Glucose Intolerance Is Associated With Altered Calcium Homeostasis: A Possible Link Between Increased Serum Calcium Concentration and Cardiovascular Disease Mortality

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Serum calcium concentration has recently been shown to predict cardiovascular mortality in a large health-screening program. Since impaired glucose tolerance (IGT) is an independent cardiovascular risk factor, we examined the association between glucose intolerance and serum calcium in a population-based cohort study. To characterize this association, we measured total serum calcium, parathyroid hormone (PTH), 25-hydroxyvitamin D (25OHD), and 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D$) levels in a cohort of 1,071 randomly selected white individuals aged 40 to 65 years in whom an oral glucose tolerance test had been completed. In multivariate analyses, the 2-hour plasma glucose was positively associated with increasing total serum calcium and PTH in men and women after adjustment for age, obesity, season, and 25OHD. The adjusted odds ratio (OR) between increasing quintiles of total serum calcium and IGT was 1.63 (95% confidence interval [CI], 1.42 to 1.88). The OR comparing the top with the bottom quintile was 8.5 (95% CI, 4.5 to 16.0). The association with quintile of serum PTH was 1.30 (95% CI, 1.14 to 1.49). These data suggest that IGT is associated with an increase in both total serum calcium and PTH that cannot be explained by confounding by aging, obesity, or 25OHD. This relationship may explain the previously observed association between serum calcium and cardiovascular mortality. Whether this association is a manifestation of a shared cellular defect or represents a common relationship with an unknown etiologic factor are important questions for further research.

IN A RECENT REPORT from a population study of 33,346 individuals in Sweden, the serum calcium concentration measured at baseline predicted survival during the subsequent 10.8 years of follow-up study.[1] In men aged less than 50 years, this excess mortality was largely attributable to cardiovascular disease. When serum calcium was 2.51 to 2.55 mmol/L, the risk of death was increased 50% compared with those for whom serum calcium was 2.31 to 2.45 mmol/L. However, this study was unable to suggest any mechanisms for this observation, nor was it able to describe the relationship between elevated serum calcium levels and other established cardiovascular risk factors.

Impaired glucose tolerance (IGT) is associated with an increased risk of coronary artery disease and with a cluster of other metabolic abnormalities in the metabolic cardiovascular syndrome (or syndrome X), a common key feature of which is insulin resistance.[2] An association between an increased serum calcium concentration and IGT would be of interest since it might explain, in part, the observed relationship between the serum calcium concentration and future risk of cardiovascular mortality. It has previously been suggested that altered calcium metabolism might be an additional feature of the metabolic cardiovascular syndrome, although the evidence for this is relatively weak. In a large cross-sectional study, a weak unadjusted correlation was observed between a random glucose concentration and serum total calcium.[3] In a separate case-control study, male subjects with IGT had elevated calcium concentrations by comparison to control subjects, although this did not reach conventional statistical significance.[4] In this latter study, subjects were not matched for confounding factors that are associated with glucose intolerance but that might also affect calcium homeostasis, most notably aging and obesity. We therefore undertook a study with the aim to describe the association between glucose intolerance and calcium homeostasis in a population in which these confounding factors were measured. Since this investigation was constructed as a population-based cohort study, we were able to describe the relationship of total serum calcium with glucose intolerance, with the latter measured as a continuous and a categorical variable.

A link between calcium and glucose metabolism could occur via a number of pathways. It has long been known, for example, that insulin secretion is a calcium-dependent process.[5] The exocytosis of secretory granules containing insulin in the β cell is dependent on the influx of calcium through voltage-dependent calcium channels. Persistent alteration of extracellular calcium concentrations could therefore have effects on β-cell secretory function. Since insulin secretion is an important determinant of glucose tolerance,[6] this might be a plausible mechanism linking abnormalities of calcium homeostasis to glucose intolerance. A further possible link has been suggested by the observation of an association between low serum 25-hydroxyvitamin D (25OHD) levels and glucose intolerance.[7] Such an association is biologically plausible, because insulin secretion has been shown to be reduced when the serum concentration of 25OHD is low.[8,9] In this study, we have therefore assayed serum 25OHD, 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D$), and parathyroid hormone (PTH), as well as measures of insulin secretion and insulin resistance, in an attempt to describe the underlying relationship between calcium homeostasis and glucose intolerance.

