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# Plasma C-Peptide Is Inversely Associated with Calcium Intake in Women and with Plasma 25-Hydroxy Vitamin D in Men[1,2]

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## Abstract

The consumption of calcium, vitamin D, and dairy products may be associated with a reduced risk of type 2 diabetes mellitus. However, whether this reduction is due to calcium, vitamin D, or other components of dairy products is not clear. We examined intakes of total calcium and vitamin D, and plasma concentrations of 25 hydroxyvitamin D [25(OH)D] in relation to fasting plasma concentrations of C-peptide in 2 cross-sectional studies among healthy men from the Health Professionals Follow-up Study and among healthy women from the Nurses' Health Study I. Intake of total calcium was modestly inversely associated with C-peptide concentration in women ($P$-trend = 0.05); however, the inverse association was not significant in men ($P = 0.7$). Concentrations of C-peptide were 20% lower among men who had plasma concentrations of 25(OH)D in the highest quartile compared with those in the lowest quartile ($P$-trend = 0.08); there was no association in women ($P = 0.3$). The inverse association between calcium intake and the plasma C-peptide concentration was stronger in hypertensive individuals of both sexes. The difference in the C-peptide concentrations between extreme quartiles of calcium intake was 17% in men and 20% in women. Plasma concentrations of C-peptide for the combination of the highest tertiles of calcium intake and plasma 25(OH)D compared with the opposite extreme were 35% lower ($P = 0.03$) in men and 12% lower ($P = 0.01$) in women. The results suggest that calcium intake or systemic vitamin D status, after adjustment for intake of dairy products, is associated with decreased insulin secretion. J. Nutr. 139: 547–554, 2009.

## Introduction

In several prospective cohort studies, higher intakes of calcium and vitamin D (1–4) and dairy foods (2,5–7) were associated with a lower risk of type 2 diabetes and metabolic syndrome. However, these studies could not determine whether this association is due to calcium, vitamin D, or any of the other numerous components of dairy foods. Moreover, calcium absorption can be influenced by systemic vitamin D status, which is partially influenced by sun exposure. Plasma concentrations of 25-hydroxyvitamin D [25(OH)D][7] are preferable to vitamin D intake as a measure of systemic vitamin D status; however, none of the studies referenced above measured 25(OH)D. Further, trials assessing the effects of supplemental calcium or vitamin D on diabetes or diabetes-related problems have yielded conflicting results (8–14). Because these trials were small and short in duration, we cannot draw definite conclusions. Identification of nutrients that may prevent type 2 diabetes is important for the formulation of new dietary recommendations. Furthermore, identifying subpopulations that are susceptible to any effects of calcium or 25(OH)D on insulin resistance would help us target high-risk populations; unfortunately, limited data are available. Only 1 small trial found that calcium supplementation can decrease fasting levels of insulin in hypertensive individuals, but whether calcium had the same effect among normotensive individuals was not examined (8).

C-peptide, cleaved from proinsulin, has a longer half-life than insulin and is preferable as a measure of insulin secretion, because it is less actively extracted by the liver (15). It also might be a better marker of insulin demand in nondiabetics (16). High levels of C-peptide or insulin were associated with insulin resistance, diabetes (17,18), and other chronic diseases that are partially related to diabetes and insulin resistance, including colon adenoma and pancreatic cancer (19,20) in both the Health Professionals Follow-up Study (HPFS) and Nurses' Health Study (NHS) cohorts. Circulating C-peptide also appears to be a useful surrogate for evaluating potential dietary predictors of diabetes. In previous

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[7] Abbreviations used: C-PEP$_{11}$, C-peptide concentrations at the lowest tertile of calcium plus 25(OH)D; C-PEP$_{13}$, C-peptide concentrations when 25(OH)D is in the highest and calcium is in the lowest tertile; C-PEP$_{31}$, C-peptide concentrations when calcium is in the highest and 25(OH)D is in the lowest tertile; C-PEP$_{33}$, C-peptide concentrations at the highest tertile of calcium plus 25(OH)D; HPFS, Health Professionals Follow-up Study; 25(OH)D, 25 hydroxyvitamin D; NHS, Nurses' Health Study.
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analyses, the identified dietary factors (high glycemic load, coffee, and cereal fiber) associated with C-peptide (21,22) were also found to be risk or protective factors for diabetes (23,24). Consequently, in nondiabetics, a high level of C-peptide is likely a good surrogate for insulin resistance in most circumstances.

The current study was designed to evaluate dietary calcium intake and plasma concentrations of 25(OH)D in relation to fasting plasma concentrations of C-peptide in 2 large cross-sectional studies (HPFS and NHS), one involving nondiabetic men and the other nondiabetic women. C-peptide was the best available biomarker of insulin resistance in these cohorts.

