

The Effects of Calcium and Vitamin D Supplementation on Blood Glucose and Markers of Inflammation in Nondiabetic Adults

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OBJECTIVE — We sought to compare the effects of combined calcium and vitamin D supplementation versus placebo on blood glucose and markers of inflammation in nondiabetic adults aged ≥ 65 years.

RESEARCH DESIGN AND METHODS — A total of 314 Caucasian adults without diabetes received either 500 mg calcium citrate and 700 IU vitamin D₃ or placebos daily for 3 years in a double-blind, randomized, controlled trial designed for bone-related outcomes. In a post hoc analysis, fasting plasma glucose (FPG), insulin sensitivity (estimated by homeostasis model assessment of insulin resistance [HOMA-IR]), plasma C-reactive protein, and interleukin-6, were measured at baseline and 3 years.

RESULTS — The effects of combined calcium–vitamin D supplementation on 3-year change in FPG depended on baseline FPG ($P = 0.02$ for interaction). Therefore, we conducted analyses separately in participants with normal fasting glucose (NFG) (FPG < 5.6 mmol/l, $n = 222$) and impaired fasting glucose (IFG) (FPG 5.6–6.9 mmol/l, $n = 92$) at baseline. Among participants with IFG at baseline, those who took combined calcium–vitamin D supplements had a lower rise in FPG at 3 years compared with those on placebo (0.02 mmol/l [0.4 mg/dl] vs. 0.34 mmol/l [6.1 mg/dl], respectively, $P = 0.042$) and a lower increase in HOMA-IR (0.05 vs. 0.91, $P = 0.031$). In the NFG subgroup, there was no difference in the change in FPG or HOMA-IR between the two treatment arms. There were no differences in C-reactive protein or interleukin-6 between the two treatment arms in either subgroup.

CONCLUSIONS — In healthy, older adults with IFG, supplementation with calcium and vitamin D may attenuate increases in glycemia and insulin resistance that occur over time. However, our findings should be considered hypothesis generating and need to be confirmed in randomized trials specifically designed for the outcomes of interest.

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Type 2 diabetes is associated with considerable morbidity and mortality, and its prevalence has been increasing nationally and worldwide with more than 1 million new cases per year diagnosed in the U.S. alone (1,2). Potentially modifiable risk factors for type 2 diabetes have been identified, the major one being obesity (3). However, although weight loss has been shown to be successful in delaying the onset of type 2 diabetes, it is difficult to achieve and maintain

long term (4). Therefore, identification of weight-independent, easily modified risk factors is urgently needed to attenuate the increase in the incidence of type 2 diabetes. There is accumulating evidence to suggest that altered calcium and vitamin D homeostasis may play a role in the development of type 2 diabetes. The role of vitamin D is suggested by cross-sectional studies showing that low serum 25-hydroxyvitamin D concentration is associated with glucose intolerance, diabetes, insulin resistance, and the metabolic syndrome (5–11). The role of calcium in the development of type 2 diabetes is suggested indirectly by cross-sectional studies in which high calcium intake has been found to be inversely associated with body weight and fatness (12–16). In the Nurses' Health Study, a large prospective observational study in women, it was recently shown that a combined high intake of calcium and vitamin D is inversely associated with risk of incident type 2 diabetes (17). Despite the supporting evidence from observational studies, the results of small intervention studies with vitamin D or calcium supplementation on glucose tolerance have been inconsistent (18–26).

The purpose of the present study was to determine the effects of combined calcium and vitamin D supplementation on glucose metabolism and systemic inflammation in nondiabetic adults.

RESEARCH DESIGN AND METHODS — The present study is an ancillary analysis using existed data and new metabolic measurements in archived samples from a completed double-blind, parallel-group, single-center, randomized, controlled 3-year clinical trial on the effects of calcium and vitamin D supplementation on osteoporosis (27).

The trial was conducted at the Human Nutrition Research Center on Aging at Tufts University with approval from the Tufts–New England Medical Center Human Investigation Review Committee and written informed consent by all participants.

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Abbreviations: FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, impaired fasting glucose; NFG, normal fasting glucose; PTH, parathyroid hormone.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Participants were healthy, ambulatory adults aged ≥ 65 years and recruited through direct mailings and presentations in the community. Criteria for exclusion included current cancer, laboratory evidence of kidney or liver disease, dietary calcium intake exceeding 1,500 mg per day, and bone-altering conditions (hyperparathyroidism; nephrolithiasis; renal disease; bilateral hip surgery; or therapy with bisphosphonate, calcitonin, estrogen, tamoxifen, or testosterone in the past 6 months or fluoride in the past 2 years).