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SUBJECTS AND METHODS

The Isle of Ely Study was established in 1990 as a longitudinal cohort study of the etiology and pathogenesis of non–insulin-dependent diabetes mellitus and related metabolic disorders. The study design and methods have been described in detail elsewhere,[10] but in brief, a sample of 1,122 white subjects were selected at random from a sampling frame consisting of all adults free of known diabetes who were registered with the single general practice in the city of Ely (response rate, 74%). The volunteers underwent for a standard 75-g oral glucose tolerance test and a clinical examination that included a dietary and medical questionnaire and standard anthropometric measurements. The volunteers were asked to fast from 10:00 PM the previous evening. Smoking status and alcohol intake were assessed using the Health and Lifestyle Survey questionnaire.[11] Leisure time physical activity was assessed using the Paffenbarger questionnaire[12] with standard coding of specific activities using published MET scores (metabolic equivalents) for recreations and sports.[13,14] The study was approved by the Cambridge Local Research Ethics Committee.

Serum and plasma samples were obtained at fasting and 30 and 120 minutes post–glucose load. Plasma samples were immediately separated in a cooled centrifuge at 4°C. Serum was removed from the clotted samples after 1 hour. All fasting samples were taken between 8:30 and 9:30 AM. They were immediately placed on ice and permanently stored at −70°C within 4 hours. Glucose concentrations were measured immediately in the routine laboratory using a hexokinase assay.[15] All other biochemical measurements were made on stored aliquots at a later date. For each analyte, assays were performed in a single batch to eliminate between-batch assay variation. Serum 25OHD concentrations were measured on fasting samples by acetonitrile extraction followed by radioimmunoassay (reported coefficient of variation [CV] of the assay at 12.8 μg/L, 14.4%). Serum 1,25-$(OH)_2$D was determined by column chromatography immunoextraction followed by radioimmunoassay (Immunodiagnostic Systems, Boldon, UK; CV at 44.5 ng/L, 12.4%). The serum intact PTH level was measured using a two-site immunoradiometric assay with an *N*-terminal monoclonal antibody as capture (Immunodiagnostic Systems; CV at 28.8 ng/L, 8.1%). The total serum calcium level was measured using a modification of the ortho-cresolphthalein compexone reaction with 8-quinolinol to reduce magnesium interference (CV at 2.3 mmol/L, 1.7%). Serum albumin was assayed using an adaptation of the bromocresol purple dye-binding method (CV at 42 g/L, 1.7%). The serum creatinine level was measured using the alkaline picrate (Jaffe reaction) (Dimension (R) clinical chemistry system; DuPont, Wilmington, MA). The plasma insulin level was measured using two-site immunometric assays with either ^{125}I or alkaline phosphatase labels.[16,17] The 30-minute insulin incremental response was calculated by dividing the difference between the 30-minute insulin and fasting insulin by the 30-minute plasma glucose.[18] Total serum calcium was adjusted for serum albumin using the equation of the linear regression between these variables derived directly from this population rather than from the routine laboratory, as has been recommended elsewhere.[19]