## Materials and Methods

*Study population.* Two ongoing cohort studies with concurrent information on all study variables provided data for analyses: the HPFS and the NHS I. For HPFS, 51,529 American male health professionals, ages 40–75 y, provided detailed medical histories and lifestyle information on a mailed questionnaire in 1986. Subsequent questionnaires have been mailed every 2 y to update information on health-related behaviors and medical events, and the questionnaire sent in 1994 provided the information on nondietary variables for the current analyses. Dietary questionnaires have been mailed every 4 y, including the one in 1994 that was used for this study. In 1993 and 1994, we sent venipuncture kits to participants, and 18,140 men returned specimens on ice using an overnight courier. The NHS I, established in 1976, was another U.S. study involving 121,700 female registered nurses 30–55 y of age who completed a mailed questionnaire at baseline and subsequent biennial mailed questionnaires to provide health and lifestyle information. Here too, dietary questionnaires were sent out every 4 y. The general questionnaire and diet questionnaire sent in 1990 were used for the analyses in the NHS. Between 1989 and 1990, we collected blood samples from 32,826 cohort members. The blood samples were shipped with an ice pack via overnight mail. None of the men or women included had previously diagnosed cancer, cardiovascular disease, or diabetes at the time of blood sample collection.

The current study included 888 men from the HPFS who had information on both fasting C-peptide and plasma 25(OH)D and served as controls in prostate and colon cancer case-control studies. In women from the NHS I, the data were merged from the controls of several case-control studies, including a hypertension case-control study, a breast cancer case-control study, a diabetes case-control study, and a colon cancer case-control study. A total of 1940 women were included. Among these women, 1085 had information on plasma 25(OH)D.

Both the Institutional Review Board of Harvard School of Public Health and Brigham and Women's Hospital in Boston, Massachusetts approved the study.

*Dietary assessment.* We used the same semiquantitative FFQ in 1994 for the HPFS cohort and in 1990 for the NHS cohort to assess average consumption of calcium and vitamin D over the previous year. Using sources of food composition, including those from the USDA, we estimated calcium and vitamin D intake from foods, multivitamins, and specific supplements listed. Calcium intake had been validated previously; the correlation between the semiquantitative FFQ and 2 1-wk diet records was 0.62 for calcium (25). Intake of vitamin D had been validated using plasma concentrations of 25(OH)D, which is influenced by both diet and sun exposure; the correlation was 0.25 ($P < 0.001$) in both a previous study (26) and the current study.

*Laboratory assays.* Fasting levels of C-peptide were measured by enzyme-linked immunosorbent assay and RIA as described previously (21). Plasma 25(OH)D was determined by RIA, as previously described (27,28). The inter-assay CV were <12% for both measurements.

In men, both C-peptide and 25(OH)D were measured in 2005. In women, C-peptide was measured at different time periods (1998–1999, 2000–2001, and 2004). Plasma 25(OH)D was measured in 1999–2000 and in 2003. We examined the potential drift over time in both studies by using the quality controls in each study to standardize any variations.

*Measurement of nondietary factors.* Height, weight, and smoking history were reported at baseline and weight and smoking status were updated biennially. The correlation coefficient between self-reported weight and weight as measured by trained personnel was 0.96 (29). We calculated BMI (kg/m$^2$) in 1994 for the HPFS cohort and in 1990 for the NHS cohort. Questionnaire data on physical activity were obtained in 1994 for the HPFS cohort and in 1988 for the NHS cohort. The reproducibility and validity of the physical activity questionnaire have been described elsewhere (30). If the data for BMI or physical activity closest to the year of blood draw were missing, they were replaced by the data collected in the previous questionnaire. The proportion of men or women who did not provide data for BMI or physical activity for the year when blood was collected was <2%.

*Assessment of hypertension.* Hypertension assessed from biennial questionnaires is highly valid (31). For our analysis, any men who self-reported hypertension in 1994 or before and any women who self-reported hypertension in 1990 or before were considered hypertensive.

