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Plasma calcium as a predictor of cardiovascular disease in a community-based cohort

Short title: Calcium and cardiovascular disease

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Abstract

Objective Primary hyperparathyroidism and calcium supplementation have been linked to cardiovascular outcomes. The study objective was to examine plasma calcium as a predictor of cardiovascular disease in the general population, as results from previous cohort studies are conflicting.

Design, participants and measurements Plasma calcium was measured in 4003 participants (aged 25-84 years) in the 1994/5 Busselton Health Survey. Using a Cox proportional hazards model, we examined albumin-corrected calcium as a predictor of total mortality, cardiovascular mortality and cardiovascular events up to the end of 2010.

Results At baseline, there were significant positive relationships between plasma calcium and each of body mass index, systolic and diastolic blood pressure, glucose and total cholesterol. During the follow-up period, 666 participants died (278 from cardiovascular disease) and 652 had incident cardiovascular events. After adjustment for age and sex, each additional 0.1 mmol/L of albumin-corrected calcium at baseline was associated with a hazard ratio [HR] of 1.09 (95% confidence interval [CI] 0.99, 1.20; P=0.062) for total mortality, 1.06 (95% CI 0.92, 1.23; P=0.41) for cardiovascular mortality and 1.13 (1.03, 1.24; P=0.012) for cardiovascular events. These associations were attenuated by further adjustment for standard cardiovascular risk factors with HR 1.03 (95% CI 0.94, 1.14), 0.99 (95% CI 0.86, 1.16) and 1.05 (95% CI 0.95, 1.15) respectively.

Conclusion After adjustment for age and sex, plasma calcium is a predictor of cardiovascular events. This appears to be mediated by conventional cardiovascular risk factors, and calcium is not an independent predictor of cardiovascular disease.

In healthy individuals, circulating concentrations of calcium are tightly regulated by homeostatic mechanisms involving the calcium-sensing receptor, parathyroid hormone and 1,25-dihydroxyvitamin D, and disorders of calcium homeostasis are associated with a range of adverse clinical outcomes, including cardiovascular disease.[1] Hypercalcaemia caused by primary hyperparathyroidism is associated with hypertension and cardiovascular dysfunction[2] and several cohort studies[3-5] (though not all[6]) report an increased risk of cardiovascular disease. In cohort studies of patients with end-stage renal failure, serum calcium is a predictor of cardiovascular outcomes, such that each additional 0.25 mmol/L of calcium at baseline is associated with a 15% increase in cardiovascular mortality.[7, 8] The pathophysiology of chronic kidney disease is complex, however, and the contribution of circulating calcium to cardiovascular risk is difficult to tease out from those of phosphate, vitamin D and parathyroid hormone.[9]

It is uncertain whether circulating calcium concentrations are a predictor of cardiovascular risk in the general population, and in particular whether the small differences in calcium which exist between normocalcaemic individuals are associated with differing risks of cardiovascular disease. Cohort studies have yielded conflicting results: in three studies of Swedish men, plasma calcium was a predictor of total mortality, cardiovascular mortality[10, 11] and myocardial infarction[12], whereas in the Framingham Study and the Atherosclerosis Risk in Communities (ARIC) Study, calcium was not a predictor of cardiovascular disease in age- and sex-adjusted[13] or multivariable-adjusted models.[13, 14] The conflicting results from these studies may reflect demographic differences between participants or differences in analysis, such as adjustment for covariates. The latter is important because plasma calcium is significantly associated with cardiovascular risk factors including body mass index (BMI), blood pressure, glucose, cholesterol and insulin resistance.[14-21]

Interest in this area has been rekindled by two meta-analyses suggesting that calcium supplementation increases the risk of cardiovascular disease in older women,[22, 23] although this remains controversial.[24-27] The putative mechanism includes an increase in circulating calcium concentrations after ingestion of calcium supplements,[28] and in a recent study of women who were all taking calcium and vitamin D supplements, serum calcium was an independent predictor of cardiovascular events.[18] In view of the conflicting evidence as to whether plasma calcium is a predictor of cardiovascular outcomes in the general population, we examined this in a well-characterized, community-based cohort.