Statistical Methods

World Health Organization (WHO) criteria for diabetes and IGT were used to classify the 1,122 subjects.[20] Fifty-one subjects were found to have newly diagnosed diabetes. We elected a priori to exclude these subjects from the analyses, since the relationship between the intermediate physiological parameters (insulin resistance and secretion) may be different in the diabetic state as a result of glucose toxicity. We also excluded subjects whose serum creatinine was 150 μmol · L^{-1} or greater. Univariate correlations were examined using Spearman rank correlation, and multiple regression analyses were conducted using the SPSS (SPSS Inc, Chicago, IL) for Windows package. These data were collected over a 2-year period, and therefore, individuals were studied under differing climatic conditions. Analysis of the effect of season was undertaken by grouping individuals according to three periods of the year, November to February, March to June, and July to October, on the basis of the observed relationship between mean serum 25OHD concentration and mean hours of daily sunshine (data supplied by the local Metrological Office). These seasons were coded for in the various multivariate models by a pair of binary indicator variables. The relationship between IGT as a binary outcome variable and calcium homeostasis was described by use of logistic regression in the EGRET epidemiological statistical package (Statistics and Epidemiology Research Corp, Seattle, WA).

RESULTS

One thousand seventy-one nondiabetic subjects were identified by the oral glucose tolerance test. Because of the possibility that renal impairment could affect calcium and PTH homeostasis, 14 subjects whose serum creatinine was 150 μmol · L^{-1} or greater were excluded from the analyses. Thus, the remaining analyses were conducted on 1,057 subjects, of whom 188 were found to have IGT by WHO criteria.

In Table 1, the arithmetic mean calcium concentration is shown by category of the 2-hour plasma glucose concentration stratified by gender. In both men and women, there was a significant increase in serum calcium with worsening glucose

Table 1. Serum Calcium, PTH, and Vitamin D Concentration by 2-Hour Plasma Glucose Concentration

			PTH (ng · mL^{-1})		25OHD (μg · L^{-1})		1,25-$(OH)_2$D (pmol · L^{-1})	
2-Hour Plasma Glucose (mmol · L^{-1})	No. of Subjects	Calcium (mmol · L^{-1})	Mean	Range	Mean	Range	Mean	Range
Men								
<5.00	111	2.27 ± 0.10	25.2	23.3-27.2	21.4	19.7-23.30	85.8	81.2-90.8
5.00-5.69	62	2.29 ± 0.11	24.8	22.6-27.2	24.5	22.3-27.0	87.5	80.8-94.8
5.70-6.49	97	2.27 ± 0.10	28.1	26.1-30.3	20.5	18.6-22.5	81.8	76.9-86.9
6.50-7.79	114	2.26 ± 0.10	28.0	26.0-30.1	21.6	20.1-23.4	89.3	84.0-95.0
≥7.78	74	2.33 ± 0.11	28.0	25.6-30.5	27.6	24.8-30.8	90.4	83.8-97.5
Significance test for linear trend		.03		.01		.018		NS
Women								
<5.00	123	2.26 ± 0.12	25.9	24.1-27.9	19.9	18.1-21.8	81.4	76.8-86.3
5.00-5.69	103	2.26 ± 0.11	25.2	23.3-27.3	20.2	18.5-22.1	76.7	70.8-82.9
5.70-6.49	126	2.28 ± 0.12	26.8	25.0-28.7	20.0	18.6-21.6	79.6	74.5-85.1
6.50-7.79	133	2.29 ± 0.12	29.4	27.2-31.7	19.1	17.5-20.8	78.9	74.6-83.4
≥7.78	114	2.36 ± 0.11	33.9	31.1-36.8	21.3	19.5-23.2	79.2	73.2-85.6
Significance test for linear trend		<.0001		<.0001		NS		NS

tolerance. Data for serum PTH, 25OHD, and 1,25-$(OH)_2$D are shown as the geometric mean (95% confidence interval [CI]), since the underlying distribution was skewed but was normalized by logarithmic transformation. Mean serum PTH also increased with worsening glucose tolerance. Individuals in the highest 2-hour plasma glucose group, ie, those with IGT, had the highest mean serum calcium and highest serum PTH concentration. In men, serum 25OHD increased across the glucose tolerance categories, but this relationship was not seen in women. In neither sex was there a significant linear relationship with serum 1,25-$(OH)_2$D.

