed (Supplementary Table 2). Within the healthy subset, after adjustment for demographic characteristics, lifestyle factors and anthropometrics, the highest corrected serum calcium quartile was positively associated with FI ($\beta = 1.3$, 95% CI: 1.2, 1.5, P-trend < 0.001) and HOMA-IR ($\beta = 1.3$, 95% CI: 1.1, 1.5, P-trend < 0.001) (**Table 4**, model 1). On further adjustment with IScore, the associations did not change (Table 4, model 2). The associations were not materially different after adjustments for either individual inflammatory markers or the combined IScore (data not shown). Similar patterns, but with slightly stronger associations, were found in the subset with vitamin-D data ($n = 687$) **(Supplemental Table 3)**. We then assessed if inflammatory score was independently associated with the glucose dysregulation outcomes FPG, FI, HOMA-IR, and prevalent diabetes, and observed significant associations (Q4 compared with Q1) for all outcomes (**Table 5**). Finally, we assessed if inflammatory score was independently associated with corrected serum calcium concentrations (**Table 6**), and observed a significant interaction between IScore and sex, revealing a positive association only in men.

Discussion

In this study, elevated corrected serum calcium concentrations were significantly associated with markers of dysregulated glucose metabolism (increased FI and HOMA-IR as well as FPG), and prevalent type II diabetes, but not prevalent prediabetes, in a rural Indian population after adjustment for all measured potential confounders. When investigated in a subset with available data, these associations were observed to be independent of endogenous regulators of calcium metabolism, including serum phosphorus, creatinine, and vitamin D. The findings were consistent, but slightly attenuated, after excluding participants who used regular medication for hypertension that could have altered calcium metabolism. Furthermore, we observed similar patterns of associations in the subset of participants who had no self-reported history of chronic disease and in whom inflammatory markers were assessed. Inclusion of IScore in this subgroup as a measure of chronic inflammation did not alter the observed measures of association between serum-corrected calcium and glucose dysregulation. However, IScore was independently associated with markers of dysregulated glucose metabolism in both sexes and with elevated serum calcium concentrations, but only in men.

Our results are consistent with previous cross-sectional studies in other populations. In South Korean adults aged ≥40 y, elevated serum calcium was positively associated with type II diabetes and metabolic syndrome (10). Although we did not find any evidence of sex-specific associations for serum calcium and glucose dysregulation in our study population, such associations have been observed in other populations but not consistently. In a healthy Canadian population significantly higher levels of FPG and HOMA-IR were observed in the high-calcium group than in the low-calcium group, and this effect was more evident in women than men (12). Conversely, in Japanese type II diabetics significant positive correlations between serum calcium and FPG and HOMA-IR were observed in men (11) but not in women. A study on oral glucose intolerance in UK participants aged 40–65 y found 2-h plasma glucose to be positively associated with total serum calcium and parathyroid hormone (PTH) both among men and women. Cohort studies (5–9) conducted so far have mostly confirmed temporal associations between elevated serum calcium and incident type II diabetes. These associations, which have been reported

TABLE 1 Participant characteristics[1]

