Baseline Serum 25-Hydroxy Vitamin D Is Predictive of Future Glycemic Status and Insulin Resistance

The Medical Research Council Ely Prospective Study 1990–2000

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OBJECTIVE—Accumulating epidemiological evidence suggests that hypovitaminosis D may be associated with type 2 diabetes and related metabolic risks. However, prospective data using the biomarker serum 25-hydroxyvitamin D [25(OH)D] are limited and therefore examined in the present study.

RESEARCH DESIGN AND METHODS—A total of 524 randomly selected nondiabetic men and women, aged 40–69 years at baseline, with measurements for serum 25(OH)D and IGF-1 in the population-based Ely Study, had glycemic status (oral glucose tolerance), lipids, insulin, anthropometry, and blood pressure measured and metabolic syndrome risk (metabolic syndrome z score) derived at baseline and at 10 years of follow-up.

RESULTS—Age-adjusted baseline mean serum 25(OH)D was greater in men (64.5 nmol/l [95% CI 61.2–67.9]) than women (57.2 nmol/l [54.4,60.0]) and varied with season (highest late summer). Baseline 25(OH)D was associated inversely with 10-year risk of hyperglycemia (fasting glucose: $\beta = -0.0023$, $P = 0.019$; 2-h glucose: $\beta = -0.0097$, $P = 0.006$), insulin resistance (fasting insulin $\beta = -0.1467$, $P = 0.010$; homeostasis model assessment of insulin resistance [HOMA-IR]: $\beta = -0.0059$, $P = 0.005$), and metabolic syndrome z score ($\beta = -0.0016$, $P = 0.048$) after adjustment for age, sex, smoking, BMI, season, and baseline value of each metabolic outcome variable. Associations with 2-h glucose, insulin, and HOMA-IR remained significant after further adjustment for IGF-1, parathyroid hormone, calcium, physical activity, and social class.

CONCLUSIONS—This prospective study reports inverse associations between baseline serum 25(OH)D and future glycemia and insulin resistance. These associations are potentially important in understanding the etiology of abnormal glucose metabolism and warrant investigation in larger, specifically designed prospective studies and randomized controlled trials of supplementation. ***Diabetes* 57:2619–2625, 2008**

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Though the most well-known role of vitamin D is the regulation of calcium absorption and bone metabolism, it is becoming clear that this hormone has pleiotropic effects with possible roles in the pathogenesis of cancer (1), cardiovascular disease (2), multiple sclerosis (3), and type 1 diabetes (4). Recent epidemiological evidence (5–9) also points to a potential association of vitamin D insufficiency with adverse metabolic risk, including that for type 2 diabetes (10,11). While the exact mechanisms that underlie the multiple effects of vitamin D on different tissues are not currently understood, one unifying factor is the expression of vitamin D receptors (VDRs) in >30 tissues, including pancreatic islet cells (12). There is some evidence that polymorphisms in the *VDR* gene may be associated with insulin resistance, insulin secretion, and fasting glucose concentrations (13–16), suggesting that vitamin D is likely to contribute to glucose metabolism (8). It is also becoming clear that there are physiological interactions between vitamin D, IGF-1, and its binding proteins IGFBP-1 and IGFBP-3 (17,18). Variations in circulating IGF-1 and its binding proteins are associated with variations in glycemia (19) and may interact with vitamin D status to influence metabolic syndrome risk (7).

Epidemiological studies examining vitamin D status and the risk of hyperglycemia or insulin resistance have thus far been suggestive of inverse associations but are inconclusive. Most studies that have used the biomarker serum 25-hydroxy vitamin D3 [25(OH)D] concentration, generally recognized as a marker of human vitamin D status, have been cross-sectional in nature; hence, the temporal sequence of associations is unclear. Need et al. previously reported that lower serum 25(OH)D was associated with higher fasting glucose throughout the range of serum 25(OH)D measured and most marked when 25(OH)D levels were <40 nmol/l (20). Ford et al. (6), in the Third National Health and Nutrition Examination Survey of 8,421 men and women, reported an inverse association between 25(OH)D and metabolic syndrome risk, particularly for hyperglycemia, hypertriglyceridemia, and abdominal obesity. Hypponen et al. (7) most recently reported in the 1958 birth cohort study of 6,810 men and women that higher serum 25(OH)D was associated with lower risk of metabolic syndrome (odds ratio 0.33 [95% CI 0.26–0.42]) and each of its individual components cross-sectionally, including an inverse association with A1C level as a marker of glucose status. They found an interaction between 25(OH)D and IGF-1 on metabolic risk, such that