Randomization and intervention

A computer-generated random number sequence was used to assign participants to either the placebo or the calcium-vitamin D supplementation group with stratification according to sex and decade of age. Participants were advised to maintain their usual diets and avoid taking supplemental calcium and vitamin D on their own for 2 months before and throughout the study. At bedtime, participants took separate pills containing 700 IU cholecalciferol (vitamin D₃) and 500 mg elemental calcium as calcium citrate or separate matching placebo tablets containing microcrystalline cellulose.

During the 3-year trial, 127 participants discontinued treatment; 4 died, 40 stopped for personal reasons (e.g., they lost interest or moved away), 46 withdrew because of illness, 17 started estrogen or glucocorticoid therapy, and 20 withdrew because of problems with the medication. These participants were encouraged to return for all subsequent follow-up evaluations. At the last visit, 389 participants (87% of the 445 randomized) returned for evaluation. Among those, we excluded nonwhite persons ($n = 14$) because in previous studies, vitamin D deficiency correlated with diabetes primarily in whites (7,17) and because nonwhites exhibit a different vitamin D, calcium, and parathyroid hormone (PTH) homeostasis (28,29). We excluded patients with diabetes ($n = 21$), based on either self-report or baseline fasting plasma glucose (FPG) ≥ 7 mmol/l (126 mg/dl) (30), to minimize confounding with diabetes therapy during the course of the trial. We also excluded participants who did not have stored specimens available for measurement of glucose tolerance outcomes ($n = 38$). The baseline characteristics of the group without specimens did not differ from the cohort with available specimens. After excluding two additional participants with very high C-reactive protein at

baseline (>30 mg/l), indicating a severe inflammatory process, 314 participants entered the analyses.

Outcomes and measurements

Ascertainment of exposures. Calcium and vitamin D intakes were estimated at baseline on the basis of a food frequency questionnaire (31). Vitamin D status was assessed by measuring serum 25-hydroxyvitamin D levels both at baseline and after the intervention to assess the efficacy of the vitamin D supplementation. **Ascertainment of outcomes.** Glucose tolerance was assessed by measuring FPG. The latest American Diabetes Association criteria (30) were used to classify participants' glucose tolerance based on baseline FPG values: normal fasting glucose (NFG) <5.6 mmol/l (100 mg/dl), impaired fasting glucose (IFG) 5.6–6.9 mmol/l (100–125 mg/dl), IFG, and diabetes ≥ 7 mmol/l (126 mg/dl). Assessment of insulin sensitivity in the basal (nonstimulated) state was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) based on fasting glucose and insulin measurements as follows: $\text{HOMA-IR} = (\text{glucose [mmol/l]} \times \text{insulin [mU/l]})/22.5$ (32). High HOMA-IR scores denote low insulin sensitivity (increased insulin resistance). HOMA-IR has a high correlation with measures of insulin sensitivity obtained from the euglycemic clamp procedure (33,34), including prediction of age-related insulin resistance in older people (35). We did not assess pancreatic β -cell function using HOMA because HOMA modeling of β -cell function is not valid in older persons in whom dynamic testing is required to estimate age-related impairment of β -cell function (35).

Assessment of potential confounders. Height (to ± 0.1 cm) was measured at baseline using a wall-mounted stadiometer, and body weight (to ± 100 g) was measured using an electronic calibrated scale (Model CN-20; DETECTO-Cardinal Scale Manufacturing, Webb City, MO). BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Data on age, sex, and smoking status (never, past, or current) were self-reported at baseline. Leisure, household, and occupational activity levels were estimated at baseline with use of the Physical Activity Scale for the Elderly questionnaire (36).

Blood analyses. Blood measurements were done in the morning after an 8-h overnight fast. Plasma and serum was

stored at -80°C . Plasma glucose was measured by an oxygen rate method using the Beckman Synchron LX System (Beckman Coulter, Fullerton, CA) with intra- and interassay coefficients of variation (CVs) of 2.0 and 3.0%, respectively. Serum insulin was measured by radioimmunoassay commercial kit (DPC Coat-A-Count Insulin assay; Diagnostic Products, Los Angeles, CA) with intra- and interassay CVs of 3.1–9.3 and 4.9–10.0%, respectively. Plasma high-sensitivity C-reactive protein was measured by the Olympus auto-analyzer (Smith-Kline Beecham Laboratories, Santa Cruz, CA) using reagent from Equal Diagnostics (Exton, PA). Assay sensitivity is 0.05 mg/l with intra- and interassay CVs of 2.0 and 3.3%, respectively, at midrange. Interleukin-6 was measured by immunoassay commercial kit (R&D Systems Quantikine HS, Minneapolis, MN) with intra- and interassay CVs of 6.9–7.8 and 6.5–9.6%, respectively. Plasma 25-hydroxyvitamin D was measured by the method of Preece et al. (37) and 1,25-dihydroxyvitamin D by a competitive protein-binding method. The CVs for the vitamin D assays ranged from 5.5 to 7.7%. PTH was measured by chemoluminescence (Bayer HealthCare, Tarrytown, NY). Assay sensitivity was 2.5 pg/ml with an intra-assay CV of 5.2% and an interassay CV of 8.2%. PTH and 25-hydroxyvitamin D were measured in a blinded fashion as the samples were collected. Measurements of 1,25-hydroxyvitamin D, glucose, insulin, C-reactive protein, and interleukin-6 were done in a blinded fashion, in duplicate, in pairs (before/after intervention) at the same time, in the same analytical run, and in random order to reduce systematic error and inter-assay variability.