Methods

The Busselton Health Study (http://busseltonhealthstudy.com) includes cross-sectional health surveys conducted between 1966 and 1987 of residents of Busselton, a rural town in south-west Western Australia (latitude 33.6ºS) with a mainly white population. In 1994/95, all surviving participants of earlier surveys were invited to participate in a follow-up survey. There were 5,909 adults who attended the survey, of whom 4,843 gave blood samples. After restricting the analysis to participants aged 25-84 years and eliminating those with missing values for key variables, there were 4,003 people in the full cohort analysis described here. All participants gave informed consent.

The conduct of the 1994/95 survey has been described previously.[29] Briefly, participants completed a health questionnaire and underwent various measurements and tests. Blood pressure was measured using a mercury sphygmomanometer after five minutes rest in a sitting position. Anthropometric measures were obtained by trained survey staff using standardised protocols. Smoking history, alcohol consumption, diabetes and medications were

obtained by questionnaire. Plasma total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides, glucose, albumin, creatinine and total calcium were determined from a fasting venous blood sample at the time of survey. Plasma calcium was measured using a Hitachi 747 analyzer (Roche Diagnostics Australia Pty Ltd, Castle Hill, New South Wales) and the manufacturer's cresolphthalein complexone method. Alcohol consumption was labelled 'light' if ≤140 g/week and 'heavy' if >140 g/week. BMI was calculated as weight (kg) divided by the square of height (m). Estimated glomerular filtration rate (eGFR) in mL/min/1.73m^2 was calculated using the Modification of Diet in Renal Disease Study Group formula: eGFR =175 x [Creatinine*0.0113]$^{-1.154}$ x [Age]$^{-0.203}$ x [0.742 if female].[30] Plasma calcium was corrected to the mean albumin concentration in study participants of 45g/L, using the formula: corrected calcium = calcium + 0.02 x (45 – albumin). The vitamin D status of Busselton Health Study participants is not known, but in a recent national Australian survey, the mean 25-hydroxyvitamin D concentration was 63 nmol/L, with 31% of participants having a level of less than 50 nmol/L.[31]

Hospital admission, cancer and death records from 1 January 1980 to 31 December 2010 were accessed using record linkage to the Western Australian Hospital Morbidity Data System, which records all hospital admissions to public and private hospitals in Western Australia, as well as cancer registrations and deaths.[32] International Classification of Diseases, 9th revision (ICD-9) codes were used for events up to 30 June 1999, and ICD-10 codes for subsequent events. History of cardiovascular disease at baseline was defined as hospital admission with a primary or secondary discharge diagnosis of cardiovascular disease (ICD-9 390-459) during the 15 years before the survey (ie from 1980 to 1994), and history of cancer as any cancer diagnosis prior to the survey. Cardiovascular events during the 15 year follow-up period from

1995 to the end of 2010 were ascertained from hospital admission and death records. Cardiovascular events were defined as hospital admission with a principal diagnosis of coronary heart disease (ICD9 410-414; ICD10 I20-25) or stroke (ICD9 430-437; ICD10 I60-68, G45 or death from cardiovascular disease (ICD9 390-459; ICD10 I00-99, G45).