	Men	Women	*P* value[2]	Total
Number (%)	1291 (47.8)	1408 (52.2)		2699
Sociodemographic and socioeconomic characteristics				
Age, y	53.6 ± 7.0	47.3 ± 5.5	<0.0001	50.3 ± 7.0
Education, *n* (%)				
No formal education	969 (75.1)	1342 (95.4)	<0.001	2311 (85.7)
Primary school (up to class IV)	228 (17.7)	52 (3.7)	—	280 (10.4)
Secondary school (class V–X/XII)	94 (7.3)	13 (0.9)	—	107 (4.0)
Occupation, *n* (%)				
Unemployed	76 (5.9)	260 (18.5)	<0.001	336 (12.5)
Manual labour (skilled/unskilled)	1166 (90.4)	1139 (80.9)	—	2305 (85.5)
Skilled nonmanual/semiprofessional	48 (3.7)	8 (0.6)	—	56 (2.1)
SLI[3]	27.4 ± 8.1	26.9 ± 8.4	0.0966	27.1 ± 8.3
Urbanization[4] (tertiles)				
Low	467 (36.2)	513 (36.4)	0.893	980 (36.3)
Medium	397 (30.7)	441 (31.3)	—	838 (31.0)
High	427 (33.1)	454 (32.2)	—	881 (32.6)
Lifestyle characteristics, *n* (%)				
Tobacco				
Never	496 (38.4)	1048 (74.5)	<0.001	1544 (57.2)
Current	753 (58.3)	350 (24.9)	—	1103 (40.9)
Former	42 (3.2)	8 (0.6)	—	50 (1.8)
Alcohol				
Never	437 (33.8)	1073 (76.4)	<0.001	1510 (56.0)
Daily/most days	98 (7.6)	5 (0.4)	—	103 (3.8)
Weekends only	158 (12.2)	13 (0.9)	—	171 (6.3)
Monthly	249 (19.3)	59 (4.2)	—	308 (11.4)
Special occasions	349 (27.0)	255 (18.1)	—	604 (22.4)
PAL				
Extremely inactive	177 (14.8)	68 (5.2)	<0.001	245 (9.8)
Sedentary	486 (40.7)	538 (41.4)	—	1024 (41.1)
Moderately active	435 (36.5)	563 (43.3)	—	998 (40.0)
Vigorously active	95 (8.0)	130 (10.0)	—	225 (9.0)
Physical characteristics				
BMI, kg/m^2	20.5 ± 3.7	21.9 ± 4.2	<0.0001	21.2 ± 4.0
Waist circumference, cm	76.8 ± 10.5	73.4 ± 10.2	<0.0001	75.0 ± 10.5
Prevalent hypertension,[5] *n* (%)	529 (41.0)	396 (28.1)	<0.001	925 (34.3)
Regular medication for hypertension, *n* (%)	108 (11.5)	80 (7.0)	<0.001	188 (9.0)
Prevalent prediabetes,[5] *n* (%)	287 (25.0)	291 (23.1)	0.274	578 (24.0)
Prevalent diabetes,[5] *n* (%)	122 (9.6)	103 (7.6)	0.060	225 (8.5)
Regular medication for diabetes, *n* (%)	51 (4.0)	46 (3.4)	0.383	97 (3.7)
Biochemical parameters				
Corrected serum calcium,[6] mg/dL	9.3 (8.9, 9.8)	9.3 (8.9, 9.7)	0.8051	9.3 (8.9, 9.7)
Serum calcium uncorrected, mg/dL	9.8 (9.3, 10.3)	9.7 (9.3, 10.3)	0.1550	9.7 (9.3, 10.3)
Serum phosphorus, mg/dL	4.0 ± 0.8	4.2 ± 0.8	<0.0001	4.1 ± 0.8
Serum vitamin D,[7] ng/mL	19.2 ± 10.1	20.8 ± 8.0	0.0001	20.0 ± 9.1
Fasting plasma glucose, mg/dL	99.1 ± 27.8	97.9 ± 27.3	0.2884	98.5 ± 27.5
Fasting insulin (μU/ml)	3.0 (1.6, 5.9)	4.8 (3.0, 7.8)	<0.0001	4.1 (2.3, 7.0)
HOMA-IR	0.7 (0.4, 1.4)	1.1 (0.7, 1.9)	<0.0001	0.9 (0.5, 1.7)
Serum creatinine, mg/dL	0.9 ± 0.2	0.7 ± 0.1	<0.0001	0.8 ± 0.2