Statistical analysis

All variables of interest were examined for normality. C-reactive protein and interleukin-6 had skewed distribution and were transformed to their natural logarithms for analyses. To examine differences in baseline characteristics between groups, we used Student's *t* test for differences in means for continuous data and the χ^2 test for differences in proportions for categorical variables. The primary end point was 3-year change from baseline in FPG. Secondary end points included 3-year changes from baseline in HOMA-IR, C-reactive protein, and interleukin-6. To compare differences between the treatment arms in outcomes over time, we used general linear models (PROC GLM

Table 1—Baseline characteristics of study participants by FPG

	NFG (n = 222)		IFG (n = 92)	
	Placebo	Calcium + Vitamin D	Placebo	Calcium + Vitamin D
n	114	108	47	45
Sex (women)	73 (64)	64 (59)	21 (45)	23 (51)
Age (years)	71.7 ± 0.4	70.6 ± 0.4	71.3 ± 0.8	71.1 ± 0.7
Weight (kg)	71.1 ± 1.2	71.6 ± 1.2	80.0 ± 2.3	77.5 ± 1.8
BMI (kg/m ²)	26.2 ± 0.3	26.1 ± 0.3	28.1 ± 0.7	27.8 ± 0.6
Vitamin D intake (IU/day)	196 ± 10	191 ± 9	186 ± 19	166 ± 15
Calcium intake (mg/day)	772 ± 35	740 ± 32	734 ± 51	614 ± 53
Physical activity score	112 ± 5	112 ± 5	126 ± 8	130 ± 8
Smoking (%)				
Yes (currently)	5	6	2	9
Yes (formerly)	53	42	66	56
Never	42	53	32	36
25-hydroxyvitamin D (nmol/l)	70.6 ± 2.8	81.4 ± 3.7	81.2 ± 4.7	71.2 ± 5.2
1,25-hydroxyvitamin D (pmol/l)	85.8 ± 1.7	85.3 ± 1.7	84.2 ± 2.7	84.5 ± 2.9
PTH (pmol/l)	4.2 ± 0.2	3.8 ± 0.2	4.0 ± 0.2	4.5 ± 0.3
FPG (mmol/l)	5.09 ± 0.03	5.10 ± 0.03	6.00 ± 0.05	6.04 ± 0.05
HOMA-IR	1.14 ± 0.06	1.27 ± 0.07	2.26 ± 0.14	2.23 ± 0.17
C-reactive protein (mg/l)	2.92 ± 0.32	2.94 ± 0.27	2.45 ± 0.37	2.51 ± 0.28
Interleukin-6 (pg/ml)	3.91 ± 0.38	3.43 ± 0.41	2.90 ± 0.37	4.28 ± 0.80

Data are means ± SE or n (%). *P* values for differences in characteristics between placebo and calcium–vitamin D groups were all nonstatistically significant (*P* > 0.05) within the two subgroups except for plasma 25-hydroxyvitamin D in the NFG subgroup (in which *P* = 0.02 between the placebo and calcium–vitamin D groups). To change from SI (mmol/l) to traditional (mg/dl) units for glucose, divide by 0.0555.

procedure; SAS Software, Cary, NC). We adjusted end points for baseline values to avoid potential bias that might result if the magnitude of the change depended on starting value, and for several risk factors for type 2 diabetes (age, sex, BMI, physical activity, and smoking).

To assess whether the effect of supplementation with calcium plus vitamin D on the primary end point, 3-year change in FPG, varied according to baseline glucose tolerance, we tested for interaction between treatment group assignment and baseline FPG. We performed stratified analyses if we found a statistically significant interaction. *P* values for Student's *t* tests are two sided, and statistical significance was set at *P* ≤ 0.05. Statistical analysis was done using SAS version 9.1.