Descriptive results are presented as mean (standard deviation [SD]) for quantitative variables and proportions (percentage) for categorical variables. Age- and sex-adjusted comparisons of variables across tertiles of albumin-corrected calcium (≤2.26, 2.27 to 2.33, and >2.33 mmol/L) at baseline were performed using regression-based trend tests. Cox regression modelling was used to estimate the effect of albumin-adjusted calcium on total mortality, cardiovascular mortality and incident cardiovascular events (fatal or non-fatal) after adjustment for age and sex, after further adjustment for measures of obesity (BMI and waist circumference), and after further adjustment for the following cardiovascular risk factors: diabetes, smoking, alcohol drinking, systolic blood pressure, diastolic blood pressure, hypertension medication, glucose, total cholesterol, HDL cholesterol, triglycerides and lipid-lowering medication. In sensitivity analyses, regression models were re-fitted using uncorrected calcium in place of albumin-corrected calcium and in three sub-cohorts: the first obtained by excluding participants with a history of cancer, cardiovascular disease or chronic kidney disease (defined as eGFR<30 mL/min/1.73m^2) at baseline, a second sub-cohort obtained by excluding people taking thiazide or thiazide-like diuretics or calcium supplements at baseline (because of the effects of these medications on plasma calcium), and the third comprised postmenopausal women (because of the effects of menopause on circulating calcium). Results from Cox models are presented as the estimated hazard ratio for each additional 0.1 mmol/L of albumin-corrected calcium at baseline (as a continuous variable) or for the middle and upper tertiles of albumin-corrected calcium compared with the lowest

tertile (as reference), together with the 95% confidence interval [CI] and associated P value. Significance was set at P<0.05.

The study was approved by the Human Research Ethics Committee of The University of Western Australia and the Confidentiality of Health Information Committee of the Department of Health of Western Australia.

Results

Table 1 shows baseline characteristics of the full cohort and of participants analysed by tertiles of albumin-corrected calcium. The mean age of the 4003 participants was 52 years and 56% were female. At baseline, most standard cardiovascular risk factors showed a significant (age- and sex-adjusted) positive trend across increasing tertiles of calcium, including BMI, waist circumference, systolic and diastolic blood pressure, plasma glucose and plasma total cholesterol.

During the 15 year follow-up period, there were 666 deaths (including 278 from cardiovascular diseases) and 652 participants experienced an incident cardiovascular event (fatal or non-fatal). The observed proportions of participants experiencing events (death from any cause, cardiovascular death, cardiovascular event) increased across tertiles of calcium (Table 1). Table 2 shows hazard ratios for the outcome measures associated with plasma calcium at baseline analysed as a continuous variable and as tertiles of calcium. After adjustment for age and sex, each additional 0.1 mmol/L of albumin-corrected plasma calcium at baseline was associated with a hazard ratio of 1.09 (95% CI 0.99, 1.20; P=0.062) for total mortality, 1.06 (95% CI 0.92, 1.23; P=0.414) for cardiovascular mortality and 1.13 (1.03,

1.24; P=0.012) for cardiovascular events. Adjustment for measures of obesity reduced the hazard ratios slightly to 1.08 (95% CI 0.98, 1.18; P=0.119) for total mortality, 1.05 (95% CI 0.91, 1.21; P=0.505) for cardiovascular mortality and 1.11 (1.01, 1.22; P=0.029) for cardiovascular events. After further adjustment for standard cardiovascular risk factors, none of these associations were significant, with attenuated hazard ratios of 1.03 (95% CI 0.94, 1.14) for total mortality, 0.99 (95% CI 0.86, 1.16) for cardiovascular mortality and 1.05 (95% CI 0.95, 1.15) for cardiovascular events. In the corresponding models that considered calcium in tertiles, there was no significant association with any of the outcome measures after adjustment for age and sex or after further adjustment for cardiovascular risk factors.

The attenuation of the hazard ratios from the age- and sex-adjusted models by multivariate adjustment suggests that any increased risk of cardiovascular events in the former is explained by associations between plasma calcium and cardiovascular risk factors. The intermediate models adjusted for age, sex and measures of obesity, and further modelling for the cardiovascular event rate showed that the attenuation could not be attributed to obesity or any other particular cardiovascular risk factor; that is, the hazard ratio progressively declined with adjustment for each additional risk factor, except for alcohol and smoking.