metabolic syndrome risk was lowest when both 25(OH)D and IGF-1 were highest. To our knowledge, there has only been one previous prospective study (21) of the association between serum 25(OH)D and the risk of type 2 diabetes, but the inverse association was attenuated and made nonsignificant after adjustment for confounders. There are no prospective studies, to the best of our knowledge, of the association between serum 25(OH)D and future continuous distribution of glycemic status or insulin resistance. Several studies (11,22,23) have examined the association of diabetes or metabolic syndrome risk with dietary and/or supplemental vitamin D intake, but this represents only a small proportion of vitamin D available in humans, especially in the U.K., where few foods are vitamin D fortified (e.g., margarine) or naturally rich in vitamin D (e.g., egg yolk and oily fish). The main source of vitamin D is from sunlight exposure of the skin, in which a narrow band of ultraviolet-B radiation converts its prehormone, endogenously, into vitamin D3 in the skin, while liver, kidneys, and other activating tissues then hydroxylate it to form 25(OH)D and 1,25-dihydroxyvitamin D3 (calcitriol), respectively. There is general agreement that serum 25(OH)D concentrations best reflect vitamin D repletion or "status," current definitions being suggested to be "sufficiency" (concentrations >75 nmol/l or 30 μg/l), "hypovitaminosis D" (<75 nmol/l or <30 μg/l), "insufficiency" (<50 nmol/l or <20 μg/l) (12), and classical "deficiency" (<25 nmol/l).

The aim of our study was to investigate the prospective association between serum 25(OH)D concentration and markers of metabolic risk, including glucose, insulin, insulin resistance, and a continuous metabolic syndrome risk z score. We further wanted to test whether any associations observed were independent of a comprehensive range of potential confounding or effect-modifying factors, including circulating IGF-1 or its binding proteins.

RESEARCH DESIGN AND METHODS

The participants in this study are a subset of the Ely Study (Cambridgeshire, U.K.), which was established in 1990 as a prospective study; detailed methodology has been described previously (24,25). In brief, from a sampling frame of all adults free of known diabetes and registered with a single practice serving Ely, 1,122 European-origin adults, aged 40–69 years, were randomly selected (response rate 74%). The baseline examination took place between 1990 and 1992. Among those who were biochemically nondiabetic (n = 1,040) at baseline (26), follow-up examination occurred at a median of 4.5 years (when n = 912 attended) and again at 10 years (when n = 683 attended). Of 683 with baseline and 10-year follow-up data, 524 participants had complete data for this analysis after exclusions [n = 5 excluded with elevated plasma creatinine >150 μmol/l, n = 126 missing blood samples for serum 25(OH)D and IGF-1 assays, and n = 28 for other missing variables] and formed the present study population. All participants gave written informed consent, and the study was approved by the local research ethics committee.

Measurements. At both the baseline (1990–1992) and the 10-year (2000–2003) health check visits, participants attended in the morning after an overnight fast. We performed anthropometric measurements to standard protocol (25), including waist circumference (cm), weight (kg), and height (m), and calculated BMI (weight in kilograms divided by the square of height in meters [kg/m^2]). Blood pressure was measured using a standard protocol while seated (25). Venous plasma glucose was measured both fasting and 120 min after a 75-g glucose load (oral glucose tolerance test) and analyzed in fresh samples using a hexokinase assay on blood collected into fluoridated tubes. All samples were cooled on ice, centrifuged into plasma/serum aliquots onsite, and stored at −70°C within 4 h of collection. Biochemical assays were carried out using frozen aliquots for lipids (fasting triglyceride and HDL cholesterol) and fasting insulin. Plasma insulin was measured using two-site immunoassays with either ^{125}I or alkaline phosphatase labels and triglycerides with the RA1000 (Bayer Diagnostics, Suffolk, U.K.), using a standard automated enzymatic method. All samples (baseline and 10-year follow-up) were handled in the same manner and analyzed at Addenbrooke's Hospital clinical chemistry laboratory.

At baseline, fasting serum 25(OH)D concentration was measured by radioimmunoassay using acetonitrile extracts of serum. Intact serum parathyroid hormone (PTH) was measured using a two-site immunoradiometric assay with an NH_2-terminal monoclonal antibody as capture. Total serum calcium concentration was measured using a modified ortho-cresolphthalein compexone reaction with 8-quinolinol to reduce magnesium interference, while serum albumin, measured with an adapted bromocresol purple dye–binding method, was used to calculate corrected calcium. Serum creatinine was assayed using the alkaline picrate Jaffe reaction. Fasting plasma concentrations of IGF-1, IGFBP-1, and IGFBP-3 (IGF binding proteins) were measured by previously reported antibody-based assays (27). All interassay coefficients of variation were <15%. Additionally, at baseline, information was collected by questionnaire on smoking habits, physical activity, and employment (25). We categorized these variables as never, ex-smoker, or current smoker and manual or nonmanual occupational social class, respectively. Physical activity was described as a continuously distributed physical activity score obtained from the modified Paffenbarger questionnaire, from which we computed estimates of energy expended in specific activities as the multiple of reported frequency and duration by the average energy cost of that activity from the Ainsworth energy expenditure compendium. The energy cost of activities is measured in metabolic equivalents (METs), which is a means of expressing the energy expenditure of that activity relative to that of resting. The units of the resulting summary score summated over all reported activities are MET hours per day (28).