RESULTS

Participant characteristics and follow-up

At baseline, the average age of the 314 participants with complete data was 71.2 years, BMI was 26.7 kg/m², and plasma 25-hydroxyvitamin D concentration was 76 nmol/l. When the entire cohort was analyzed, there was no difference between placebo and calcium–vitamin D supplementation in the primary outcome,

change in FPG (0.18 vs. 0.12 mmol/l, respectively, *P* = 0.29), or change in HOMA-IR (0.42 vs. 0.18, respectively, *P* = 0.08). However, in the multivariate prediction model, we found an interaction between baseline FPG and treatment group on the primary outcome (*P* = 0.02). Therefore, we stratified the cohort into two subgroups separated by glucose tolerance status at baseline, as defined by the American Diabetes Association criteria based on FPG (NFG [*n* = 222] vs. IFG [*n* = 92]) and examined the effect of supplementation on 3-year changes in metabolic outcomes in each subgroup.

As expected, the IFG subgroup was, on average, heavier than the NFG cohort (78.8 vs. 71.3 kg, respectively, *P* < 0.001) and had higher FPG (6.0 mmol/l [108.5 mg/dl] vs. 5.1 mmol/l [91.9 mg/dl], *P* < 0.001) and HOMA-IR (2.24 vs. 1.21, *P* < 0.001). The IFG subgroup reported higher daily physical activity scores compared with the NFG group (128 vs. 112, respectively, *P* = 0.016). The baseline characteristics of the two treatment arms within each subgroup were well balanced (Table 1).

Intervention

The supplements were generally well tolerated, but 11 participants discontinued treatment due to difficulty swallowing the

pill, and 9 discontinued because of side effects (3 in the placebo and 6 in the calcium–vitamin D arm). As compared with those who took placebo, participants in the calcium–vitamin D arm in both subgroups had a statistically significant increase in plasma 25-hydroxyvitamin D concentration (*P* < 0.001) and a decrease in PTH (*P* < 0.001) after supplementation (Table 2). During the intervention period, participants maintained a relatively stable BMI without any statistically significant differences between treatment arms in either subgroup (Table 2).

Metabolic outcomes by subgroup

FPG. Among participants with IFG at baseline, those randomized to calcium–vitamin D supplementation had a smaller increase in FPG at 3 years than those who took placebo after adjustment for baseline values and risk factors for type 2 diabetes (0.02 ± 0.09 vs. 0.34 ± 0.11 mmol/l, respectively, *P* = 0.042) (Fig. 1A). At the end of the follow-up period, 81% of participants taking placebo remained with IFG or had developed diabetes (by self-report or FPG ≥ 7 mmol/l [126 mg/dl]) vs. 70% of those taking calcium–vitamin D (*P* for χ^2 = 0.28).

In the NFG subgroup, there was no difference in FPG change at 3 years between the two treatment arms (0.15 ±

Table 2—Effects (change from baseline) of combined calcium and vitamin D supplementation on 3-year change in metabolic parameters in subgroups by baseline glucose tolerance status

	NFG			IFG		
	Placebo	Calcium + vitamin D	P for between-group comparison	Placebo	Calcium + vitamin D	P for between-group comparison
n	114	108		47	45	
25-hydroxyvitamin D (nmol/l)	-0.92 ± 2.36	29.6 ± 3.44	<0.001	-7.8 ± 2.9	31.2 ± 4.4	<0.001
1,25-hydroxyvitamin D (pmol/l)	-13.8 ± 1.94	-13.2 ± 2.21	0.84	-12.9 ± 3.0	-15.0 ± 4.1	0.70
PTH (pmol/l)	0.66 ± 0.12	-0.47 ± 0.14	<0.001	0.48 ± 0.16	-0.84 ± 0.25	<0.001
BMI (kg/m ²)	0.01 ± 0.13	0.04 ± 0.11	0.85	0.14 ± 0.18	0.04 ± 0.16	0.67
C-reactive protein (mg/l)	0.79 ± 0.70	0.50 ± 0.32	0.72	1.19 ± 0.56	1.46 ± 1.56	0.87
Interleukin-6 (pg/ml)	0.48 ± 0.59	0.14 ± 0.39	0.61	0.48 ± 0.64	0.78 ± 0.84	0.78

Data are means ± SE after adjustment for baseline values. To change from SI (mmol/l) to traditional (mg/dl) units for glucose, divide by 0.0555.

0.03 mmol/l in calcium–vitamin D vs. 0.12 ± 0.04 mmol/l in placebo, $P = 0.55$.) (Fig. 1A). The proportion of participants who progressed to IFG or diabetes was 19% in the placebo vs. 20% in the calcium–vitamin D group (P for $\chi^2 = 0.84$).

Changes in FPG at 3 years in either the NFG or IFG subgroups were not influenced by baseline plasma 25-hydroxyvitamin D concentration (data not shown).

Insulin resistance. In the IFG subgroup, the effects of calcium–vitamin D on insulin resistance paralleled those on FPG; HOMA-IR increased in the placebo arm while it remained essentially unchanged in the calcium–vitamin D arm (0.91 ± 0.31 vs. 0.05 ± 0.19, respectively, $P = 0.031$) (Fig. 1B). In contrast, in the NFG subgroup, there was no difference in 3-year change in HOMA-IR between placebo and calcium–vitamin D arms (Fig. 1B).