In sensitivity analyses, regression modelling was repeated using uncorrected calcium which gave very similar results to albumin-corrected calcium; in particular, the age- and sex-adjusted hazard ratio for cardiovascular events was 1.10 (95% CI 1.01, 1.20; P=0.028) for each additional 0.1 mmol/L of calcium, whereas after multivariate adjustment the hazard ratio was 1.02 (95% CI 0.93, 1.11; P=0.745). We also analysed three subcohorts. The first comprised 3142 individuals obtained by excluding 861 participants with a history of cardiovascular disease (N=724), cancer (N=210) or eGFR<30 mL/min/1.73m^2 (N=16) at

baseline. (Some participants satisfied more than one exclusion criteria; hence the numbers excluded add up to more than 861). In this subgroup, none of the associations were significant, with age- and sex-adjusted hazard ratio of 1.04 (95% CI 0.92, 1.19; P=0.508) for total mortality, 0.95 (95% CI 0.78, 1.17; P=0.656) for cardiovascular mortality and 1.10 (95% CI 0.97, 1.25; P=0.133) for cardiovascular events for each additional 0.1 mmol/L of albumin-corrected calcium. As in the primary analysis, these associations were not significant after further adjustment for cardiovascular risk factors. The second subcohort comprised 3802 individuals obtained by excluding 201 participants who were taking thiazide or thiazide-like diuretics (N=145), calcium supplements (N=51) or both (N=5) at baseline and gave similar results, with hazard ratios of 1.09 (95% CI 0.98, 1.21; P=0.101) for total mortality, 1.02 (95% CI 0.87, 1.20; P=0.812) for cardiovascular mortality and 1.08 (95% CI 0.97, 1.20; P=0.151) for cardiovascular events. After further adjustment for cardiovascular risk factors, none of these associations were significant. The third subcohort comprised 1211 post-menopausal women in whom the results were very similar to those for the full cohort; in particular, the hazard ratio for cardiovascular events was 1.10 (95% CI 1.01, 1.20; P=0.016) after adjustment for age, and 1.08 (95% CI 0.95, 1.23; P=0.232) after further adjustment for cardiovascular risk factors.

Discussion

In this large, community-based cohort, plasma calcium was a significant predictor of cardiovascular events after adjustment for age and sex, such that each additional 0.1 mmol/L of baseline calcium was associated with a 13% increase in cardiovascular events. This relative risk was slightly attenuated by adjustment for measures of obesity and was no longer statistically significant in the multivariable-adjusted models, suggesting that any increased risk is mediated by obesity and other standard cardiovascular risk factors. Consistent with

this, there was a strong association at baseline between calcium and cardiovascular risk factors including BMI, blood pressure, glucose and cholesterol, as has been previously reported.[14-21] Thus, although there was evidence of plasma calcium as a predictor of cardiovascular events, it had no independent predictive value when those factors were taken into account.

In sensitivity analyses, the age-and sex-adjusted hazard ratios for cardiovascular events were attenuated and no longer significant after exclusion of participants taking calcium-sparing diuretics or calcium supplements, and after excluding those with preexisting cardiovascular disease, cancer or renal impairment. This may reflect a loss of statistical power, since many of the participants excluded were at increased risk of cardiovascular disease, but could also be interpreted as indicating that plasma calcium has no predictive value in the non-medicated, general population.

Previous studies have generated conflicting results for both age-adjusted and multivariate-adjusted relative risks for calcium as a predictor of cardiovascular disease.[10-14] If calcium were an independent predictor of outcomes, this would manifest in significantly increased relative risks in both age-adjusted and multivariate-adjusted models. In two studies of Swedish men, calcium was a predictor of cardiovascular outcomes after adjustment for age.[10, 11] In one of these[11] and a third Swedish study,[12] calcium had independent predictive value in multivariate-adjusted models, which appears inconsistent with our data. The Framingham Study reported that serum calcium did not predict cardiovascular events in either age-adjusted and multivariate-adjusted models,[13] whereas the ARIC Study reported that calcium was not an independent predictor of cardiovascular outcomes in a multivariate model.[14] Differences between studies in participant demographics, recruitment strategies and analysis techniques

may account for these inconsistencies; a meta-analysis with substantially increased statistical power over individual studies might clarify the relationship between calcium and cardiovascular outcomes. In a further recent study from Germany, serum calcium was an independent predictor of total mortality but not cardiovascular events.[33] All participants in this study had coronary heart disease at baseline and more than half were hypercalcaemic, making comparison with our results difficult.