Statistical methods. From the date of blood draw, we derived the season of measurement of serum 25(OH)D (April through September coded as spring/summer; October through March as autumn/winter). As sex-specific associations with outcomes were similar, and there was no interaction between sex and 25(OH)D, analyses were pooled for men and women. Four categories of 25(OH)D (created to reflect deficiency [<25 nmol/l], insufficiency [25–49.9 nmol/l], hypovitaminosis but not insufficiency [50–74.9 nmol/l], and sufficiency [≥75 nmol/l]) were used to examine the distribution of baseline parameters adjusted for age and sex. We tested differences in proportions using χ^2 tests and linear trends for continuous traits across 25(OH)D categories using generalized linear models. For nonnormally distributed variables (fasting insulin, triglycerides, IGFBP-1, PTH, and homeostasis model assessment of insulin resistance [HOMA-IR]) we applied logarithmic transformations and examined geometric means and 95% CIs.

The outcomes were continuous quantitative metabolic traits, including fasting glucose, 2-h glucose, and fasting insulin. Additionally, we calculated a marker of insulin resistance in the form of the HOMA-IR as follows: ([fasting insulin × fasting glucose]/22.5) (29). We also derived a continuous metabolic syndrome risk z score as the mean of the following five sex-specific standardized continuous indexes of obesity (BMI + waist circumference/2), hypertension (systolic blood pressure + diastolic blood pressure/2), insulin resistance (fasting insulin), hyperglycemia (2-h plasma glucose), and dyslipidemia (inverted fasting HDL + fasting triglycerides/2) (30). We did not study the outcome of diabetes incidence a priori, as we had only 54 events by 10 years of follow-up among this cohort.

We used multiple linear regression to assess the relationship between baseline circulating 25(OH)D (continuous) and metabolic status (continuous) at the 10-year follow-up. We adjusted for the potential confounding variables, as determined a priori. Three models were constructed. Model 1 was adjusted for age, sex, smoking status, season, BMI, and baseline status of the metabolic parameter being assessed as the outcome variable in the linear regression (for instance adjusting for fasting glucose at baseline when examining fasting glucose at the 10-year follow-up). Model 2 was additionally adjusted for PTH level, corrected calcium, and IGF-1. Model 3 was additionally adjusted for physical activity score and for social class. The variables age, BMI, waist circumference, and physical activity score were entered in all models as continuous variables, while family history, sex, season, and smoking status were used as categorical variables. We tested for statistical interaction of 25(OH)D with BMI, IGF-1, and the IGF-1 binding proteins IGFBP-1 and IGFBP-3 by using product terms and likelihood ratio tests. All statistical analyses were performed using Stata/SE 9.2 (Stata, College Station, TX). All P values were based on two-sided tests. The cutoff for statistical significance was 0.05.

RESULTS

Among 524 initially nondiabetic participants with complete data at baseline and 10-year follow-up, there were 214 men and 310 women, mean (±SD) age 52.9 ± 7.7 years at baseline. Serum 25(OH)D concentration was higher in

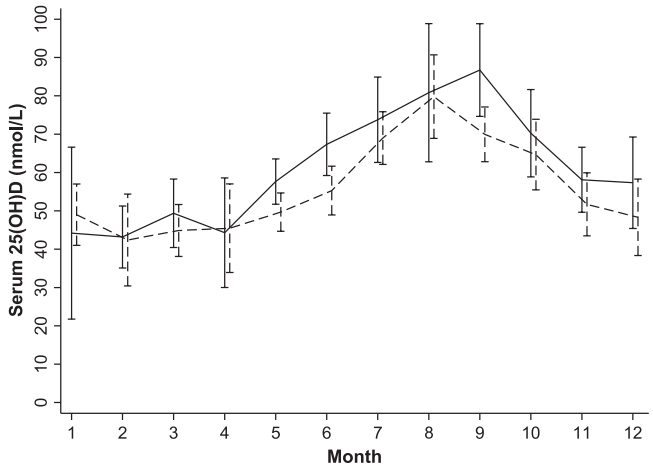

FIG. 1. The distribution of serum 25(OH)D by month in men (solid line) and women (broken line) in the Ely study. Vertical lines are the 95% CIs around the mean values at each month point. Month 1 represents January, while month 12 represents December.

men (64.7 ± 27.3 nmol/l) than in women (57.1 ± 23.4 nmol/l; $P = 0.001$); the age- and sex-adjusted mean 25(OH)D concentration was 60.2 ± 25.3 nmol/l. Serum 25(OH)D was strongly related to the season in which it was measured, with the lowest levels found in early spring and highest in late summer in both men and women (Fig. 1). For most ($n = 10$) months of the year, serum 25(OH)D concentrations were <75 nmol/l, and for 4 months of the year, the mean concentration was <50 nmol/l (i.e., in the hypovitaminosis D and insufficiency range, respectively). Twenty-nine participants (72% women) were vitamin D deficient [25(OH)D <25 nmol/l].