Systemic inflammation. There were no statistically significant differences in 3-year changes in plasma C-reactive protein or interleukin-6 between the two treatment arms in either subgroup (Table 2).

CONCLUSIONS— In this post hoc analysis of a randomized, controlled trial designed for skeletal primary outcomes, we found that daily supplementation with 500 mg calcium citrate and 700 IU vitamin D₃ for 3 years prevented increases in plasma glucose and insulin resistance in the subgroup of participants with IFG at baseline but had no apparent effect among those with NFG. There was no effect of supplementation on markers of systemic inflammation among either subgroup.

Our results are consistent with cross-sectional studies that have reported an inverse association between calcium and vitamin D status, as measured by serum 25-hydroxyvitamin D concentration and

calcium intake, respectively, and prevalence of type 2 diabetes (5–11). Consumption of dairy products, a rich source of both calcium and vitamin D, has also been associated with decreased risk of type 2 diabetes (38,39). Our findings are also in accord with recently reported findings from the Nurses' Health Study, in which a reported daily intake of >800 IU vitamin D and >1,200 mg calcium was associated with a 33% lower risk of type 2 diabetes as compared with a daily intake of <400 IU and 600 mg calcium and vitamin D, respectively (17).

Previously reported trials with vitamin D supplementation in patients with type 2 diabetes have shown conflicting results (18–20,24,25). Although firm conclusions from these trials cannot be drawn because the studies were short in duration, included few subjects, and used various formulations of vitamin D, there is limited evidence to suggest that vitamin D supplementation at an early stage in the

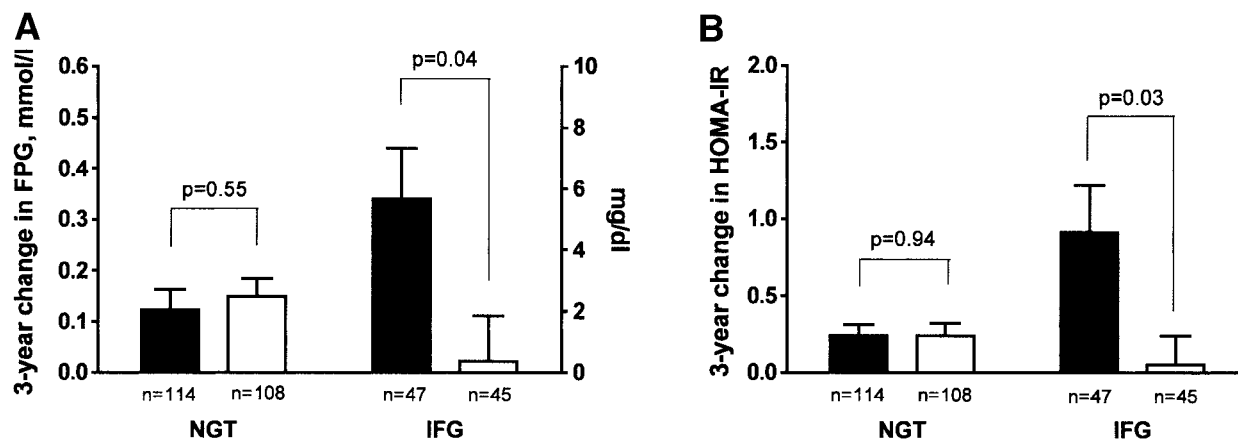


Figure 1—Effects of combined calcium and vitamin D supplementation on FPG (A) and HOMA-IR (B) in subgroups by baseline glucose tolerance status. Changes in FPG and HOMA-IR are expected means ± SEM by general linear model analysis after adjustment for baseline values and risk factors for type 2 diabetes (age, sex, BMI, physical activity, and smoking). ■, placebo; □, calcium and vitamin D supplementation.

pathogenesis of diabetes (i.e., glucose intolerance) may be of benefit in delaying progression to clinical diabetes (20).