[1]Participants were rural Indian men and women aged 40–84 y ($n = 2699$) in the Andhra Pradesh Children and Parents Study (APCaPS, 2010–12). Values are *n* (%), means ± SDs, or median (IQR). FPG, fasting plasma glucose; PAL, physical activity level; SLI, Standard of Living Index.
[2]*P*values are from *t* test, Wilcoxon rank-sum, or chi-square test for difference in means, medians, or proportions.
[3]Standardized score based on household assets.
[4]Based on composite score indicating degree of urbanization of the area.
[5]Hypertension: doctor-diagnosed disease, systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg at the time of the interview; diabetes: doctor-diagnosed disease or FPG >126 mg/dL at the time of the interview; prediabetes: FPG = 100–125 mg/dL at the time of the interview.
[6]Total serum calcium corrected for albumin through the use of the formula: (serum calcium mg/dL) + [0.8 × (4 – serum albumin g/dL)].
[7]Data were available for 63.4% of participants ($n = 1713/2699$). Missing data for all other variables were <5% except for PAL (7.7%).

TABLE 2 Distribution of population characteristics by quartiles of corrected serum calcium concentrations[1]

	Quartiles of corrected serum calcium concentrations[2]				
	Q1	Q2	Q3	Q4	*P* value[3]
Serum calcium concentration, mg/dL	8.7 (8.5, 8.8)	9.1 (9.0, 9.2)	9.5 (9.3, 9.6)	10.1 (9.9, 10.5)	—
Number	654	650	651	651	—
Sociodemographic and socioeconomic characteristics					
Age, y	50.1 ± 7.0	50.1 ± 7.1	50.4 ± 7.0	50.7 ± 7.0	0.34
Sex, *n* (%)					
Men	329 (50.3)	302 (46.5)	302 (46.4)	327 (50.2)	0.28
Education, *n* (%)					
No formal education	555 (84.9)	552 (84.9)	561 (86.2)	560 (86.2)	0.50
Primary school (up to class IV)	72 (11.0)	63 (9.7)	70 (10.8)	66 (10.2)	—
Secondary school (class V–X/XII)	27 (4.1)	35 (5.4)	20 (3.1)	24 (3.7)	—
Occupation, *n* (%)					
Unemployed	60 (9.2)	89 (13.7)	96 (14.7)	76 (11.7)	—
Manual labour	579 (88.5)	550 (84.6)	542 (83.3)	552 (86.1)	—
Skilled nonmanual/semiprofessional	15 (2.3)	11 (1.7)	13 (2.0)	14 (2.2)	0.078
SLI[4]	26.8 ± 7.9	27.6 ± 8.3	27.9 ± 8.1	26.4 ± 8.7	0.003
Urbanization[5] (tertiles)					
Low	198 (30.3)	208 (32.0)	231 (35.5)	308 (47.3)	<0.001
Medium	269 (41.1)	251 (38.6)	185 (28.4)	87 (13.4)	—
High	187 (28.6)	197 (29.4)	235 (36.1)	256 (39.3)	—
Lifestyle characteristics, *n* (%)					
Tobacco					
Never	378 (57.9)	385 (59.2)	360 (55.3)	365 (56.2)	0.48
Ever	275 (42.1)	265 (40.8)	291 (44.7)	285 (43.8)	—
Alcohol					
Never	346 (53.0)	389 (59.8)	353 (54.3)	357 (54.9)	0.12
Daily/weekly	70 (10.7)	50 (7.7)	71 (10.9)	74 (11.4)	—
Monthly/special Occasions	237 (36.3)	211 (32.5)	226 (34.8)	219 (33.7)	—
PAL					
Extremely inactive	39 (7.1)	59 (10.1)	65 (10.3)	72 (11.3)	0.005
Sedentary	217 (39.7)	243 (41.4)	282 (44.8)	247 (38.6)	—
Moderately active	249 (4.5)	235 (40.0)	232 (36.9)	244 (38.1)	—
Vigorously active	42 (7.7)	50 (8.5)	50 (7.9)	77 (12.0)	—
Physical characteristics					
BMI, kg/m^2	20.9 ± 3.7	21.3 ± 3.9	21.5 ± 4.3	21.2 ± 4.1	0.025
Waist circumference, cm	74.2 ± 10.1	75.2 ± 10.5	75.8 ± 10.8	75.1 ± 10.3	0.044
Prevalent hypertension,[6] *n* (%)	200 (30.6)	216 (33.2)	240 (36.9)	234 (35.9)	0.071
Prevalent prediabetes,[6] *n* (%)	137 (22.3)	136 (22.6)	164 (27.9)	132 (22.7)	0.071
Prevalent diabetes,[6] *n* (%)	40 (6.1)	48 (7.4)	61 (9.4)	69 (10.6)	0.016
Biochemical parameters					
Serum phosphorus, mg/dL	4.0 ± 1.2	4.0 ± 0.6	4.2 ± 0.6	4.4 ± 0.6	<0.001
Serum vitamin D,[7] ng/mL	20.3 ± 10.2	19.9 ± 8.4	19.3 ± 8.4	20.5 ± 9.3	0.25
Fasting plasma glucose, mg/dL	95.7 ± 20.0	96.6 ± 24.3	100.6 ± 29.2	100.6 ± 33.1	<0.001
Fasting insulin, μU/mL	3.6 (2.1, 6.0)	3.8 (2.2, 6.8)	4.3 (2.5, 7.5)	4.5 (2.4, 7.8)	<0.001
HOMA-IR score	0.8 (0.5, 1.4)	0.9 (0.5, 1.6)	1.0 (0.6, 1.9)	1.1 (0.5, 1.9)	<0.001
Serum creatinine, mg/dL	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	<0.001

