# Prospective Associations of Vitamin D With β-Cell Function and Glycemia

## The PROspective Metabolism and ISlet cell Evaluation (PROMISE) Cohort Study

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**OBJECTIVE**—To examine the prospective associations of baseline vitamin D [25-hydroxyvitamin D; 25(OH)D] with insulin resistance (IR), β-cell function, and glucose homeostasis in subjects at risk for type 2 diabetes.

**RESEARCH DESIGN AND METHODS**—We followed 489 subjects, aged 50 ± 10 years, for 3 years. At baseline and follow-up, 75-g oral glucose tolerance tests (OGTTs) were administered. IR was measured using the Matsuda index ($IS_{OGTT}$) and the homeostasis model assessment of IR (HOMA-IR), β-cell function was determined using both the insulinogenic index divided by HOMA-IR (IGI/IR) and the insulin secretion sensitivity index-2 (ISSI-2), and glycemia was assessed using the area under the glucose curve ($AUC_{glucose}$). Regression models were adjusted for age, sex, ethnicity, season, and baseline value of the outcome variable, as well as baseline and change in physical activity, vitamin D supplement use, and BMI.

**RESULTS**—Multivariate linear regression analyses indicated no significant association of baseline 25(OH)D with follow-up $IS_{OGTT}$ or HOMA-IR. There were, however, significant positive associations of baseline 25(OH)D with follow-up IGI/IR (β = 0.005, $P$ = 0.015) and ISSI-2 (β = 0.002, $P$ = 0.023) and a significant inverse association of baseline 25(OH)D with follow-up $AUC_{glucose}$ (β = −0.001, $P$ = 0.007). Progression to dysglycemia (impaired fasting glucose, impaired glucose tolerance, or type 2 diabetes) occurred in 116 subjects. Logistic regression analyses indicated a significant reduced risk of progression with higher baseline 25(OH)D (adjusted odds ratio 0.69 [95% CI 0.53–0.89]), but this association was not significant after additional adjustment for baseline and change in BMI (0.78 [0.59–1.02]).

**CONCLUSIONS**—Higher baseline 25(OH)D independently predicted better β-cell function and lower $AUC_{glucose}$ at follow-up, supporting a potential role for vitamin D in type 2 diabetes etiology.
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Emerging evidence suggests that vitamin D [25-hydroxyvitamin D; 25(OH)D] may play a role in the etiology of type 2 diabetes (1,2). Vitamin D levels are lower in those with type 2 diabetes and impaired glucose tolerance (IGT) compared with those with normal glucose tolerance (NGT) (3–5). In addition, most (6–9), but not all (10,11), prospective studies have shown a significant inverse association of baseline serum 25(OH)D with incident diabetes. To date, however, the exact mechanisms through which vitamin D affects diabetes risk are not yet fully known, particularly whether vitamin D plays a role in insulin resistance (IR) and/or β-cell dysfunction, the main pathophysiological disorders underlying type 2 diabetes.

Previous studies have reported significant inverse associations of vitamin D with IR (12–16) and β-cell dysfunction (17,18), including our cross-sectional study in the current cohort (19), although findings have been inconsistent (14,16,20–22). These inconsistencies may be attributed to the cross-sectional design of these studies or the use of less precise measures of outcomes. Only two prospective studies have been conducted to date, both of which reported significant inverse associations of baseline 25(OH)D with IR after 5 and 10 years of follow-up, respectively, in largely white cohorts (6,23). No study has yet examined β-cell function prospectively in relation to vitamin D. The objective of this study, therefore, was to examine the prospective association of baseline serum 25(OH)D with IR, β-cell function, and glucose homeostasis in a cohort of 489 subjects at high risk for type 2 diabetes.

## RESEARCH DESIGN AND METHODS

A detailed methodology of the PROspective Metabolism and ISlet cell Evaluation (PROMISE) cohort study has been published previously (19,24). In brief, PROMISE study participants, aged ≥30 years, were recruited from Toronto and London, Ontario, Canada, between May 2004 and December 2006. Participants were at high risk for type 2 diabetes, as they were recruited on the basis of the presence of one or more risk factors for diabetes, including obesity, hypertension, a family history of diabetes, and/or a history of gestational diabetes or birth of a macrosomic infant (24). Participants were contacted annually after the baseline visit to update contact information and collect data on major health events. Participants were invited to return to the clinic examination centers after 3 years for follow-up assessments.