In subsequent analyses, we examined the possibility that the association between serum total calcium and glucose intolerance was due to confounding. We have previously demonstrated the association between increasing age and obesity and glucose intolerance in this population.[10] Therefore, we examined the relationship between serum calcium, PTH, and vitamin D and age and obesity. Table 2 shows that the mean total corrected serum calcium and PTH increased with age in men and women. In women in this population, the mean concentration of serum 25OHD increased across age strata, but this effect was not seen in men. Conversely, the concentration of serum 1,25-$(OH)_2$D decreased with increasing age in men, but there was no significant relationship in women. Table 3 shows the rank correlation coefficients between these variables and markers of obesity. There was a positive correlation between body mass index (BMI) and total serum calcium and PTH in men, and a similar but weaker relationship in women. The concentration of serum 1,25-$(OH)_2$D was strongly negatively correlated with increasing obesity. Overall, these data demonstrate that an older and more obese group would be characterized by elevated serum calcium and elevated PTH. Thus, it is possible that the observed relationship between worsening glucose tolerance and serum calcium could be due to confounding by age and obesity. Subsequent analyses examined the extent to which the relationship was independent of these confounding factors.

Table 4 shows regression coefficients for serum calcium in sex-specific multiple regression analyses with 2-hour plasma glucose as the dependent variable. The first model included age, BMI, serum 25OHD, and the indicator variables for season. We have included season as a covariate because it is possible that the season of the year could be a confounder in the relationship between calcium and glucose tolerance, and that this effect might be mediated by a pathway that is independent of exposure to sunlight and hence higher serum 25OHD concentration. It has been reported, for example, that plasma glucose concentrations after a glucose load increase with the ambient temperature.[21] The data demonstrate that there is a strong association between serum calcium and 2-hour plasma glucose that is independent of age, obesity, serum 25OHD, or season. In the second model, the following additional potential confounding factors were added: smoking (as a pair of indicator variables coding for smokers, ex-smokers, and nonsmokers) and physical activity. The effect of total serum calcium in the model remained significant. Since the age group studied included premenopausal and postmenopausal women, we examined the possibility of confounding by menopausal status. Since we had neither self-reported nor biochemical menopausal status, we stratified women by age into groups aged less than 50 and older than 50, as the mean age of the menopause in women in the United Kingdom is 50 years.[22] We examined the association between serum calcium and glucose tolerance in the two groups separately, and demonstrated that the association among women less than 50 was the same as for women over 50 (data not shown).

Figure 1 shows the arithmetic mean total serum calcium and the geometric mean serum PTH by 2-hour plasma glucose after adjustment for age, BMI, season, serum 25OHD, and gender. The data show that both total serum calcium and PTH increase with the 2-hour plasma glucose, and that this increase is independent of age, BMI, gender, season, and serum 25OHD concentration. The adjusted means suggest that there is possibly a threshold in this association, with the greatest increase in calcium occurring at the transition from normal glucose tolerance to IGT. Therefore, the data were analyzed as a case-control study so that the strength of the relationship could be assessed by examining the association between increasing quintiles of serum PTH and total calcium and the risk of having IGT. This risk is expressed as an odds ratio (OR), the measure of association between an exposure and a binary outcome variable. If there is no association between the exposure and the disease, the OR would be 1. If the exposure increases the risk of having