Table 1 shows that at baseline, across categories of increasing serum 25(OH)D, there was a significantly greater proportion of men, and both systolic and diastolic blood pressure were lower. BMI was not significantly different across vitamin D categories, while waist circumference was lowest in the highest category of 25(OH)D. With increasing 25(OH)D, there was a significant decrease in fasting glucose, fasting insulin, HOMA-IR, and metabolic syndrome risk z score, while there was an increase in IGF-1 levels and in both its binding proteins. Notably, serum PTH was significantly lower and serum calcium higher across increasing categories of 25(OH)D.

Table 2 shows that baseline 25(OH)D was significantly inversely associated with 10-year risk of increase in fasting glucose, 2-h glucose, fasting insulin, HOMA-IR, and metabolic syndrome risk z score in analyses adjusted for age, sex, smoking, BMI, season, and for the baseline status of the metabolic outcome variable (model 1). We did not find any significant association with lipids, blood pressure, or waist circumference, suggesting that the association with

TABLE 1
Age- and sex-adjusted baseline characteristics by categories of baseline 25(OH)D: the Ely Study 1990–2000

	25(OH)D categories at baseline				
	<25 nmol/l	25–49.9 nmol/l	50–74.9 nmol/l	≥75 nmol/l	*P* value
n	29	155	219	121	
Age (years)	50 (47.2–52.8)	52 (50.8–53.2)	53.5 (52.5–54.5)	53.7 (52.3–55.1)	0.007
Women	21 (72.4)	106 (68.4)	128 (58.5)	55 (45.5)	0.001
Family history of diabetes	5 (17.2)	27 (17.4)	35 (16.0)	14 (11.6)	0.578
Smoking					
Former	10 (34.5)	32 (20.6)	66 (30.1)	44 (36.4)	
Current	4 (13.8)	32 (20.6)	33 (15.1)	25 (20.7)	0.058
Social class (manual)	13 (44.8)	55 (35.5)	78 (35.6)	51 (42.1)	0.446
BMI (kg/m^2)	24.6 (23.3–25.9)	25.7 (25.1–26.3)	25.5 (25–25.9)	25.0 (24.3–25.6)	0.449
Waist circumference (cm)	80.9 (77.6–84.2)	83.9 (82.5–85.4)	81.9 (80.7–83.1)	80.1 (78.5–81.7)	0.010
Systolic blood pressure (mmHg)	130.7 (124.9–136.5)	129.4 (126.8–131.9)	127.3 (125.2–129.5)	122.8 (119.8–125.74	0.0008
Diastolic blood pressure (mmHg)	78.8 (75.2–82.5)	79.1 (77.5–80.7)	77.8 (76.5–79.2)	74.73 (72.85–76.61)	0.002
Total MET hours per day score	12.5 (8.4–16.6)	12.2 (10.5–13.9)	14 (12.5–15.4)	14.4 (12.5–16.4)	0.090
IGF-1	148.6 (129–168.2)	147.5 (139–156)	165.9 (158.8–173)	161.7 (152–171.3)	0.013
IGFBP-1*	20.7 (16.3–26.4)	22.5 (20.2–25)	24.2 (22.1–26.4)	25.2 (22.4–28.4)	0.075
IGFBP-3	3.81 (3.53–4.1)	3.81 (3.69–3.94)	3.94 (3.84–4.05)	3.98 (3.84–4.13)	0.059
Fasting glucose (mmol/l)	5.72 (5.55–5.9)	5.69 (5.62–5.77)	5.66 (5.6–5.73)	5.57 (5.49–5.66)	0.036
2-h glucose (mmol/l)	5.85 (5.32–6.37)	6.16 (5.93–6.39)	6.02 (5.83–6.21)	6.14 (5.89–6.4)	0.754
Fasting plasma insulin (pmol/l)*	45.1 (37.4–54.4)	39.8 (36.7–43.2)	37.9 (35.4–40.6)	33.5 (30.6–36.8)	0.001
Total cholesterol (mmol/l)	6.43 (6.01–6.85)	6.4 (6.22–6.59)	6.46 (6.3–6.61)	6.52 (6.31–6.72)	0.466
HDL cholesterol (mmol/l)	1.5 (1.37–1.62)	1.43 (1.38–1.49)	1.52 (1.48–1.57)	1.51 (1.45–1.57)	0.098
Fasting triglyceride (mmol/l)*	1.16 (0.99–1.37)	1.17 (1.09–1.25)	1.1 (1.03–1.16)	1.12 (1.03–1.21)	0.343
HOMA-IR index*	1.64 (1.35–2.00)	1.44 (1.32–1.57)	1.37 (1.27–1.47)	1.19 (1.08–1.31)	0.0007
Metabolic syndrome risk z score	0.04 (−0.18 to 0.25)	0.11 (0.02–0.21)	−0.01 (−0.09 to 0.07)	−0.13 (−0.24 to −0.02)	0.003
PTH (μg/l)*	37.7 (32.6–43.5)	30.2 (28.4–32.2)	26.6 (25.2–28.0)	24.3 (22.7–26.1)	1.5E-09
Calcium (mmol/l)	2.12 (2.08–2.16)	2.11 (2.09–2.12)	2.13 (2.12–2.15)	2.15 (2.13–2.17)	0.008