[1] Participants were rural Indian men and women aged 40–84 y (*n* = 2699) in the Andhra Pradesh Children and Parents Study (APCaPS, 2010–12). Values are *n* (%), means ± SDs, or median (IQR). FPG, fasting plasma glucose; PAL, physical activity level; Q, quartile; SLI, Standard of Living Index.
[2] Total serum calcium corrected for albumin through the use of the formula: (calcium mg/dL) + [(0.8 × (4 – albumin g/dL)].
[3] *P* values are from ANOVA, Kruskal-Wallis ANOVA, or chi-square test for difference in means, medians or proportions.
[4] Standardized score based on household assets.
[5] Based on composite score indicating degree of urbanization of the area.
[6] Hypertension: doctor-diagnosed disease, a systolic blood pressure ≥140 mm Hg, or a diastolic blood pressure ≥90 mm Hg at the time of the interview; diabetes: doctor-diagnosed disease or FPG >126 mg/dL at the time of the interview; prediabetes: FPG = 100–125 mg/dL at the time of the interview.
[7] Data were available for 63.4% of participants (*n* = 1713/2699). Missing data for all other variables were <5% except for PAL (7.7%).

TABLE 3 Associations of corrected serum calcium concentrations with fasting plasma glucose, fasting insulin, insulin resistance, prevalent prediabetes and diabetes[1]

Corrected serum calcium quartiles	FPG, mg/dL (β; 95% CI)	FI,[2] μU/ml (β; 95% CI)	HOMA-IR[2] (β; 95% CI)	Prevalent prediabetes (OR; 95% CI)	Prevalent diabetes (OR; 95% CI)
Model 1	$n = 2605$	$n = 2604$	$n = 2603$	$n = 2385$	$n = 2605$
Q1	0.00 (ref)	0.00 (ref)	0.00 (ref)	1.00 (ref)	1.00 (ref)
Q2	0.7 (−2.2, 3.6)	1.1 (1.0, 1.2)	1.1 (1.0, 1.3)	1.0 (0.8, 1.4)	1.2 (0.8, 1.8)
Q3	4.6 (1.6, 7.5)	1.3 (1.1, 1.4)	1.3 (1.2, 1.5)	1.3 (1.0, 1.8)	1.6 (1.0, 2.4)
Q4	4.6 (1.6, 7.6)	1.4 (1.3, 1.6)	1.5 (1.3, 1.6)	0.9 (0.7, 1.3)	1.9 (1.2, 2.9)
P-trend	<0.001	<0.001	<0.001	0.749	0.001
Model 2	$n = 2394$	$n = 2393$	$n = 2392$	$n = 2190$	$n = 2394$
Q1	0.00 (ref)	0.00 (ref)	0.00 (ref)	1.00 (ref)	1.00 (ref)
Q2	−1.4 (−4.4, 1.6)	1.1 (1.0, 1.2)	1.1 (1.0, 1.2)	0.9 (0.7, 1.2)	0.9 (0.5, 1.5)
Q3	1.3 (−1.6, 4.3)	1.2 (1.1, 1.3)	1.2 (1.1, 1.3)	1.0 (0.8, 1.4)	1.1 (0.7, 1.8)
Q4	2.1 (−0.9, 5.2)	1.4 (1.2, 1.5)	1.4 (1.2, 1.5)	0.7 (0.5, 1.1)	1.6 (1.0, 2.6)
P-trend	0.058	<0.001	<0.001	0.206	0.020
P-interaction by sex[3]	0.6589	0.3144	0.2690	0.8267	0.4047