Table 2. Serum Calcium, PTH, and Vitamin D Concentration by Age Group

Age Group	No. of Subjects	Calcium (mmol · L^{-1})	PTH (ng · mL^{-1})		25OHD (μg · L^{-1})		1,25-$(OH)_2$D (pmol · L^{-1})	
			Mean	Range	Mean	Range	Mean	Range
Men								
40-44	73	2.25 ± 0.12	24.8	22.5-27.4	22.1	19.5-25.0	91.9	85.5-98.4
45-49	84	2.28 ± 0.10	26.4	24.1-29.0	21.8	20.0-23.9	87.9	82.2-94.0
50-54	85	2.25 ± 0.10	25.8	23.8-27.9	22.0	19.8-24.3	89.6	84.0-95.5
55-59	81	2.29 ± 0.10	27.1	25.0-29.3	22.5	20.4-24.8	85.0	79.2-91.2
60-65	135	2.30 ± 0.10	28.7	27.1-30.5	23.7	22.1-25.4	82.7	78.2-87.6
P for linear trend		.001		.008		NS		.015
Women								
40-44	111	2.23 ± 0.10	25.6	23.5-27.8	19.1	17.5-20.9	81.8	76.3-87.7
45-49	119	2.26 ± 0.11	26.2	24.3-28.3	18.5	17.0-20.2	74.1	69.1-79.5
50-54	115	2.27 ± 0.14	27.1	25.0-29.3	19.1	17.3-21.0	75.9	71.1-81.1
55-59	91	2.33 ± 0.10	29.2	26.6-32.1	21.4	19.4-23.7	79.8	74.0-85.9
60-65	163	2.34 ± 0.11	31.3	29.3-33.3	21.7	20.3-23.2	83.6	79.1-88.3
P for linear trend		<.0001		<.0001		.002		NS

Table 3. Rank Correlation Between Age and Obesity and Biochemical Measures by Gender

Variable	Calcium (mmol · L^{-1})	PTH (ng · mL^{-1})	25OHD (μg · L^{-1})	1,25-$(OH)_2D$ (pmol · L^{-1})
Age (yr)				
Men	.13‡	.15‡	.06	−.10*
Women	.38‡	.19‡	.13‡	.04
BMI (kg · m^{-2})				
Men	.13†	.22‡	−.08*	−.19‡
Women	.07	.11*	.06	−.13†
WHR				
Men	.04	.16‡	−.05	−.10‡
Women	.10*	.18‡	−.03	−.09*

*$P < .05$.
†$P < .01$.
‡$P < .001$.

the disease, the OR is greater than 1, whereas a protective exposure would have an OR less than 1. Figure 2 shows that there was a strong linear relationship between the OR for IGT and quintile of total serum calcium. The overall association per quintile of total serum calcium was 1.75 (95% CI, 1.53 to 1.99). Thus, the transition from one quintile to the next highest is associated with a 75% increase in the odds of having IGT. When compared with the lowest quintile, the absolute risk in the highest quintile of total serum calcium (where total serum calcium is >2.38 mmol/L) was 8.5 (95% CI, 4.5 to 16.0). When the data were adjusted for age, BMI, and gender, the overall OR was reduced from 1.75 to 1.64 (95% CI, 1.43 to 1.88), suggesting that there is some degree of confounding. The addition of season and serum 25OHD to this model did not alter the OR (OR, 1.63; 95% CI, 1.42 to 1.88). In this model, the term for serum 25OHD was itself weakly positively associated with the outcome (OR, 1.03; 95% CI, 1.01 to 1.05). The indicator variables for season were also significantly associated with an apparent excess risk of IGT in individuals studied in the summer months. The fact that season and serum 25OHD were both significant terms in this model suggests that the effect of season is probably mediated via another pathway, perhaps ambient temperature.

The relationship between PTH and IGT was weaker than that for total serum calcium (Fig 3). However, the overall association was 1.30 (95% CI, 1.15 to 1.46). This effect was reduced by adjustment for age, BMI, and gender (OR, 1.19; 95% CI, 1.05 to 1.34). However, addition of serum 25OHD and season to this model increased the effect size (OR, 1.30; 95% CI, 1.14 to 1.49). Inclusion of physical activity in the models for serum PTH or calcium did not affect the OR, nor did it alter the

Table 4. Regression Coefficients for Serum Calcium in Sex-Specific Models With 2-Hour Plasma Glucose as Dependent Variable

	Other Factors Included in Model	Regression Coefficient for Serum Calcium	
Model 1	Age, BMI, season, 25OHD	Male	1.34*
		Female	2.17†
Model 2	Age, BMI, season, 25OHD, smoking, physical activity	Male	1.76*
		Female	1.67*

*$P < .05$.
†$P < .001$.