Data are *n* (%) or arithmetic or *geometric mean (95% CI), adjusted for age and sex (except age, adjusted for sex only). *P* values are the test for proportions for categorical variables or the test for linear trend for continuous traits.

TABLE 2
Multiple linear regression analysis of the association between baseline 25(OH)D (nmol/l) and 10-year follow-up metabolic syndrome outcomes: the Ely Study 1990–2000

Outcome per unit increase in baseline 25(OH)D	Model 1		Model 2		Model 3	
	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value
Fasting glucose (mmol/l)	−0.0023 (−0.004 to −0.0004)	0.019	−0.0018 (−0.004 to 0.0002)	0.082	−0.0019 (−0.004 to 0.0002)	0.074
2-h glucose (mmol/l)	−0.0097 (−0.017 to −0.003)	0.006	−0.0094 (−0.017 to −0.002)	0.011	−0.01 (−0.018 to −0.002)	0.013
Fasting insulin (pmol/l)	−0.1467 (−0.258 to −0.035)	0.010	−0.1447 (−0.261 to −0.028)	0.015	−0.1682 (−0.3 to −0.037)	0.012
Fasting triglyceride (mmol/l)	−0.0009 (−0.003 to 0.002)	0.47	−0.0008 (−0.003 to 0.002)	0.52	−0.0009 (−0.003 to 0.002)	0.47
HDL cholesterol (mmol/l)	0.0004 (−0.0005 to 0.001)	0.38	0.0004 (−0.001 to 0.001)	0.40	0.0004 (−0.001 to 0.001)	0.49
Diastolic blood pressure (mmHg)	0.0005 (−0.031 to 0.032)	0.98	0.0016 (−0.031 to 0.034)	0.92	−0.0001 (−0.036 to 0.036)	1
Systolic blood pressure (mmHg)	−0.0006 (−0.051 to 0.05)	0.98	0.0023 (−0.05 to 0.055)	0.93	−0.0067 (−0.065 to 0.052)	0.82
Waist circumference (cm)	0.0028 (−0.023 to 0.028)	0.83	0.0062 (−0.02 to 0.033)	0.65	0.015 (−0.013 to 0.043)	0.3
HOMA-IR index	−0.0059 (−0.01 to −0.002)	0.005	−0.0055 (−0.01 to −0.001)	0.01	−0.0063 (−0.011 to −0.002)	0.009
Metabolic syndrome risk z score	−0.0016 (−0.003 to −0.00001)	0.048	−0.0015 (−0.003 to 0.0002)	0.075	−0.0018 (−0.004 to 0.0001)	0.06

Model 1: adjusted for baseline outcome variable, age, sex, smoking status, season, and BMI. Model 2: adjusted as in model 1 plus PTH, calcium, and IGF-1. Model 3: adjusted as in model 2 plus physical activity score and occupational social class.

metabolic syndrome risk is driven largely by the glucose and insulin component. Additionally, adjusting for IGF-1, PTH, and calcium level (model 2), or including additional adjustment for physical activity score and social class (model 3), made no material difference to the associations between 25(OH)D and 2-h glucose, fasting insulin, and HOMA-IR, but the associations were attenuated, not abolished, for fasting glucose and metabolic syndrome risk z score. Per 25 nmol/l increase in serum 25(OH)D at baseline [which is equivalent to 1 SD of the 25(OH)D distribution], there was a decrease in 10-year follow-up of 0.05 mmol/l in fasting glucose, 0.25 mmol/l in 2-h glucose, 4.2 pmol/l in fasting insulin, 0.16 units in HOMA-IR, and 0.05 units in metabolic syndrome risk z score, in adjusted analyses.