[1] Participants were rural Indian men and women aged 40–84 y from the Andhra Pradesh Children and Parents Study (APCAPS, 2010–12). Associations were determined by multilevel random-effects linear (for FPG, FI and HOMA-IR) and logistic (for prediabetes and diabetes prevalence) regression models. Median serum calcium concentrations (mg/dL) corrected for albumin by quartiles: Q1 = 8.7 (IQR: 8.5, 8.8); Q2 = 9.1 (IQR: 9.0, 9.2); Q3 = 9.5 (IQR: 9.3, 9.6); Q4 = 10.1 (IQR: 9.9, 10.5). Model 1: adjusted for age (y), sex, SLI (tertiles), urbanization score (tertiles). Model 2: adjusted for age (y), sex, SLI (tertiles), urbanization score (tertiles), tobacco (never/ever), alcohol (categories), physical activity (categories), BMI (kg/m^2), waist circumference (cm). FI, fasting insulin; FPG, fasting plasma glucose; Q, quartile; ref, reference; SLI, Standard of Living Index.
[2] β coefficient and CI values were backtransformed (e^{β}) as outcome variables and were natural log transformed before analyses.
[3] Likelihood-ratio test for interaction of sex with serum calcium quartiles.

across different populations, were also unaffected by endogenous regulators of calcium metabolism including vitamin D, phosphorus, magnesium, serum creatinine or glomerular filtration rate, as well as PTH. However, a recent prospective study among southeast Asians reported no association between serum calcium and incident type II diabetes, but on the contrary reported higher dietary calcium to be associated with a reduced risk of type II diabetes development (24).

Vitamin D and PTH may also be independently involved in diabetes risk (8, 25, 26). We did not have any information available on PTH, a deficiency that limits our interpretation in terms of some residual

TABLE 4 Associations of corrected serum calcium concentrations with fasting plasma glucose, fasting insulin, insulin resistance and prevalent prediabetes[1]

Corrected serum calcium quartiles	FPG, mg/dL (β; 95% CI)	FI,[2] μU/mL (β; 95% CI)	HOMA-IR[2] (β; 95% CI)	Prevalent prediabetes (OR; 95% CI)
Model 1	$n = 917$	$n = 917$	$n = 917$	$n = 882$
Q1	0.00 (ref)	0.00 (ref)	0.00 (ref)	1.00 (ref)
Q2	−1.6 (−5.2, 2.0)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)	0.8 (0.5, 1.4)
Q3	−0.13 (−3.8, 3.5)	1.1 (0.9, 1.2)	1.1 (0.9, 1.2)	1.0 (0.6, 1.7)
Q4	1.5 (−2.2, 5.2)	1.3 (1.2, 1.5)	1.3 (1.1, 1.5)	0.8 (0.5, 1.4)
P-trend	0.334	<0.001	<0.001	0.627
P-interaction by sex[3]	0.9972	0.0361	0.0634	0.7121
Model 2	$n = 914$	$n = 914$	$n = 914$	$n = 879$
Q1	0.00 (ref)	0.00 (ref)	0.00 (ref)	1.00 (ref)
Q2	−1.6 (−5.2, 2.0)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)	0.8 (0.5, 1.4)
Q3	−0.5 (−4.2, 3.1)	1.1 (0.9, 1.2)	1.1 (0.9, 1.2)	0.9 (0.5, 1.6)
Q4	0.7 (−3.0, 4.4)	1.3 (1.1, 1.5)	1.3 (1.1, 1.5)	0.7 (0.4, 1.3)
P-trend	0.613	<0.001	<0.001	0.368
P-interaction by sex[3]	0.9973	0.0467	0.0818	0.6351
P-interaction by IScore[4]	0.6647	0.6499	0.7164	0.6182