*Statistical analysis.* We used linear regression models with a robust variance estimate to evaluate the association between intake of calcium or plasma 25(OH)D concentrations and plasma concentrations of C-peptide, without the assumption of normal distribution in the dependent variable (32). We conducted all statistical analyses using SAS software (version 8; SAS Institute). $P$-values $< 0.05$ were considered significant. In the multivariate models, we adjusted for age (<40, 40–49.9, 50–59.9, 60–64.9, ≥65 y), BMI (continuous), physical activity (quintiles), smoking history (current, past, and nonsmokers), hours since last meal, laboratory batch, state of residence, race, and dietary variables including total energy (quintiles), consumption of caffeinated coffee (continuous) and decaffeinated coffee (continuous), cereal fiber (continuous), retinol (continuous), glycemic load (continuous), and alcohol consumption (0, 0.1–4.9, 5–14.9, 15–29.9, ≥30 g/d). Because consumption of alcohol (33), cereal fiber, and coffee were inversely associated with C-peptide levels (21,22) and glycemic load was positively associated with C-peptide (21), we included these variables. We also adjusted for retinol intake, because high intake can antagonize vitamin D action at the receptor level (34). We included state of residence, race, and month of blood draw in the final model, because sunlight exposure and skin pigmentation can influence vitamin D status (35,36). For women, we additionally adjusted for menopausal status (premenopausal, perimenopausal, and postmenopausal) and use of hormones (never, past, and current hormone use). We simultaneously adjusted for dietary calcium or supplemental calcium in the multivariate model to determine whether any association between these variables was due to dietary calcium or supplemental calcium. To better examine the shape of the relationships of calcium or 25(OH)D with C-peptide, we performed a stepwise restricted cubic spline analysis (37), which is flexible for capturing a dose-response relationship of any shape, even though it can be sensitive to influential points.

To examine the combined association of calcium plus 25(OH)D with C-peptide, we created joint tertile groups for calcium and vitamin D (e.g. highest tertile of calcium and highest tertile of vitamin D, etc.). To assess whether the combination of calcium and 25(OH)D has any synergistic association with C-peptide on an additive scale, we used the Wald $P$-value for the interaction term in a model that also included main effects.

Because hypertension, obesity, menopausal status, and hormone use are known to be related to risk of insulin resistance and diabetes, we conducted subgroup analysis stratified by hypertension, BMI (in men and women), postmenopausal status, and use of hormones (in women). To evaluate effect modification, we used the Wald $P$-value for the interaction term in a model that also included main effects.

## Results

Men who had higher calcium intakes were also likely to take multivitamins and calcium supplements (**Table 1**). Calcium intake was positively associated with retinol, vitamin D, and dairy consumption and inversely associated with fasting plasma

**TABLE 1** Age-adjusted baseline characteristics according to quartiles of daily calcium intakes and plasma concentrations of 25(OH)D in healthy men and women[1]