We found no significant association between baseline IGF-1 and 10-year continuously distributed quantitative metabolic trait outcomes. There was no interaction between baseline 25(OH)D and IGF-1 on any of the metabolic outcomes. When testing for the effect of IGF-1 binding proteins, we found a significant interaction between 25(OH)D and IGFBP-1 for the 10-year outcomes of fasting glucose ($P = 0.0005$) and 2-h glucose ($P = 0.029$). There was also a significant interaction between 25(OH)D and BMI on the risk for 10-year HOMA-IR ($P = 0.011$) but not for any other outcome. Figure 2 shows how the inverse association between follow-up fasting and 2-h glucose was modified in participants with values of IGFBP-1 below and above the median (Fig. 2*A–D*). Thus, the decreasing fasting or 2-h glucose levels across increasing categories of 25(OH)D was significant among those with below, but not above, median IGFBP-1. Figure 2 combines data for the two lowest categories of 25(OH)D because of small numbers ($n = 29$) in the vitamin D deficient group, but the results are identical in magnitude and direction if four categories of vitamin D (as in Table 1) are used instead of three (as in Fig. 2).

DISCUSSION

We report, using the Ely population-based prospective study, that baseline vitamin D status [25(OH)D concentration] in nondiabetic participants is inversely associated with glucose status, insulin resistance, and metabolic syndrome risk at the 10-year follow-up. Among these individuals, higher baseline 25(OH)D was associated with significant decreases in 10-year follow-up 2-h glucose, fasting insulin, HOMA index, and metabolic syndrome risk z score in analyses adjusted for a comprehensive range of potential confounders and mediators. These findings appear to be novel, with no previous study we can find of prospective associations between baseline serum 25(OH)D and future risk of disturbed glucose metabolism or metabolic syndrome risk as assessed using continuous quantitative metabolic traits.

Our findings confirm previously reported cross-sectional observations. For instance, among 8,421 U.S. adults, those with metabolic syndrome had lower 25(OH)D concentrations (67.1 nmol/l) than those without (75.9 nmol/l) (6). Among 6,810 Caucasians in the 1958 birth cohort, aged 45 years, serum 25(OH)D was associated inversely with metabolic syndrome risk cross-sectionally, with independent associations for high A1C, blood pressure, and triglycerides after adjustment (7). Although we found an inverse association between baseline serum 25(OH)D and incident continuous metabolic syndrome risk z score, we did not