The mechanisms underlying the associations between circulating calcium, cardiovascular risk factors and cardiovascular disease appear to be multiple and complex. The calcium-sensing receptor is expressed in vascular smooth muscle and endothelial cells, and mediates at least some of the effect of circulating calcium on vascular tone and blood pressure.[1, 34] Vascular calcification is now recognised to be a complex and actively regulated process involving matrix Gla protein, fetuin A, pyrophosphate, bone morphogenetic proteins and osteoprotegerin,[9, 28, 34] and it is biologically plausible that increasing serum calcium concentrations might alter the balance of this system in favour of calcification.[28] Consistent with this, in a recent observational study of 7553 patients undergoing coronary angiography, serum calcium was a significant predictor of calcified coronary artery plaque and coronary artery disease, but not of noncalcified plaque. [35]

Strengths of our study include the relatively large size and community-based nature of the cohort. A limitation is that outcome measures were derived by record linkage rather than direct follow-up of the cohort, but this data linkage system has been shown to be reliable for cardiovascular event ascertainment.[32] A further limitation is that we measured total calcium rather than ionised calcium, which is thought to be the biologically active fraction. We corrected calcium to the mean plasma albumin concentration of the participants, as is

common practice in epidemiological studies of this type, but we recognize that the correlation between ionised total and calcium is imperfect even after correction for albumin.[36]

In conclusion, we report that plasma calcium may be a predictor of cardiovascular events in the general population, but has little or no additional predictive value when standard cardiovascular risk factors are taken into account. This has important implications for the prediction of cardiovascular disease in epidemiological studies, and to further understanding the complex associations between calcium supplementation, primary hyperparathyroidism, vascular calcification and cardiovascular disease.

Table 1. Characteristics of the total cohort.

Variable	All (n=4,003)	Albumin-corrected calcium, mmol/L Ca ≤ 2.26 (n=1,281)	2.26 < Ca ≤ 2.33 (n=1,495)	Ca > 2.33 (n=1,227)	P value*
Calcium, mmol/L	2.31 (0.08)	2.24 (0.06)	2.30 (0.05)	2.38 (0.07)	
Albumin, g/L	45.1 (2.7)	45.8 (2.6)	45.1 (2.6)	44.4 (2.7)	
Albumin-corrected calcium, mmol/L	2.30 (0.08)	2.22 (0.03)	2.30 (0.02)	2.39 (0.06)	
Sex (female), %	56.1	54.5	54.0	60.1	
Age, years	52.1 (15.3)	48.3 (14.2)	51.3 (15.1)	57.1 (15.4)	
BMI, kg/m^2	26.2 (4.2)	25.8 (4.0)	26.2 (4.2)	26.6 (4.3)	< 0.001
Waist circumference, cm	86.6 (12.7)	85.4 (12.6)	86.6 (12.8)	87.9 (12.4)	0.001
SBP, mmHg	124.3 (17.8)	121.0 (16.5)	123.7 (17.1)	128.5 (19.0)	< 0.001
DBP, mmHg	74.9 (10.1)	74.0 (9.7)	74.9 (10.3)	75.9 (10.2)	< 0.001
Hypertension medication, %	18.2	12.0	17.0	26.1	0.005
Diabetes, %	6.0	5.5	5.2	7.7	0.557
Alcohol intake, %					0.198
Never	6.6	4.8	6.8	8.3	
Former	9.6	8.8	8.9	11.4	
Light	60.3	61.8	59.6	59.5	
Heavy	23.4	24.5	24.7	20.8	
Smoking, %					0.084
Never	51.3	51.5	52.1	50.1	
Former	35.6	36.4	34.0	36.6	
Current	13.1	12.1	13.8	13.3	
Glucose, mmol/L	5.00 (1.32)	4.85 (1.14)	5.00 (1.24)	5.16 (1.55)	< 0.001
Cholesterol, mmol/L	5.63 (1.08)	5.39 (1.00)	5.60 (1.08)	5.91 (1.11)	< 0.001
HDL cholesterol, mmol/L	1.40 (0.39)	1.40 (0.39)	1.38 (0.38)	1.41 (0.40)	0.380
Lipid-lowering medication, %	2.8	1.6	2.9	3.8	0.145
Triglycerides, mmol/L	1.32 (0.92)	1.17 (0.83)	1.32 (0.93)	1.49 (0.98)	< 0.001
Cancer history, %	5.2	4.6	4.7	6.5	0.456
CVD at baseline, %	18.1	15.1	17.7	21.7	0.856
eGFR (%)					< 0.001
< 30.0 mL/min/1.73m^2	0.4	0.1	0.4	0.7	
30.0 – 59.9 mL/min/1.73m^2	21.5	13.0	20.1	32.1	
≥ 60.0 mL/min/1.73m^2	78.1	86.9	79.5	67.2	
Total deaths, N (%)	666	149 (11.6)	233 (15.6)	284 (23.1)	
CVD deaths, N (%)	278	59 (4.6)	105 (7.0)	114 (9.3)	
CVD events, N (%)	652	159 (12.4)	219 (14.6)	274 (22.3)	