The mechanisms by which vitamin D or calcium may affect risk of type 2 diabetes are not clear. Impaired pancreatic β -cell function, insulin resistance, and low-grade systemic inflammation are important risk factors of developing glucose intolerance and type 2 diabetes (40,41). In both animal and human studies, impaired pancreatic β -cell function has been reported with vitamin D insufficiency (7,9,42–45), while vitamin D supplementation restores insulin secretion (18,20,25,42,46,47). Given a recent report that 25-hydroxyvitamin D is converted to its active form, 1,25-hydroxyvitamin D, within the pancreatic β -cell by 25-hydroxyvitamin D 1- α -hydroxylase (48), in our study the lack of difference in 1,25-hydroxyvitamin D levels between the two treatment arms does not rule out an important role for 1,25-hydroxyvitamin D. There is also limited evidence of an association between vitamin D deficiency and insulin resistance (7,9,19,45), but the effect of vitamin D supplementation on insulin resistance from short-term, nonrandomized, non-controlled human trials is conflicting (25,49,50). The mechanisms by which calcium intake may alter diabetes risk are even less clear. Abnormal regulation of intracellular calcium affecting both insulin sensitivity and insulin release has been suggested as a potential mechanism to explain the putative association between calcium insufficiency and risk of type 2 diabetes (51,52). Our finding that HOMA-IR improved with calcium and vitamin D supplementation supports the hypothesis that calcium and vitamin D affect type 2 diabetes risk by modifying insulin resistance. Our study did not assess the effect of supplementation on insulin secretion while there was no effect on inflammatory markers. Two recent observational studies have reported higher C-reactive protein concentrations among patients with hypovitaminosis D (11,53), whereas two trials of vitamin D supplementation versus placebo showed conflicting results; after vitamin D supplementation, C-reactive protein declined among patients in the intensive care unit (54), whereas it remained unchanged in outpatients with congestive heart failure (55).

Our study cannot separate the independent effects of calcium and vitamin D. Only a few observational studies have examined the individual contributions of

calcium and vitamin D in relation to type 2 diabetes (17,39). In the Nurses' Health Study, total (dietary plus supplemental) calcium intake was inversely associated with incident type 2 diabetes while total vitamin D intake (after adjustment for calcium intake) was not. Of particular interest, however, was the observation that women who reported a high intake of combined calcium and vitamin D had a much lower risk of developing type 2 diabetes compared with women with lower intakes of only one of these nutrients. In cross-sectional analysis from the Women's Health Study, a randomized, controlled trial of vitamin E and aspirin in the primary prevention of cardiovascular disease in 39,876 women aged ≥ 45 years, the prevalence of type 2 diabetes was inversely associated with intake of calcium intake but not vitamin D (39). Given their close interrelationship, deciphering the individual effects of calcium and vitamin D in observational studies is challenging, but the overall evidence suggests that both nutrients play a role in relation to type 2 diabetes.

The strengths of our study include its randomized, double-blind, placebo-controlled design; the use of 25-hydroxyvitamin D and PTH to document the success of the intervention; and the adjustment in our analyses for a variety of important confounders for type 2 diabetes. It is important to note that the study was designed for skeletal primary outcomes and that we had single measurements of outcomes at baseline and 3 years only. We excluded nonwhite persons because there were too few nonwhite participants to draw any conclusions, because in previous studies, vitamin D deficiency correlated with diabetes primarily in whites (7,17) and because of evidence to suggest that nonwhites exhibit a different vitamin D, calcium, and parathyroid homeostasis (28,29). Therefore, we cannot directly extrapolate our findings to the nonwhite population. Finally, there may be potential unintended bias because we did not have available specimens for measuring the outcomes of interest in the entire cohort. To explore this possibility, we compared the baseline characteristics between the excluded group because of unavailable stored specimens ($n = 38$) and the cohort with available specimens, and we found no differences.

In conclusion, in older individuals with IFG, combined calcium and vitamin D supplementation attenuates the increases in glycemia and insulin resistance

that occur with aging. Because of the post hoc nature of our analysis, our findings should be considered hypothesis generating, and they need to be replicated in clinical trials specifically designed for diabetes-related primary outcomes in populations at high risk for type 2 diabetes. Nevertheless, our results provide preliminary support for an important role of calcium and vitamin D supplementation to lower risk of progression to clinical diabetes in individuals with glucose intolerance but not in those with NFG.

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References

- Zimmet P, Alberti KG, Shaw J: Global and societal implications of the diabetes epidemic. *Nature* 414:782–787, 2001
- Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, Marks JS: Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA* 289:76–79, 2003
- Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC: Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med* 345:790–797, 2001
- Wing RR, Phelan S: Long-term weight loss maintenance. *Am J Clin Nutr* 82 (Suppl. 1):222S–225S, 2005
- Scragg R, Holdaway I, Singh V, Metcalf P, Baker J, Dryson E: Serum 25-hydroxyvitamin D3 levels decreased in impaired glucose tolerance and diabetes mellitus. *Diabetes Res Clin Pract* 27:181–188, 1995
- Isaia G, Giorgino R, Adami S: High prevalence of hypovitaminosis D in female type 2 diabetic population (Letter). *Diabetes Care* 24:1496, 2001
- Scragg R, Sowers M, Bell C: Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care* 27:2813–2818, 2004
- Need AG, O'Loughlin PD, Horowitz M, Nordin BE: Relationship between fasting serum glucose, age, body mass index and serum 25 hydroxyvitamin D in postmenopausal women. *Clin Endocrinol (Oxf)*