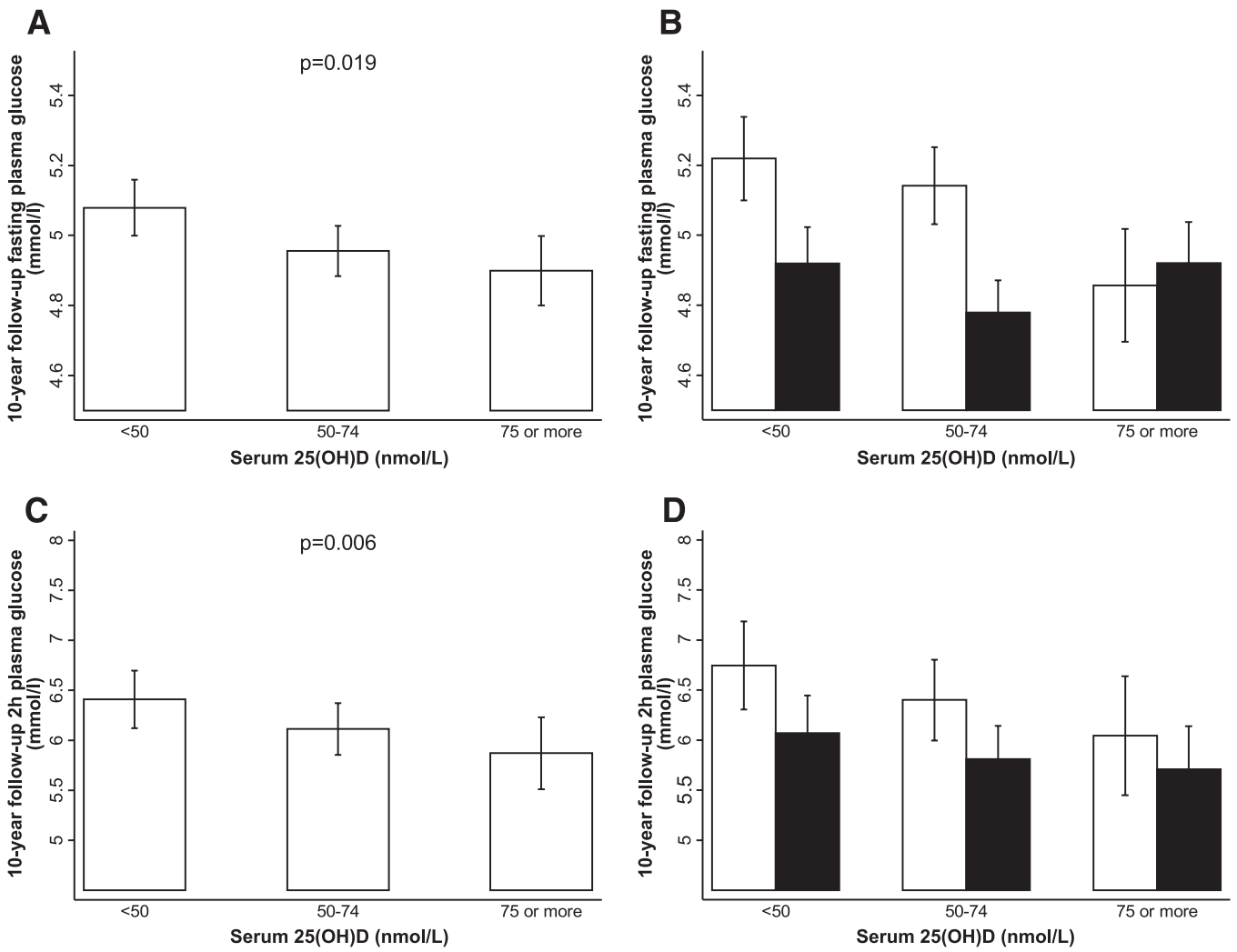

FIG. 2. Association between baseline serum 25(OH)D and 10-year follow-up fasting glucose (*A* and *B*) and 2-h glucose (*C* and *D*). *A* and *C*: The association in the entire cohort. *B* and *D*: The association was modified in participants with below and above median values of IGFBP-1. *B*: □, lower IGFBP-1, $P = 0.0028$; ■, higher IGFBP-1, $P = 0.6802$; IGFBP-1–vitamin D interaction, $P = 0.00047$. *C*: □, lower IGFBP-1, $P = 0.0031$; ■, higher IGFBP-1, $P = 0.4935$; IGFBP-1–vitamin D interaction, $P = 0.0286$. The MRC Ely Study 1990–2000.

observe this association for individual components, such as for blood pressure. The association of blood markers of vitamin D with blood pressure or hypertension is complex, and there are inconsistent findings ranging from no association to inverse or positive association (previously discussed by Scragg et al. [31]). A recent study (32) of the association of plasma 25(OH)D with incident hypertension reported an inverse association, but study limitations included using predicted vitamin D levels and self-reported hypertension rather than actual measurement in some of the included cohorts in that study. Notably, we did report a significant inverse association between serum 25(OH)D and systolic and diastolic blood pressure at baseline but did not find this prospectively. Further study of such associations is warranted in larger cohorts.

While there was evidence of interaction between 25(OH)D and IGF-1 on metabolic syndrome risk in a previous study [metabolic syndrome risk being least when both 25(OH)D and IGF-1 were highest] (7), we found no such interaction for 10 year risk of metabolic syndrome measured as a continuous risk z score, or for any individual metabolic component. We did find significant interaction between baseline IGFBP-1 and 25(OH)D on fasting and 2-h glucose at 10 years when significantly lower glycemia with increasing 25(OH)D was found in those with low, but not high, IGFBP-1. Notably, in the Ely cohort we have previously reported interaction between IGF-1 and IGFBP-1 on 4.5-year follow-up 2-h glucose concentration; those with lower, but not higher, IGFBP-1 having decreasing concentrations of 2-h glucose with increasing tertiles of baseline IGF-1 (19). Since IGF-1 increases with 25(OH)D at baseline in our cohort, as in the 1958 birth cohort (7), this may explain our 4.5-year findings. Our present 10-year follow-up data suggest, as previously reported (17,18), that biological interactions between IGFBP-1 axis and 25(OH)D are potentially important in glucose homeostasis long term and that IGF-1 axis and vitamin D axis interactions should be studied further. For example, IGFBP-1 concentrations may reflect variations in insulin secretion or hepatic insulin sensitivity, important components in glucose regulation (33).

Studies are emerging on vitamin D supplementation and glucometabolic risk. A post hoc analysis within a trial designed for bone health reported that calcium and vitamin D supplementation had no effect on fasting glucose or HOMA-IR over 3 years in 314 individuals (34). This study did, however, find that among those with impaired fasting glucose (but not with normoglycemia), there was a modest

attenuation of the rise over time in fasting glucose and in HOMA-IR (*P* for treatment vs. placebo, 0.042 and 0.031, respectively) (34). That study measured serum 25(OH)D in a subset, which increased with intervention, but did not report on its associations with the metabolic outcomes. Since dietary intake, including supplementation, provides little vitamin D and sunlight exposure providing the major source through skin synthesis, it is important that further studies include assessment of individual vitamin D status [serum 25(OH)D concentration]. A recent larger, clinical trial (23) of vitamin D plus calcium supplementation (400 IU and 1,000 mg, respectively, daily versus placebo) in ~34,000 nondiabetic women (the Women's Health Initiative Study) also included 2,020 women for whom repeat measures of fasting glucose and fasting insulin were available. There was no significant effect of supplementation on these metabolic parameters at 3 or 6 years of follow-up. Notably, there was also no significant association with incident diabetes risk over 7 years (hazard ratio 1.01 [95% CI 0.94–1.10], with 2,291 cases of incident type 2 diabetes). However, the independent effect of vitamin D could not be assessed as the supplementation was in combination with calcium, and, as the authors suggest, the dose of vitamin D given (400 IU) was modest and likely to be inadequate (23). Furthermore, since dietary intake is a poor source of vitamin D, compared with sunlight-induced endogenous skin production, such studies require serum 25(OH)D data as well, in at least a representative subsample.