| | Total calcium intake, *mg/d* | | | | Plasma 25(OH)D, *mmol/L* | | | |
|---|---|---|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 |
| Men from HPFS cohort | | | | | | | | |
| *n* | 221 | 222 | 222 | 223 | 222 | 224 | 222 | 220 |
| Median total Ca intake or plasma 25(OH)D | 580 | 760 | 952 | 1408 | 38 | 55 | 68 | 88 |
| Age, *y* (not adusted) | 65 | 64 | 65 | 68 | 67 | 67 | 68 | 68 |
| Plasma C-peptide, *μg/L* | 2.25 | 2.09 | 2.19 | 2.03 | 2.32 | 2.15 | 2.25 | 1.82 |
| BMI, *kg/m²* | 25.9 | 26.0 | 25.7 | 25.6 | 26.5 | 26.1 | 26.0 | 25.2 |
| Hypertension, *%* | 28 | 29 | 24 | 26 | 31 | 28 | 29 | 22 |
| Physical activity, *MET/wk* | 44 | 61 | 47 | 58 | 59 | 45 | 61 | 61 |
| Use of multivitamin, *%* | 38 | 48 | 50 | 61 | 42 | 49 | 43 | 53 |
| Alcohol, *g/d* | 14.7 | 15.1 | 12.6 | 11.2 | 8.1 | 10.1 | 12.0 | 12.5 |
| Dietary intake | | | | | | | | |
| Cereal fiber, *g/d* | 6.6 | 8.0 | 8.2 | 7.6 | 7.1 | 7.9 | 7.7 | 8.0 |
| Glycemic load | 131 | 132 | 134 | 137 | 137 | 139 | 135 | 135 |
| Retinol, *μg/d* | 1110 | 1033 | 1335 | 1999 | 1201 | 1344 | 1315 | 1638 |
| Calcium, *mg/d* | 552 | 753 | 968 | 1522 | 863 | 922 | 932 | 1038 |
| Calcium supplement user, *%* | 16 | 29 | 40 | 68 | 34 | 40 | 34 | 39 |
| Vitamin D, *μg/d* | 7.7 | 10.0 | 12.0 | 15.4 | 9.3 | 11.0 | 11.2 | 12.4 |
| Dairy foods,[3] *servings/d* | 0.8 | 1.8 | 2.1 | 3.7 | 1.8 | 1.9 | 2.1 | 2.3 |
| Women from NHS cohort | | | | | | | | |
| *n* | 272 | 274 | 270 | 269 | 271 | 272 | 270 | 272 |
| Median total Ca intake or plasma 25(OH)D | 548 | 768 | 1103 | 1675 | 44 | 67 | 85 | 112 |
| Age, *y* (not standardized) | 57 | 57 | 59 | 60 | 59 | 60 | 58 | 60 |
| C-peptide, *μg/L* | 2.04 | 1.96 | 1.80 | 1.79 | 2.04 | 1.79 | 1.89 | 1.60 |
| BMI, *kg/m²* | 26.0 | 25.9 | 25.5 | 25.2 | 26.3 | 25.6 | 25.0 | 24.2 |
| Hypertension, *%* | 14 | 15 | 16 | 16 | 17 | 18 | 17 | 18 |
| Postmenopausal women, *%* | 80 | 81 | 85 | 85 | 86 | 87 | 85 | 84 |
| Hormone use among postmenopausal women, *%* | 31 | 31 | 35 | 51 | 33 | 44 | 39 | 44 |
| Physical activity,[2] *MET/wk* | 13 | 15 | 21 | 18 | 14 | 15 | 18 | 20 |
| Use of multivitamin, *%* | 22 | 32 | 44 | 59 | 28 | 35 | 52 | 44 |
| Alcohol, *g/d* | 6.5 | 4.7 | 5.2 | 5.5 | 4.6 | 4.8 | 5.5 | 7.0 |
| Dairy dietary intake | | | | | | | | |
| Cereal fiber, *g/d* | 4.9 | 5.5 | 5.5 | 5.6 | 5.4 | 5.4 | 5.6 | 5.5 |
| Glycemic load | 106 | 106 | 105 | 102 | 106 | 105 | 105 | 104 |
| Retinol, *μg/d* | 823 | 1008 | 1225 | 1792 | 1039 | 1237 | 1373 | 1338 |
| Intake of calcium, *mg/d* | 536 | 777 | 1101 | 1777 | 945 | 1060 | 1160 | 1110 |
| Calcium supplement user, *%* | 12 | 28 | 60 | 93 | 42 | 51 | 59 | 53 |
| Intake of vitamin D, *μg/d* | 5.7 | 7.7 | 9.9 | 12.9 | 7.1 | 9.1 | 10.2 | 9.9 |
| Dairy consumption,[3] *servings/d* | 1.2 | 2.1 | 2.8 | 2.4 | 1.9 | 2.2 | 2.3 | 2.4 |

[1] Values are age-adjusted means or percentages unless otherwise noted.
[2] MET, Metabolic equivalent task.
[3] 50 g of cheese or 175 g of yogurt.

concentrations of C-peptide and alcohol intake (Table 1). The overall pattern for the associations between 25(OH)D and covariates in men shared some similarity to that of calcium with other covariates. In addition, men who had high levels of 25(OH)D were more likely to drink alcohol and less likely to be hypertensive. Levels of 25(OH) were positively associated with physical activity. In women, the association between calcium intake and other covariates was similar to that in men. Additionally, women with higher calcium intakes were more likely to be postmenopausal and hormone users. The pattern of the association between 25(OH)D and covariates in women were similar to that in men.

Total calcium intake was inversely associated with fasting plasma concentrations of C-peptide in women (**Table 2**). Further adjustment of total vitamin D intake or plasma concentrations of 25(OH)D and dairy foods did not materially change this association. After adjustment, the fasting concentrations of C-peptide were 5.1% lower in women in the highest quartile of calcium intake compared with women in the lowest quartile; for men, the difference was 6.7% in the same direction but it was not significant ($P$-trend = 0.7). We further divided calcium intake into several categories (<500, 500–800, 800–1200, and ≥1200 mg/d) to identify a threshold for the beneficial biologic effect in women. On multivariate analysis limited to women, compared calcium intake < 500 mg/d, total calcium intakes >800 mg/d was associated with 11% lower concentrations of C-peptide ($P$-trend = 0.05). Calcium intake ≥1200 mg/d was not associated with lower C-peptide concentrations. In men, we did not identify any threshold or dose-response relationship between calcium and C-peptide ($P = 0.7$).