[1] Participants were a random sample of healthy rural Indian men and women aged 40–84 y ($n = 1000$) from the Andhra Pradesh Children and Parents Study (APCaPS, 2010–12). Participants with a self-reported history of any chronic disease including hypertension, diabetes, cardiovascular disease, chronic obstructive respiratory disease, and cancer were excluded. Associations were determined with multilevel random-effects linear (for FPG, FI and HOMA-IR) and logistic (for prevalent prediabetes) regression models. Median serum calcium concentrations (mg/dL) corrected for albumin by quartiles: Q1 = 8.7 (IQR: 8.5, 8.8); Q2 = 9.1 (IQR: 9.0, 9.2); Q3 = 9.5 (IQR: 9.3, 9.6); Q4 = 10.1 (IQR: 9.9, 10.5). Model 1: adjusted for age (y), sex, SLI (tertiles), urbanization score (tertiles), tobacco (never/ever), alcohol (categories), physical activity (categories), BMI (kg/m^2), and waist circumference (cm); Model 2: Model 1 plus overall inflammatory score. FI, fasting insulin; FPG, fasting plasma glucose; IScore, inflammatory score; Q, quartile; ref, reference; SLI, Standard of Living Index.
[2] β coefficient and CI values were back-transformed (e^{β}) as outcome variables and were ln-transformed before analyses.
[3] Likelihood-ratio test for interaction of sex and serum calcium quartiles.
[4] Likelihood-ratio test for interaction of IScore and serum calcium quartiles [IScore was categorized as an anti-inflammatory score or a proinflammatory score based on negative and positive values, respectively (21)].

TABLE 5 Associations of inflammatory score with fasting plasma glucose, fasting insulin, insulin resistance and prevalent prediabetes[1]

IScore quartiles[2]	FPG (mg/dL) (β; 95% CI) (n = 913)	FI[3] (μU/ml) (β; 95% CI) (n = 913)	HOMA-IR[3] (β; 95% CI) (n = 913)	Prevalent prediabetes (OR; 95% CI) (n = 878)
Q1	Ref	Ref	Ref	Ref
Q2	0.01 (−0.01, 0.04)	1.1 (1.01, 1.3)	1.1 (1.01, 1.3)	1.5 (0.9, 2.8)
Q3	0.02 (−0.006, 0.05)	1.08 (0.96, 1.2)	1.1 (0.96, 1.2)	1.2 (0.6, 2.1)
Q4	0.07 (0.04, 0.1)	1.3 (1.1, 1.5)	1.4 (1.2, 1.6)	2.8 (1.5, 5.4)
P-trend	<0.001	0.001	<0.001	0.003
P-interaction by sex[4]	0.2701	0.7967	0.7707	0.1256