- 62:738–741, 2005
9. Chiu KC, Chu A, Go VL, Saad MF: Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 79:820–825, 2004
 10. Ford ES, Ajani UA, McGuire LC, Liu S: Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. *Diabetes Care* 28:1228–1230, 2005
 11. Cigolini M, Iagulli MP, Miconi V, Galiotto M, Lombardi S, Targher G: Serum 25-hydroxyvitamin D3 concentrations and prevalence of cardiovascular disease among type 2 diabetic patients. *Diabetes Care* 29:722–724, 2006
 12. McCarron DA, Morris CD, Henry HJ, Stanton JL: Blood pressure and nutrient intake in the United States. *Science* 224:1392–1398, 1984
 13. Lind L, Lithell H, Hvarfner A, Pollare T, Ljunghall S: On the relationships between mineral metabolism, obesity and fat distribution. *Eur J Clin Invest* 23:307–310, 1993
 14. Davies KM, Heaney RP, Recker RR, Lappe JM, Barger-Lux MJ, Rafferty K, Hinders S: Calcium intake and body weight. *J Clin Endocrinol Metab* 85:4635–4638, 2000
 15. Lovejoy JC, Champagne CM, Smith SR, de Jonge L, Xie H: Ethnic differences in dietary intakes, physical activity, and energy expenditure in middle-aged, premenopausal women: the Healthy Transitions Study. *Am J Clin Nutr* 74:90–95, 2001
 16. Jacqmain M, Doucet E, Despres JP, Bouchard C, Tremblay A: Calcium intake, body composition, and lipoprotein-lipid concentrations in adults. *Am J Clin Nutr* 77:1448–1452, 2003
 17. Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, Hu FB: Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care* 29:650–656, 2006
 18. Gedik O, Akalin S: Effects of vitamin D deficiency and repletion on insulin and glucagon secretion in man. *Diabetologia* 29:142–145, 1986
 19. Lind L, Pollare T, Hvarfner A, Lithell H, Sorensen OH, Ljunghall S: Long-term treatment with active vitamin D (1,25-dihydroxyvitamin D₃) in middle-aged men with impaired glucose tolerance: effects on insulin secretion and sensitivity, glucose tolerance and blood pressure. *Diabetes Res* 11:141–147, 1989
 20. Orwoll E, Riddle M, Prince M: Effects of vitamin D on insulin and glucagon secretion in non-insulin-dependent diabetes mellitus. *Am J Clin Nutr* 59:1083–1087, 1994
 21. Heaney RP, Davies KM, Barger-Lux MJ: Calcium and weight: clinical studies. *J Am Coll Nutr* 21:152S–155S, 2002
 22. Gunther CW, Legowski PA, Lyle RM, McCabe GP, Eagan MS, Peacock M, Teegarden D: Dairy products do not lead to alterations in body weight or fat mass in young women in a 1-y intervention. *Am J Clin Nutr* 81:751–756, 2005
 23. Zofkova I, Stolba P: Effect of calcitriol and trifluoperazine on glucose stimulated B cell function in healthy humans. *Exp Clin Endocrinol* 96:185–191, 1990
 24. Fliser D, Stefanski A, Franek E, Fode P, Gudarzi A, Ritz E: No effect of calcitriol on insulin-mediated glucose uptake in healthy subjects. *Eur J Clin Invest* 27:629–633, 1997
 25. Borissova AM, Tankova T, Kirilov G, Dakovska L, Kovacheva R: The effect of vitamin D3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. *Int J Clin Pract* 57:258–261, 2003
 26. Zemel MB, Thompson W, Milstead A, Morris K, Campbell P: Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. *Obes Res* 12:582–590, 2004
 27. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE: Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *N Engl J Med* 337:670–676, 1997
 28. Bell NH, Greene A, Epstein S, Oexmann MJ, Shaw S, Shary J: Evidence for alteration of the vitamin D-endocrine system in blacks. *J Clin Invest* 76:470–473, 1985
 29. Harris SS: Vitamin D and African Americans. *J Nutr* 136:1126–1129, 2006
 30. American Diabetes Association: Diagnosis and classification of diabetes mellitus (Position Statement). *Diabetes Care* 28 (Suppl. 1):S37–S42, 2005
 31. Krall EA, Sahyoun N, Tannenbaum S, Dallal GE, Dawson-Hughes B: Effect of vitamin D intake on seasonal variations in parathyroid hormone secretion in postmenopausal women. *N Engl J Med* 321:1777–1783, 1989
 32. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
 33. Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M: Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 23:57–63, 2000
 34. Garcia-Estevez DA, Araujo-Vilar D, Fiestras-Janeiro G, Saavedra-Gonzalez A, Cabezas-Cerrato J: Comparison of several insulin sensitivity indices derived from basal plasma insulin and glucose levels with minimal model indices. *Horm Metab Res* 35:13–17, 2003
 35. Chang AM, Smith MJ, Bloem CJ, Galecki AT, Halter JB, Supiano MA: Limitation of the homeostasis model assessment to predict insulin resistance and beta-cell dysfunction in older people. *J Clin Endocrinol Metab* 91:629–634, 2006
 36. Washburn RA, Smith KW, Jette AM, Janney CA: The Physical Activity Scale for the Elderly (PASE): development and evaluation. *J Clin Epidemiol* 46:153–162, 1993
 37. Preece MA, O'Riordan JL, Lawson DE, Kodicek E: A competitive protein-binding assay for 25-hydroxycholecalciferol and 25-hydroxyergocalciferol in serum. *Clin Chim Acta* 54:235–242, 1974
 38. Choi HK, Willett WC, Stampfer MJ, Rimm E, Hu FB: Dairy consumption and risk of type 2 diabetes mellitus in men: a prospective study. *Arch Intern Med* 165:997–1003, 2005
 39. Liu S, Song Y, Ford ES, Manson JE, Buring JE, Ridker PM: Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* 28:2926–2932, 2005
 40. Pittas AG, Joseph NA, Greenberg AS: Adipocytokines and insulin resistance. *J Clin Endocrinol Metab* 89:447–452, 2004
 41. Stumvoll M, Goldstein BJ, van Haeften TW: Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* 365:1333–1346, 2005
 42. Norman AW, Frankel JB, Heldt AM, Grodsky GM: Vitamin D deficiency inhibits pancreatic secretion of insulin. *Science* 209:823–825, 1980
 43. Chertow BS, Sivitz WI, Baranetsky NG, Clark SA, Waite A, Deluca HF: Cellular mechanisms of insulin release: the effects of vitamin D deficiency and repletion on rat insulin secretion. *Endocrinology* 113:1511–1518, 1983
 44. Soliman AT, Aref MK, Rogol AD: Arginine-induced insulin and growth hormone secretion in children with nutritional rickets. *J Pediatr Gastroenterol Nutr* 6:589–592, 1987
 45. Baynes KC, Boucher BJ, Feskens EJ, Kromhout D: Vitamin D, glucose tolerance and insulinaemia in elderly men. *Diabetologia* 40:344–347, 1997
 46. Tanaka Y, Seino Y, Ishida M, Yamaoka K, Yabuuchi H, Ishida H, Seino S, Seino Y, Imura H: Effect of vitamin D3 on the pancreatic secretion of insulin and somatostatin. *Acta Endocrinol (Copenh)* 105:528–533, 1984
 47. Inomata S, Kadowaki S, Yamatani T, Fukase M, Fujita T: Effect of 1 alpha (OH)-vitamin D3 on insulin secretion in diabetes mellitus. *Bone Miner* 1:187–192, 1986
 48. Bland R, Markovic D, Hills CE, Hughes SV, Chan SL, Squires PE, Hewison M: Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. *J Steroid Biochem Mol Biol* 89–90:121–125, 2004