**TABLE 2** Fasting plasma concentrations of C-peptide in relation to total daily calcium intakes in healthy men and women[1,2]

| | Q1 | Q2 | Q3 | Q4 | *P*-trend |
|---|---|---|---|---|---|
| Men, *n* | 221 | 222 | 222 | 223 | |
| Median calcium intake, *mg/d* | 580 | 760 | 952 | 1408 | |
| C-peptide, $\mu g/L$ | | | | | |
| Multivariate | 2.09 ± 0.16 | 1.94 ± 0.16 | 2.02 ± 0.15 | 1.95 ± 0.16 | 0.6 |
| Multivariate + BMI | 2.06 ± 0.16 | 1.91 ± 0.16 | 1.99 ± 0.15 | 1.92 ± 0.16 | 0.6 |
| Multivariate + BMI, vitamin D intake | 2.03 ± 0.16 | 1.88 ± 0.16 | 1.98 ± 0.15 | 1.89 ± 0.16 | 0.5 |
| Multivariate + BMI, vitamin D intake, dairy foods | 2.02 ± 0.11 | 1.89 ± 0.17 | 2.01 ± 0.15 | 1.88 ± 0.17 | 0.7 |
| Women,[3] *n* | 272 | 274 | 270 | 269 | |
| Median calcium intake, *mg/d* | 548 | 768 | 1103 | 1675 | |
| C-peptide, $\mu g/L$ | | | | | |
| Multivariate | 2.01 ± 0.07 | 1.95 ± 0.07 | 1.83 ± 0.07 | 1.85 ± 0.07 | 0.01 |
| Multivariate + BMI | 1.98 ± 0.07 | 1.94 ± 0.06 | 1.84 ± 0.06 | 1.86. ± 0.06 | 0.03 |
| Multivariate + BMI, vitamin D intake | 1.98 ± 0.06 | 1.93 ± 0.06 | 1.84 ± 0.06 | 1.87 ± 0.06 | 0.03 |
| Multivariate + BMI, vitamin D intake, dairy foods | 1.97 ± 0.07 | 1.93 ± 0.01 | 1.84 ± 0.06 | 1.87 ± 0.06 | 0.05 |

[1] Values are means ± SEM.
[2] Other variables adjusted in the model include: age, smoking, physical activity, race, state of residence, hours of fasting, laboratory batch, time of blood draw, caffeinated and decaffeinated coffee consumption, alcohol, cereal fiber, retinol, and glycemic load intake, and total energy intake.
[3] For women, we additionally adjusted for postmenopausal status and hormone use.

In a multivariate analysis, we observed an inverse association between plasma concentrations of vitamin D and fasting C-peptide in men and in women, but after further adjustment for BMI, the inverse association persisted only in men. In men, the difference in plasma concentrations of C-peptide between the extreme quartiles was 20% (**Table 3**).

Overall, results for calcium intake and plasma 25(OH)D from our spline analyses were similar to those obtained with our indicator approach using categorical variables (Tables 2 and 3) (graph not shown).

We examined the joint association of total calcium intake plus plasma concentrations of 25(OH)D with fasting plasma concentrations of C-peptide (**Table 4**). For the combined highest levels of calcium intake and plasma 25(OH)D, compared with the opposite extreme, plasma concentrations of C-peptide were 35% lower ($P = 0.03$) among men and 12% lower ($P = 0.01$) among women.

Furthermore, we examined the effect of the modification for the combination of calcium intake plus plasma 25(OH)D with C-peptide using an additive scale. Under the null assumption, the differences between the C-peptide concentrations at the highest tertile of calcium intake plus plasma 25(OH)D ($C\text{-}PEP_{33}$) and at the lowest tertile of calcium intake plus plasma 25(OH)D ($C\text{-}PEP_{11}$) can be computed by summing the following 2

**TABLE 3** Fasting plasma concentrations of C-peptide in relation to plasma concentrations of 25(OH)D in healthy men and women[1–3]

| | Plasma vitamin D, 25(OH)D | | | | |
|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | *P*-trend |
| Men, *n* | 222 | 224 | 222 | 220 | |
| Median plasma 25(OH)D, *mmol/L* | 38 | 55 | 68 | 88 | |
| C-peptide, $\mu g/L$ | | | | | |
| Multivariate + calcium intake | 2.26 ± 0.23 | 2.10 ± 0.23 | 2.22 ± 0.28 | 1.75 ± 0.24 | 0.03 |
| Multivariate + calcium intake + BMI | 2.25 ± 0.23 | 2.09 ± 0.25 | 2.22 ± 0.28 | 1.76 ± 0.24 | 0.04 |
| Multivariate + calcium intake + BMI + dairy foods | 2.15 ± 0.23 | 2.02 ± 0.23 | 2.19 ± 0.28 | 1.70 ± 0.25 | 0.08 |
| Women, *n* | 271 | 272 | 270 | 272 | |
| Median plasma 25(OH)D, *mmol/L* | 44 | 67 | 85 | 112 | |
| C-peptide, $\mu g/L$ | | | | | |
| Multivariate + calcium intake | 2.47 ± 0.25 | 2.28 ± 0.26 | 2.39 ± 0.23 | 2.05 ± 0.23 | 0.0003 |
| Multivariate + calcium intake + BMI | 2.23 ± 0.23 | 2.13 ± 0.25 | 2.33 ± 0.22 | 2.09 ± 0.23 | 0.3 |
| Multivariate + calcium intake + BMI + dairy foods | 2.24 ± 0.23 | 2.13 ± 0.25 | 2.33 ± 0.22 | 2.09 ± 0.23 | 0.3 |