At baseline, 654 individuals without diabetes participated; the mean age was 50.19 ± 9.67 years, 357 (73.01%) were female, and 142 (29%) were nonwhites (12% Hispanic, 7% South Asian, and 10% other). Of 654 participants, contact was maintained with 549 (84%), and 496 (76%) participants attended

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the 3-year follow-up clinic visits. Those who attended the follow-up examination were more likely to be older, female, and white ($P < 0.02$) than those who did not attend, but there were no significant differences in BMI or measures of IR, β-cell function, or glucose homeostasis ($P \geq 0.07$).

**Measures.** As part of the baseline and 3-year clinic assessment, fasting blood samples were collected and 75-g oral glucose tolerance tests (OGTTs) were conducted, with additional blood samples collected at 30 and 120 min for glucose and insulin measurements. Fresh fasting and 30- and 120-min blood samples were immediately processed for the determination of serum glucose, and remaining samples were processed and frozen at −70°C. Glucose was measured using an enzymatic hexokinase method on the Roche Modular platform. Specific insulin was measured using the Elecsys 1010 immunoassay analyzer (Roche Diagnostics, Basel, Switzerland) and the electrochemiluminescence immunoassay. This assay shows 0.05% cross-reactivity to intact human proinsulin and the Des 31,32 circulating split form. Parathyroid hormone (PTH) was measured using an electrochemiluminescence immunoassay on the Roche Modular E170 Analyzer (Laval, Quebec, Canada), which has a detection range from 0.127 to 530 pmol/L. Baseline C-reactive protein also was measured in fasting samples using Roche Modular's particle-enhanced immunoturbidimetric assay, with a minimum detection range of 0.03 mg/L.

Vitamin D status, specifically 25(OH)D, was measured in serum using the DiaSorin 25 OH Vitamin D TOTAL competitive chemiluminescent immunoassay on the automated LIAISON Analyzer (Stillwater, MN). This assay has 100% specificity for both $25(OH)D_2$ and $25(OH)D_3$. The detection limit of the assay is 10 nmol/L, and we found that it has an intra-assay coefficient of variation of 6.7% and an interassay coefficient of variation of 11.6%. The 25(OH)D TOTAL method has been validated against the DiaSorin radioimmunoassay ($r = 0.92$), which is the first test approved for clinical diagnosis by the Food and Drug Administration and also is the most widely used method (25). In addition, the laboratory in which this assay was conducted participates in the International External Quality Assessment Scheme for Vitamin D Metabolites (DEQAS, Northwest Thames, U.K.), and it has been reported that the 25(OH)D results from this laboratory were consistently within 1 SD of the group mean in the international DEQAS proficiency surveys (25).

Anthropometric measurements were performed with participants in light clothing and with shoes removed. Height and weight were measured to the nearest 10th of a cm and kg, respectively, and BMI was calculated as weight in kilograms divided by the square of height in meters ($kg/m^2$). Waist circumference was measured at the natural waist, defined as the narrowest part of the torso, as viewed from behind, or the minimal circumference between the umbilicus and xiphoid process, as viewed from the front. Blood pressure was measured twice in the right arm with the subject seated after a 5-min rest using an automated sphygmomanometer. Each measure was determined twice using standardized procedures, with the average used in the analysis. Physical activity level was determined using a version of the Modifiable Activity Questionnaire (MAQ) (26), which collects information on both leisure and occupational activity over the past year (including measures of frequency and duration). The MAQ has been shown to have good reliability and validity (26). Each reported activity from the MAQ is weighted by its relative intensity, referred to as a metabolic equivalent (MET) of the task, thereby deriving MET hours per week (MET h/week) as the final unit of expression. Season was defined using the date participants completed their baseline assessment and was categorized as follows: May through October (summer/early fall); November through April (winter/early spring). Supplement use, specifically any vitamin or multivitamin containing vitamin D, was obtained through an open-ended question on current medication use. Ethnicity, smoking, and the participant's family history of diabetes were assessed using structured questionnaires.