49. Taylor AV, Wise PH: Vitamin D replacement in Asians with diabetes may increase insulin resistance. *Postgrad Med J* 74:365–366, 1998
50. Kumar S, Davies M, Zakaria Y, Mawer EB, Gordon C, Olukoga AO, Boulton AJ: Improvement in glucose tolerance and beta-cell function in a patient with vitamin D deficiency during treatment with vitamin D. *Postgrad Med J* 70:440–443, 1994
51. Zemel MB: Nutritional and endocrine modulation of intracellular calcium: implications in obesity, insulin resistance and hypertension. *Mol Cell Biochem* 188: 129–136, 1998
52. Fujita T, Palmieri GM: Calcium paradox disease: calcium deficiency prompting secondary hyperparathyroidism and cellular calcium overload. *J Bone Miner Metab* 18:109–125, 2000
53. Timms PM, Mannan N, Hitman GA, Noonan K, Mills PG, Syndercome-Court D, Aganna E, Price CP, Boucher BJ: Circulating MMP9, vitamin D and variation in the TIMP-1 response with VDR genotype: mechanisms for inflammatory damage in chronic disorders? *Qjm* 95: 787–796, 2002
54. Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R: Bone turnover in prolonged critical illness: effect of vitamin D. *J Clin Endocrinol Metab* 88:4623–4632, 2003
55. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R: Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. *Am J Clin Nutr* 83:754–759, 2006
56. Food and Nutrient Board IoM: *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride*. Washington, DC, National Academy Press, 1997
57. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R: Estimates of optimal vitamin D status. *Osteoporos Int* 16:713–716, 2005
58. Heaney RP: The Vitamin D requirement in health and disease. *J Steroid Biochem Mol Biol* 97:13–19, 2005