[1] Other variables adjusted in the model include: age, smoking, physical activity, race, state of residence, month of blood draw, fasting hours, laboratory batches, time of blood draw, caffeinated and decaffeinated coffee consumption, alcohol, cereal fiber, retinol, and glycemic load intake, and total energy intake.
[2] Postmenopausal status and hormone use were additionally adjusted for women.
[3] Concentrations of C-peptide are presented as means ± SE.

**TABLE 4** Fasting plasma concentrations of C-peptide according to different levels of daily calcium intakes and plasma 25(OH)D in healthy men and women[1–3]

| Tertiles of plasma 25(OH)D (median) | Tertiles of total calcium intake (median) | | | | | |
|---|---|---|---|---|---|---|
| | Tertile 1 (593 mg/d) | | Tertile 2 (831 mg/d) | | Tertile 3 (1279 mg/d) | |
| Men | *n* | *μg/L* | *n* | *μg/L* | *n* | *μg/L* |
| Tertile 1 (40 mmol/L) | 76 | 2.50 ± 0.33 | 61 | 2.10 ± 0.26 | 52 | 1.92 ± 0.26 |
| Tertile 2 (60 mmol/L) | 59 | 2.12 ± 0.33 | 67 | 1.90 ± 0.26 | 65 | 2.36 ± 0.48 |
| Tertile 3 (83 mmol/L) | 49 | 1.63 ± 0.29 | 62 | 1.83 ± 0.25 | 80 | 1.65 ± 0.28 |
| Women | Tertile 1 (593 mg/d) | | Tertile 2 (953 mg/d) | | Tertile 3 (1543 mg/d) | |
| Tertile 1 (50 mmol/L) | 162 | 2.51 ± 0.26 | 105 | 2.51 ± 0.26 | 94 | 2.37 ± 0.24 |
| Tertile 2 (75 mmol/L) | 93 | 2.50 ± 0.26 | 139 | 2.34 ± 0.24 | 131 | 2.43 ± 0.25 |
| Tertile 3 (83 mmol/L) | 106 | 2.19 ± 0.25 | 118 | 2.16 ± 0.25 | 137 | 2.20 ± 0.23 |

[1] Multivariates include: age, BMI, smoking, physical activity, race, state of residence, month of blood draw, fasting hours, laboratory batches, time of blood draw, caffeinated and decaffeinated coffee consumption, alcohol, cereal fiber, retinol, and glycemic load intake, total energy intake, and dairy foods.
[2] Postmenopausal status and hormone use were additionally adjusted for women.
[3] Concentrations of C-peptide are presented as means ± SE.

differences: *1*) $C\text{-}PEP_{31} - C\text{-}PEP_{11}$, which is the difference in C-peptide between calcium intake in the highest and lowest tertiles, when plasma 25(OH)D is in the lowest tertile; and *2*) $C\text{-}PEP_{13} - CPEP_{11}$, which is the difference in C-peptide between plasma 25(OH)D in the highest and lowest tertiles when calcium intake is in the lowest tertile. The resulting equation can be written as: $C\text{-}PEP_{33} - C\text{-}PEP_{11} = (C\text{-}PEP_{31} - C\text{-}PEP_{11}) + (C\text{-}PEP_{13} - C\text{-}PEP_{11})$. This equation did not hold for men, which indicated an effect modification (Table 4); however, the interaction was not significant ($P = 0.1$). In women, no significant modification effect was found ($P = 0.7$).

We further conducted subgroup analyses according to hypertension and BMI for men and women and according to postmenopausal status and use of hormones for women only. These subgroups did not differ, except for an association between calcium intake and C-peptide among hypertension strata. Calcium intake was significantly inversely associated with the fasting plasma concentrations of C-peptide in hypertensive men (17% difference between extreme quartiles) and hypertensive women (20% difference between extreme quartiles) but not among nonhypertensive men ($P$-trend = 0.7) or women ($P$-trend = 0.3) (**Table 5**). Plasma concentrations of vitamin D in relation to C-peptide did not vary significantly according to hypertension status (not shown).