[1]Participants were a random sample of healthy rural Indian men and women aged 40–84 y (n = 1000) from the Andhra Pradesh Children and Parents Study (APCaPS, 2010–12). Participants with a self-reported history of any chronic disease including hypertension, diabetes, cardiovascular disease, chronic obstructive respiratory disease, and cancer were excluded. Associations were adjusted for age (years), sex, SLI (tertiles), urbanization score (tertiles), tobacco (never/ever), alcohol (categories), physical activity (categories), BMI (kg/m^2), waist circumference (cm), serum calcium (mg/dL), serum phosphorus (mg/dL), and serum creatinine (mg/dL) with a random-effects linear/logistic multilevel model. CRP, C-reactive protein; FI, fasting insulin; FPG, fasting plasma glucose; IScore, inflammatory score; Q, quartile; Ref, reference; sICAM-1, soluble intercellular adhesion molecule 1; SLI, Standard of Living Index.
[2]IScore was calculated by summing the z scores of markers to create a standardized overall inflammatory marker score for each participant as follows: [(z score IL-6) + (z score CRP) + (z score IL-18) + (z score sICAM-1)] – (z score adiponectin) (21).
[3] β coefficient and CI values were back-transformed (e^{β}) as outcome variables and were ln-transformed before analyses.
[4]Likelihood-ratio test for interaction of sex and inflammatory score quartiles.

confounding. However, the dose-response association (Q4 compared with Q1) was consistent in the overall study population as well as across several subsets within the study population (i.e., a healthy subset, a subset excluding participants with regular hypertensive medication use, as well as a subset with vitamin D data). The associations with markers of dysregulated glucose metabolism and prevalent type II diabetes were strengthened in the subset with vitamin D data. This subset included young men and women living in locations with higher urbanization scores and low SLIs. This might have been a chance finding due to the relatively smaller sample size but could possibly be indicating high-risk individuals within this population.

The probable causal role of elevated serum calcium for type II diabetes is becoming established through cohort studies and consistent cross-sectional findings across different populations worldwide (4–13, 27). Randomized clinical trials report that calcium-channel blockers or other medications prevent or stabilize type II diabetes compared with the use of diuretics in patients with other disease conditions including hypertension (14, 28).

The proposed mechanisms for the associations of elevated serum calcium on glucose metabolism dysregulation include the role of calcium in reducing the function of glucose transporters on adipocytes (15), altering β cell function, and increasing insulin resistance. Serum calcium concentrations could be elevated by underlying chronic low levels of inflammation (4, 27). Inflammation and elevated calcium could synergistically contribute to organelle dysfunction and damage, leading to dysregulated glucose metabolism (4, 27). In our study, chronic inflammation did not appear to mediate associations between serum calcium and dysregulated glucose metabolism. Inadequate measurement of inflammation could have influenced these associations. However, although we did not have information on TNF-α and IL-10, important pro- and anti-inflammatory markers, respectively (29), the use of a composite inflammatory score has been shown previously to adequately characterize the overall inflammatory status of the participants (21). The role of chronic inflammation in the pathogenesis of type II diabetes is well established, (4, 27). We found independent positive associations of chronic inflammation with

TABLE 6 Associations of inflammatory score with corrected serum calcium concentrations[1]

IScore quartiles[2]	Corrected serum calcium concentration (β; 95% CI)		
	Overall (n = 914)	Men (n = 461)	Women (n = 453)
Q1	Ref	Ref	Ref
Q2	0.09 (−0.02, 0.2)	0.3 (0.1, 0.5)	−0.07 (−0.2, 0.09)
Q3	0.1 (0.02, 0.2)	0.3 (0.2, 0.5)	−0.07 (−0.2, 0.1)
Q4	0.2 (0.1, 0.4)	0.4 (0.2, 0.6)	0.2 (−0.01, 0.3)
P-trend	<0.001	<0.001	0.113
P-interaction by sex[3]	0.0258	—	—