**Outcome variables.** IR was quantified using both the $IS_{OGTT}$ index of Matsuda and DeFronzo (27) and the homeostasis model assessment of IR (HOMA-IR) of Matthews et al. (28). The $IS_{OGTT}$ index, which is a measure of insulin sensitivity (27), is defined as follows: $10{,}000/\sqrt{(FPG \times FPI) \times (G \times I)}$, where FPG refers to fasting plasma glucose, FPI refers to fasting plasma insulin, G refers to mean glucose during the OGTT, and I refers to mean insulin during the OGTT. This index reflects whole-body insulin sensitivity and has been validated against the euglycemic-hyperinsulinemic clamp technique (27). HOMA-IR, which is defined as FPG × FPI/22.5 (28), largely reflects hepatic IR and also has been validated against the clamp (28). β-Cell dysfunction was calculated by taking the insulinogenic index (IGI) divided by HOMA-IR (IGI/IR) (29), which is a widely used measure of β-cell function. The IGI is calculated by taking the ratio of 30 min insulin minus fasting insulin to 30 min glucose minus fasting glucose (29) and has been validated against gold-standard measures of insulin secretion (first-phase insulin secretion on intravenous glucose tolerance testing [IVGTT]). The insulin secretion sensitivity index-2 (ISSI-2), which is a more recently proposed measure of β-cell function that is analogous to the disposition index but derived from the OGTT (30), also was calculated. This index, which has been validated against directly measured disposition index (31), is defined as the ratio of the area under the insulin curve ($AUC_{insulin}$) to the area under the glucose curve ($AUC_{glucose}$), multiplied by $IS_{OGTT}$ (31). Glycemia was assessed using the $AUC_{glucose}$ during the OGTT, calculated using the trapezoidal rule. Progression to dysglycemia was defined as having developed impaired fasting glucose (IFG), IGT, or incident type 2 diabetes based on the OGTT at the 3-year follow-up. IFG, IGT, and diabetes were categorized using 1999 World Health Organization criteria (32). In addition, participants who reported being diagnosed with type 2 diabetes between baseline and follow-up during either the annual telephone contacts or the clinical examinations also were considered to have incident diabetes at follow-up. Verification of self-reported diabetes was obtained from the participant's physician through a supplementary form requesting information on the date of diagnosis, blood glucose levels supporting the diagnosis, and current treatment.

**Statistical analysis.** SAS version 9.1 was used for all analyses. Continuous variables were reported as means ± SD or median (interquartile range) in the case of skewed distributions, whereas categorical variables were reported as *n* (%). Natural logarithmic transformations were applied for all non–normally distributed variables. $\chi^2$ Tests and Student *t* tests were used to examine differences between those who did and did not attend the 3-year follow-up examination. Changes in participant characteristics between baseline and follow-up were tested using the McNemar test for categorical variables and the paired Student *t* test or Wilcoxon signed-rank test for normally distributed or non–normally distributed continuous variables, respectively. Percentage change was calculated as the follow-up value minus the baseline value divided by the baseline value, multiplied by 100. Multiple linear regression analyses were conducted to investigate the association of baseline serum 25(OH)D as the independent variable with measures of IR ($IS_{OGTT}$ and HOMA-IR), β-cell function (IGI/IR and ISSI-2), and glycemia ($AUC_{glucose}$) at the 3-year follow-up as the dependent variables. Separate models were used for each outcome variable, which included adjustment for the baseline value of the outcome measure being assessed. Differences in baseline serum 25(OH)D, according to glycemic progression status (NGT vs. IFG, IGT, or type 2 diabetes at follow-up), were evaluated using *t* tests. The association of baseline serum 25(OH)D with progression to dysglycemia was assessed using multivariate logistic regression analysis. Odds ratios are presented to indicate the risk of progression to dysglycemia per SD increase in 25(OH)D. Potential confounders were identified on the basis of the results of previous cross-sectional analyses in the PROMISE study cohort (19). Significant positive associations of baseline serum 25(OH)D levels with age, vitamin D supplement use, and physical activity and negative associations of 25(OH)D with BMI and waist circumference were documented. On the basis of these findings, staged multivariate regression models were constructed for the current analysis. Model 1 was adjusted for age, sex, ethnicity, and season of the 25(OH)D measurement; model 2 was additionally adjusted for baseline and change in physical activity and vitamin D supplement use; and model 3 was additionally adjusted for baseline and change in BMI. Possible effect modifiers including sex, ethnicity, BMI, and season also were investigated.