To explore whether the association between calcium intake and fasting C-peptide was due to dietary calcium or supplemental calcium, we adjusted dietary calcium and supplemental calcium simultaneously in the same model and found that only supplemental calcium remained significant. The medians of quartiles 1–4 for dietary calcium were 440, 656, 843, and 1259 mg/d in men and 458, 656, 860, and 1253 mg/d in women. The median calcium intake of supplemental users was 200 mg/d in men and 500 mg/d in women. The differences in C-peptide

**TABLE 5** Total daily calcium intakes based on hypertension status and dietary and supplemental calcium intakes of hypertensive men and women in relation to fasting plasma concentrations of C-peptide[1–4]

| | Q1 | Q2 | Q3 | Q4 | *P*-trend | *P*-interaction |
|---|---|---|---|---|---|---|
| Men | | | | | | |
| Normotensive, *n* | 147 | 146 | 145 | 148 | | |
| Total calcium intake, *mg/d* | 1.99 ± 0.24 | 1.59 ± 0.17 | 2.00 ± 0.16 | 1.96 ± 0.22 | 0.7 | 0.09 |
| Hypertensive, *n* | 76 | 75 | 76 | 75 | | |
| Total calcium intake, *mg/d* | 2.19 ± 0.30 | 2.28 ± 0.27 | 2.02 ± 0.26 | 1.78 ± 0.30 | 0.02 | |
| Dietary calcium, *mg/d* | 2.17 ± 0.30 | 2.02 ± 0.26 | 2.22 ± 0.28 | 1.81 ± 0.40 | 0.6 | |
| Women | | | | | | |
| Normotensive, *n* | 411 | 412 | 410 | 412 | | |
| Total calcium intake, *mg/d* | 1.86 ± 0.07 | 1.83 ± 0.06 | 1.73 ± 0.06 | 1.81 ± 0.06 | 0.3 | 0.05 |
| Hypertensive, *n* | 74 | 73 | 74 | 74 | | |
| Total calcium intake, *mg/d* | 2.76 ± 0.2 | 2.61 ± 0.2 | 2.46 ± 0.2 | 2.2 ± 0.2 | 0.003 | |
| Dietary calcium, *mg/d* | 2.65 ± 0.25 | 2.35 ± 0.21 | 2.62 ± 0.22 | 2.01 ± 0.26 | 0.3 | |
| | Supplemental calcium, *mg/d* | | | | | |
| | *n* | Nonuser | *n* | User | *P*-value | |
| Hypertensive men | 172 | 2.19 ± 0.24 | 130 | 1.93 ± 0.25 | 0.02 | |
| Hypertensive women | 140 | 2.61 ± 0.18 | 155 | 2.29 ± 0.18 | 0.02 | |

[1] Other variables adjusted in the model include: age, BMI, smoking, physical activity, race, state of residence, hours of fasting, laboratory batches, time of blood draw, caffeinated and decaffeinated coffee consumption, alcohol, cereal fiber, retinol, and glycemic load intake, total energy intake, vitamin D intake, and dairy foods.
[2] For women, we additionally adjusted for postmenopausal status and hormone use.
[3] Dietary calcium and supplemental calcium were adjusted simultaneously.
[4] Concentrations of C-peptide are presented as means ± SE.

concentrations between calcium supplement users and nonusers was 18% in men ($P = 0.02$) and 12% in women ($P = 0.02$) (Table 5).

Finally, we examined total dairy foods and lactose consumption relative to fasting plasma concentrations of C-peptide after controlling for calcium intake and vitamin D intake or plasma concentrations of 25(OH)D, but uncovered no significant associations between dairy food consumption and C-peptide or lactose intake and C-peptide in either women ($P = 0.2$) or men ($P = 0.6$) (data not shown).

## Discussion

In this large cross-sectional dietary study, individuals with the highest calcium intake and plasma concentrations of 25(OH)D had significantly lower fasting C-peptide concentrations (35% in men and 12% in women) than individuals at the opposite extreme. Importantly, among hypertensive individuals, the differences in fasting plasma concentrations of C-peptide between the extreme quartiles of calcium intake were 17% in men and 20% in women. Furthermore, calcium itself, rather than the othelr components of a high-dairy diet, seems to account for the association.

Insulin secretion is calcium dependent (38). Calcium is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscle and adipose tissue (39). Previous intervention studies focusing on the effect of calcium on insulin secretion or diabetes-related parameters have usually been small and the results inconsistent. In comparing high (≥1200 mg/d) to low (500–800 mg/d) calcium intake, some studies found a reduction in insulin concentrations (40) and others found no difference (11,41). Because these studies used dairy foods instead of calcium, the role of calcium cannot be separated out. Cohort studies with women have consistently revealed an inverse association between calcium intake and the incidence of type 2 diabetes or the prevalence of the metabolic syndrome (1–4). The lack of published studies with men could be due to publication bias against null results. Our findings lend some indirect support to a stronger association between calcium and C-peptide in women than in men.