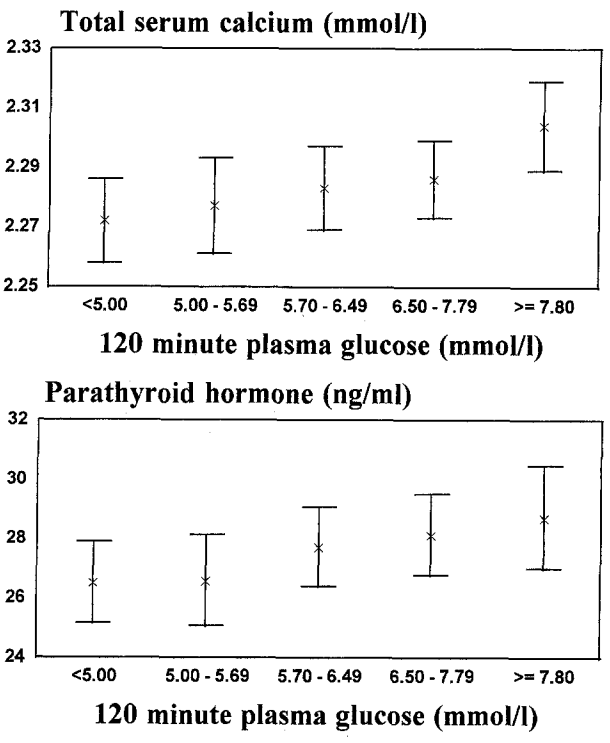

Fig 1. Adjusted arithmetic mean serum total calcium and geometric mean serum PTH by category of 2-hour plasma glucose. The model included age, obesity, season, and 25OHD concentration as covariates.

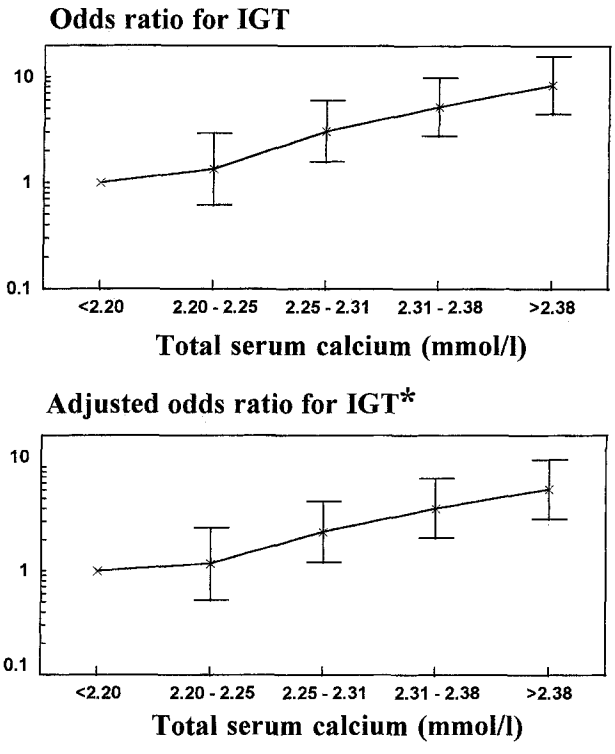

Fig 2. Unadjusted and adjusted OR for IGT for increasing quintiles of total serum calcium. *Adjusted for age, BMI, gender, season, and 25OHD.