What mechanisms may explain the associations of lower serum vitamin D status with risks of greater levels of glycemia and insulin resistance we have observed? Possibilities include direct effects of vitamin D on pancreatic β-cell secretory function through their nuclear VDRs, effects on insulin sensitivity through stimulation of insulin receptor expression regulation of intracellular calcium since an effector part of the vitamin D pathway is the vitamin D–dependent calcium-binding protein required for postinsulin receptor effects in insulin-responsive tissues, and also indirect effects through inflammatory processes (35,36). Furthermore, inadequate vitamin D usually leads to increased serum PTH, which in turn has been found to be inversely associated with insulin sensitivity in healthy adults (37). Finally, *VDR* gene polymorphisms have been associated with variation in insulin secretion among a British Bangladeshi South Asian population (14,16) and in glycemia among community-based older American adults (38), and other gene polymorphisms may affect glucose metabolism, as recently reviewed (39).

Limitations of our study include that it was of modest size, and the included cohort with complete data comprised ~50% of the initial cohort, which could have potentially biased our results. However, when comparing baseline characteristics among included and excluded participants, included participants tended to be younger and healthier (lower blood pressure, lower BMI, lower fasting and 2-h glucose, and lower fasting insulin) than nonparticipants. This is in keeping with the frequently observed "healthy participant effect" in epidemiological studies, which, if anything, would lead to a more conservative estimate of association. On an a priori basis, we did not study the association with clinical outcomes such as diabetes, as we had inadequate statistical power (<20% power with 54 incident events of diabetes) but restricted our outcomes to continuous quantitative traits. While we could study association with future glucose concentration, we could not examine association with A1C, a longer-term marker of glycemic status, as we did not measure A1C at baseline in our study. While we measured baseline serum 25(OH)D, we do not have data for sun exposure (including holidays in the sun), for supplement use, nor any interval measures of vitamin D status. We have, however, previously found remarkably close correlations between measures of vitamin D status for each month over 2–3 years in British South Asians from east London (Boucher BJ, Mannan N, and Noonan K, unpublished data). Though stability of vitamin D status across the years is unknown for European Caucasians, as in our study, it is likely to be similar in view of the regularity of seasonal variations over time. Our findings, valid within a population of European Caucasians, cannot be extrapolated to other ethnic groups, given the previously reported heterogeneity by ethnicity (10). Despite these limitations, strengths of our study include the population-based nature of the study; the use of a direct, objective measure of vitamin D status, rather than relying on self-reported vitamin D intake or sunlight exposure; and the comprehensive range of quantitative metabolic markers (at baseline and at 10-year follow-up), including glucose measurement with oral glucose tolerance tests, fasting insulin, and derived markers of insulin resistance (HOMA-IR) and a continuous metabolic syndrome risk z score. Furthermore, we have been able to adjust our analyses for a robust range of potential confounders and mediators, including PTH and calcium status (rarely included in other studies of such associations), as well as for smoking, physical activity, season, obesity, social class, age, and sex, which increases confidence in the independence of the observed associations. However, we acknowledge that residual confounding from measured and unmeasured factors cannot be excluded. Our data also demonstrate that mean serum 25(OH)D concentrations were <75 nmol/l (hypovitaminosis D) for 10 months of the year and <50 nmol/l for 4 months of the year (vitamin D insufficiency) in this cohort. There is evidence of a high prevalence of hypovitaminosis D worldwide (40), and our data are indicative of the chronically inadequate vitamin D repletion common to northern latitudes (41). This widespread problem, combined with our finding that higher vitamin D status [25(OH)D] may have a beneficial effect on future glucometabolic risk profile, raises a matter of public health importance deserving serious further investigation.

In conclusion, we have demonstrated inverse associations of baseline serum vitamin D concentration with future glucose levels and insulin resistance in a prospective population-based study, such that higher baseline vitamin D is associated with significantly lower future glucose, insulin, and HOMA-IR. These associations are independent of risk factors and potential confounders and are potentially important in understanding the etiology of metabolic disturbances associated with type 2 diabetes. Despite mounting evidence linking inadequate vitamin D repletion with abnormalities of glucose and insulin metabolism, the role of vitamin D is not fully understood and deserves further investigation in both larger, specifically designed prospective studies and with randomized controlled trials of supplementation. Demonstration of a causal role for hypovitaminosis D in these disorders would lead to new targets for efforts to prevent type 2 diabetes at the population level and, possibly, for its treatment.

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