Vitamin D may improve insulin sensitivity either directly by enhancing the insulin response for glucose transport or indirectly by regulating extracellular calcium and maintaining an adequate intracellular calcium pool (42). Two large observational studies in women identified an inverse association between vitamin D intake and diabetes that was greatly attenuated or disappeared after adjustment for calcium intake (1,4). Most importantly, plasma 25(OH)D was not measured. Although small cross-sectional studies relating plasma 25(OH)D to diabetes or insulin secretion have uncovered inverse associations (43–48), many did not fully adjust for confounding factors, particularly physical activity and BMI (45–48). Physical activity and BMI are important confounders, because they are associated with both 25(OH)D and are strong risk factors for insulin resistance (49–51). The effect of vitamin D treatment on diabetes-related parameters revealed no significant associations in 2 intervention studies (12,13). The different doses or types of vitamin D used in these studies, their small sample sizes, and the gender of the subjects may partly explain the null results; other reasons need to be determined in future investigations.

Although the reasons for the stronger association between calcium and C-peptide in women are unclear, we did find a consistent inverse association between calcium and C-peptide in women and hypertensive men. Calcium has been shown to reduce the risk of hypertension in several prospective studies (29,52) and to slightly reduce systolic blood pressure in meta-analyses of clinical trials (53,54). One small clinical trial with hypertensive patients conducted by Sanchez et al. (53,54) found that intake of 1500 mg/d calcium for 8 wk decreased fasting levels of insulin and improved insulin sensitivity. This trial supports our findings. In addition, our results complement those of Sanchez et al., as we also found calcium not to be associated with insulin secretion among nonhypertensive women and men.

This study supports an association between intakes of calcium plus vitamin D and insulin secretion in healthy individuals. The combination appears to produce a stronger effect than calcium or vitamin D alone in men, but not in women. Few studies have evaluated the association of insulin secretion with calcium intake and plasma concentrations of vitamin D. Post hoc analyses of a trial testing bone-related outcomes demonstrated that calcium plus vitamin D improved fasting glycemia and insulin secretion in nondiabetic individuals but only if they had impaired fasting glucose tolerance (10). There was no calcium alone or vitamin D alone arm in that study and, thus, the individual effect of calcium or vitamin D cannot be assessed. A cohort study of women demonstrated that calcium plus vitamin D reduced diabetes; however, plasma 25(OH)D was not measured (1). Assessment of the effect of calcium and 25(OH)D on diabetes-related problems in large prospective cohort studies and randomized trials is clearly warranted.

The associations between calcium intake or plasma concentrations of vitamin D and C-peptide differed between men and women. We do not know why the inverse association between 25(OH)D and C-peptide concentrations was noted primarily among men. This finding could represent a true biological gender difference, or it could be caused by the older mean age of the men, chance, or a residual confounding in men (although we tested the same variables in men and women).

The strengths of the study include the relatively large sample size, plasma 25(OH)D and C-peptide measurements for both genders, control for dietary and other confounders, and the high intake of calcium supplements, which allowed us to detect the independent associations of calcium and dairy intake with C-peptide. The 2 cohorts allowed us to compare results from subanalyses in both genders (e.g. the association with calcium in hypertensive individuals).

There are several important limitations of our study. First, it was observational. The potential for residual confounding remains, even though we had more extensive covariate data compared with other studies. Although the analyses were cross-sectional, we would not expect (unknown) C-peptide concentrations to influence diet and sun exposure behavior in nondiabetics. There were measurement errors for both dietary measures and biomarkers, although these would attenuate only true associations. In both cohorts, the blood and diet records were collected at the same time period; thus, no causal relationship can be established. Although C-peptide and 25(OH)D were measured at different periods, pooled quality controls were used to standardize the potential laboratory drift over time of the results of the same assay, due to the change of reagents and instruments, or modification of the measurement method. Finally, the fact that the majorities of both cohorts were white limits generalizability, because individuals with darker skin, who synthesize vitamin D less efficiently (35) and have lower 25(OH)D levels, may be at higher risk for insulin resistance.

In summary, our study provides evidence that insulin sensitivity may be associated with both calcium intake and vitamin

D and suggests that levels of C-peptide are especially high in hypertensive individuals, but not normotensive individuals, with inadequate calcium intake. Because calcium and vitamin D insufficiency are common in the U.S. population, our results would have important clinical implications if confirmed in other populations using other study designs. We think that the next steps must include large prospective studies examining the association between plasma concentrations of 25(OH)D and calcium intake, and the incidence of diabetes in diverse populations, especially people with hypertension.

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