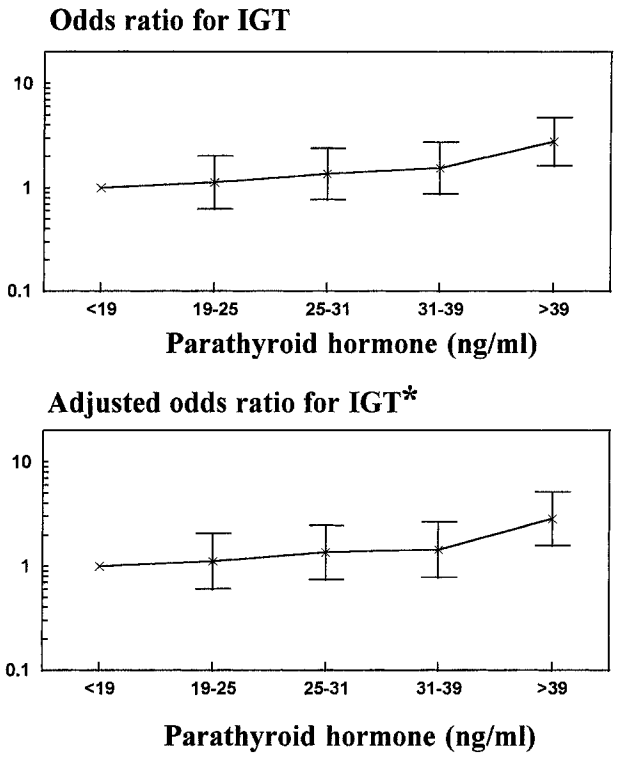

Fig 3. Unadjusted and adjusted OR for IGT for increasing quintiles of serum PTH. *Adjusted for age, BMI, gender, season, and 25OHD.

association between any of the covariates and the outcome measure.

We have previously demonstrated[23] that glucose tolerance in this population is associated positively with the fasting insulin, a measure of insulin resistance,[24] and negatively with the 30-minute insulin increment, a measure of rapid insulin secretion.[18] Since these two factors are possible pathways leading to glucose intolerance, we examined their relationship with serum calcium. A strong association between serum calcium and fasting insulin would, for example, suggest that the association between calcium and glucose intolerance could be mediated via an effect on insulin resistance. Partial correlation coefficients (adjusted for age and BMI) between these markers of insulin resistance and secretion and serum calcium are shown in Table 5. No association was demonstrated with serum calcium, suggesting that the association of serum calcium and 2-hour plasma glucose cannot be explained by an effect on insulin resistance or insulin secretion.

Table 5. Partial Correlation Coefficients Between Measures of Insulin Resistance and Secretion and Calcium and PTH Adjusted for Age and BMI

Variable	Calcium (mmol · L^{-1})	PTH (ng · mL^{-1})
Men		
Fasting insulin (pmol · L^{-1})	.03	.11*
30-minute insulin increment (pmol · L^{-1}/mmol · L^{-1})	.01	.02
Women		
Fasting insulin (pmol · L^{-1})	.02	.10*
30-minute insulin increment (pmol · L^{-1}/mmol · L^{-1})	−.07	−.03

*$P < .05$.

DISCUSSION

This study demonstrated that there is a strong association between the total corrected serum calcium concentration and the risk of IGT, and a weaker but still significant positive association with increasing concentrations of serum PTH. Before accepting that these observed associations imply that there is a true relationship between calcium homeostasis and IGT, the role of chance, bias, and confounding as alternative explanations must be considered.

Because this study was established as a population-based cohort study with a good response rate, it is unlikely that this finding could be attributed to selection bias. The strength of the associations is such that chance is a very unlikely explanation. Therefore, in seeking to find an explanation for this observation, one must concentrate on the possibility of confounding. The major factors that have an association with calcium and PTH in the normal population are age and obesity. The observation in this study of increasing serum PTH concentrations with age is consistent with other studies,[25,26] as is the observation that serum PTH concentrations are independently increased by obesity.[27,28] Although the effect of obesity on total calcium has been observed in other studies, the reported effect of aging is more variable, with a positive independent association of age and calcium found in some[3] and a nonsignificant or significantly negative association found in others.[25,26,29] The diversity may reflect true differences between populations in the relationship between calcium and increasing age, perhaps as a result of differences in the availability of serum 25OHD.