Data are shown as mean (SD) or percent. *P value for trend, after adjustment for age and sex
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; CVD, cardiovascular disease; eGFR estimated glomerular filtration rate

Table 2. Hazard ratios [HR] for albumin-corrected calcium for total cohort after adjusting for sex and age and after further adjustment for cardiovascular risk factors.

	Total deaths		CVD deaths		CVD events	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Adjusted for age and sex						
Calcium as continuous variable*	1.09 (0.99 , 1.20)	0.062	1.06 (0.92 , 1.23)	0.414	1.13 (1.03 , 1.24)	0.012
Calcium tertiles (mmol/L)						
Ca ≤ 2.26 (reference)	1.00	0.634	1.00	0.345	1.00	0.217
2.26 < Ca ≤ 2.33	1.10 (0.89 , 1.35)		1.22 (0.88 , 1.68)		0.97 (0.79 , 1.19)	
Ca > 2.33	1.09 (0.89 , 1.34)		1.03 (0.74 , 1.41)		1.13 (0.92 , 1.38)	
Adjusted for age, sex, BMI and waist circumference						
Calcium as continuous variable*	1.08 (0.98 , 1.18)	0.119	1.05 (0.91 , 1.21)	0.505	1.11 (1.01 , 1.22)	0.029
Calcium tertiles (mmol/L)						
Ca ≤ 2.26 (reference)	1.00	0.655	1.00	0.298	1.00	0.313
2.26 < Ca ≤ 2.33	1.10 (0.89 , 1.35)		1.23 (0.89 , 1.70)		0.97 (0.79 , 1.19)	
Ca > 2.33	1.08 (0.88 , 1.32)		1.02 (0.74 , 1.41)		1.11 (0.91 , 1.35)	
Adjusted for age, sex, BMI, waist circumference and other cardiovascular risk factors†						
Calcium as continuous variable*	1.03 (0.94 , 1.14)	0.518	0.99 (0.86 , 1.16)	0.950	1.05 (0.95 , 1.15)	0.316
Calcium tertiles (mmol/L)						
Ca ≤ 2.26 (reference)	1.00	0.753	1.00	0.226	1.00	0.689
2.26 < Ca ≤ 2.33	1.05 (0.86 , 1.30)		1.16 (0.84 , 1.61)		0.94 (0.77 , 1.16)	
Ca > 2.33	0.99 (0.80 , 1.21)		0.92 (0.66 , 1.28)		1.02 (0.83 , 1.25)	

* HR shown is per 0.1 mmol/L of additional albumin-corrected calcium at baseline

† Diabetes, smoking, alcohol intake, systolic blood pressure, diastolic blood pressure, hypertension medication, (log) glucose, total cholesterol, HDL cholesterol, (log) triglycerides and lipid-lowering medication

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