[1]Participants were a random sample of healthy rural Indian men and women aged 40–84 y (n = 1000) from the Andhra Pradesh Children and Parents Study (APCaPS, 2010–12). Participants with a self-reported history of any chronic disease including hypertension, diabetes, cardiovascular disease, chronic obstructive respiratory disease, and cancer were excluded. Association were adjusted for age (y), sex, SLI (tertiles), urbanization score (tertiles), tobacco (never/ever), alcohol (categories), physical activity (categories), BMI (kg/m^2), and waist circumference (cm) through the use of a random-effects linear multilevel model. The associations followed a similar pattern when adjusted for serum concentrations of phosphorous, creatinine and vitamin D in a subset with vitamin D data (n = 618). CRP, C-reactive protein; FI, fasting insulin; FPG, fasting plasma glucose; SLI, Standard of Living Index; IScore, inflammatory score; Q, quartile; Ref, reference; sICAM-1, soluble intercellular adhesion molecule 1.
[2]IScore was calculated summing the z scores of markers to create a standardized overall inflammatory marker score for each participant as follows: [(z score IL-6) + (z score CRP) + (z score IL-18) + (z score sICAM-1)] – (z score adiponectin) (21).
[3]Likelihood-ratio test for interaction of sex and inflammatory score quartiles.

markers of dysregulated glucose metabolism in the healthy subset of participants, consistent with the existing evidence.

There is a large body of preclinical data (in vitro and animal models) on the elevation of serum calcium concentrations by chronic inflammation (4, 27). We found positive associations of chronic inflammation (IScore) with elevated corrected serum calcium among men, indicating a role for chronic inflammation in elevating serum calcium concentrations. We did not find similar associations among women, suggesting that the factors for elevated serum calcium in women could be other than inflammation. Similar sex-specific differences have been observed with coronary artery calcium deposition and inflammatory markers wherein adiponectin was found to be inversely associated (30) and hsCRP positively associated with coronary artery calcium in men (31) but not among women. It has been established that inflammatory responses and their influence on various chronic diseases, including type II diabetes, are gender specific due to sex hormones, genetic factors, and differences in lifestyle risk exposures (28), but further investigations are required to understand the mechanisms better. Inadequate measurement of inflammatory markers specific for women, such as IFN-γ and IL-10 (28), could also have influenced these associations; however, this is less likely as chronic inflammation was associated with markers of dysregulated glucose metabolism.

Our results suggest that chronic inflammation may not be on the causal pathway between serum calcium and glucose dysregulation in our study population, but it remains possible that it may be involved in early elevation of serum calcium concentrations among men. In this population, serum calcium concentrations and chronic inflammation are associated with glucose metabolism but act independently of each other. Cautious interpretation of these results is required for the following reasons: *1*) the inherent limitations of a cross-sectional study design such as reverse causation and temporality; *2*) the potential measurement error of chronic inflammation, although the inflammatory markers used in our study were among the markers most commonly used to examine disease endpoints, have been validated for calculating inflammatory scores in other populations (21), and were previously associated with calcium and glucose metabolism (4, 27, 30, 31); and *3*) the association between elevated serum calcium and prevalent prediabetes was not evident in this population.

Despite these limitations, this study possesses considerable merits. This was a rural Indian population-based study with an adequate sample size to test the dose response. The dose-response results for the association of elevated serum calcium with markers of dysregulated glucose metabolism and type II diabetes were stable across different subsets. Our results are in line with scientific evidence available to date and are biologically plausible. Furthermore, we observed independent associations of chronic inflammation with dysregulated glucose metabolism and serum calcium concentrations. To the best of our knowledge, this is the first report of the associations of elevated serum calcium concentrations and chronic inflammation on markers of glucose metabolism dysregulation, evaluating them as independent risk factors, confounders, and mediators in a representative rural Indian population.

In conclusion, consistent with previous observational studies, elevated serum calcium is positively associated with markers of dysregulated glucose metabolism and prevalent type II diabetes in a rural Indian population. This association was not mediated through chronic inflammation, but chronic inflammation was independently associated with glucose metabolism dysregulation. It remains possible that inflammation might be responsible for elevation of serum calcium early in the pathway, but only among men. Large-scale, population-based studies to validate these findings, and clinical studies to address their clinical relevance and understand potential sex-specific effects are recommended.

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