The associations of serum 25OHD and 1,25-$(OH)_2$D with age and obesity and the interrelationships between the two are variable between studies.[30,31] In our study, obesity was associated with lower serum 1,25-$(OH)_2$D, but an inconsistent effect on serum 25OHD. Most studies that have a wide age range have suggested that aging is associated with decreased serum 25OHD. However, given the strong environmental determinants of serum 25OHD,[32] whether these observations are explicable on the basis of the aging process itself or are a reflection of behaviors that are correlated with increasing age is unclear. If the latter is the case, then variation between populations might be expected. Such variation between populations in the relationship between serum 25OHD and other risk factors could also explain why we have been unable to replicate the observation of an association between low serum 25OHD and IGT that has been seen in other populations.[7,33] Another reason may be that only 1.1% of the population studied in Ely had levels of serum 25OHD that were considered indicative of deficiency (ie, <5 μg/L). This is in marked contrast to studies in the East Asian population in London[33] and the New Zealand Polynesians,[7] for example, where low levels of 25OHD were common. The absence of an association of serum 25OHD and IGT in the Ely study could imply that vitamin D concentrations must be in the truly deficient range to have an effect on glucose tolerance. This suggestion might explain the apparently discrepant observations seen in small supplementation studies.[34,35] It is also possible that a gene-environment interaction is necessary for

expression of the relationship between serum 25OHD and abnormal glucose tolerance.

The pattern of these interrelationships between aging, obesity, vitamin D, and calcium suggests that these are important potential confounding factors for the association between calcium and glucose intolerance. However, in this study, adjustment for these confounding factors does not eliminate this association. Previous studies have not controlled for confounding to such a degree. One demonstrated that serum calcium was elevated in patients with type II diabetes by comparison to age- and sex-matched controls,[36] but this observation was not shown in a less well-controlled study.[37] This report is the first study to demonstrate an independent association with IGT of total serum calcium and serum PTH. The two processes might be independent of each other in a pathophysiological sense but share a common cause, as has been suggested for diabetes and heart disease.[38,39] Alternatively, calcium homeostasis and IGT could be linked more directly by a common biochemical abnormality that affects both systems. It has been observed, for example, that insulin action is abnormal in individuals with primary hyperparathyroidism[40] and is normalized following surgery. Although it has been speculated that calcium homeostasis, glucose intolerance, insulin resistance, and hypertension may be linked by a common defect in cellular ion handling,[41] we were unable to find a correlation between markers of either insulin resistance or insulin secretion and serum calcium. This may suggest that our measures of these intermediate physiological variables were too imprecise or that the association with glucose intolerance occurs via a different mechanism.

Although the analysis of the role of chance, bias, and confounding leads us to strengthen our conclusions about the association between glucose tolerance and serum calcium concentration, measurement of calcium levels in this study was not free of error. Because of the size of the study, we measured total rather than ionized calcium levels. Since the latter is of greater physiological significance, a change in the total to ionized calcium ratio would complicate the interpretation of changes in total calcium. This could introduce a bias into this type of study, if the ratio itself were associated with the outcome variable or with a powerful confounding factor. In one study, a negative association between ionized calcium and both the BMI and waist to hip ratio was demonstrated, but no significant association was found between albumin-corrected calcium and BMI. This implies that there is a relationship between the total to ionized calcium ratio and obesity.[42] In morbid obesity, there is an increase in nonionized calcium in the serum as a consequence of binding to plasma proteins and free fatty acids. Thus, any changes in total serum calcium might be attributable to altered plasma binding and not to a true variation in ionized calcium.[43] If serum total calcium is a true manifestation of serum ionized calcium, then the positive association of calcium and PTH with IGT might imply that there is an alteration in the calcium-PTH set point in this condition. Alternatively, the association with total serum calcium could be a reflection of markedly altered protein binding in IGT.

In conclusion, we have demonstrated a strong and independent association between total serum calcium and glucose intolerance, measured both as a continuous variable and as a category. Since glucose intolerance is an independent risk factor for ischemic heart disease, it is possible that this association may explain, in part, the observed link between total serum calcium and the future risk of cardiovascular disease mortality. However, a clear pathophysiological explanation for these observations remains elusive.

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