## RESULTS

Baseline vitamin D measurements were available for 489 (99%) of 496 participants who came back for their follow-up visit. Of 489 participants, 116 (23.72%) progressed to dysglycemia, of which 11 (2.25%) had IFG, 75 (15.34%) had IGT, and 30 (6.13%) were classified as having type 2 diabetes. Of 30 participants with diabetes at follow-up, 6 were diagnosed between baseline and follow-up and the remaining 24 were classified as having diabetes on the basis of the OGTT at the 3-year clinic assessment. Participant characteristics at baseline and at follow-up, as well as percentage change in these characteristics over the follow-up period, are presented in Table 1. The mean baseline serum 25(OH)D concentration was 58.01 ± 23.26 nmol/L. Based on the Institute of Medicine's 2011 Dietary Reference Intakes for Vitamin D (33), we found that ~11, 22, and 63% of our cohort had deficient (<30 nmol/L), insufficient (<40 nmol/L), and

TABLE 1
Participant characteristics at baseline and at the 3-year follow-up

| Variable | Baseline | Follow-up | Δ | % Change | *P* |
|---|---|---|---|---|---|
| Vitamin D (nmol/L) | 58.01 ± 23.26 | | | | |
| PTH (pmol/L) | 4.55 ± 1.68 | | | | |
| Anthropometry | | | | | |
| Weight (kg) | 85.75 ± 19.70 | 86.27 ± 19.67 | 0.9 (−2.15 to 4.20) | 1.06 | 0.0003 |
| BMI (kg/m$^2$) | 30.33 (26.72–34.57) | 30.43 (26.93–34.58) | 0.39 (−0.70 to 1.56) | 1.23 | <0.0001 |
| Waist circumference (cm) | 98.43 ± 15.43 | 99.12 ± 15.60 | 0.50 (−3.00 to 4.70) | 0.93 | 0.0199 |
| Physical activity (MET h/week) | 19.59 (7.39–53.52) | 23.13 (9.62–59.66) | 1.56 (−10.92 to 15.29) | 3.94 | 0.1087 |
| Smoking (% current) | 30 (6.29) | 25 (5.13) | −5 (1.16) | −16.67 | 0.09 |
| Blood pressure | | | | | |
| Systolic blood pressure (mmHg) | 125.92 ± 16.03 | 125.91 ± 15.02 | 0.00 (−7.5 to 9.5) | 0.80 | 0.79 |
| Diastolic blood pressure (mmHg) | 80.16 ± 10.32 | 80.17 ± 10.10 | 1.00 (−5.5 to 6.5) | 0.86 | 0.76 |
| Vitamin D supplement use | 212 (43.35) | 262 (53.58) | 50 (10.23) | 24.04 | <0.0001 |
| Fasting glucose (mmol/L) | 4.95 ± 0.53 | 5.20 (4.8–5.6) | 0.30 (0.0–0.6) | 6.90 | <0.0001 |
| 2-h glucose (mmol/L) | 5.72 ± 1.37 | 6.10 (5.1–7.6) | 0.65 (−0.45 to 1.80) | 11.87 | <0.0001 |
| Insulin sensitivity | | | | | |
| $IS_{OGTT}$ index | 13.45 (8.52–20.79) | 11.54 (6.89–18.85) | −1.65 (−5.64 to 1.62) | −16.13 | <0.0001 |
| IR | | | | | |
| HOMA-IR | 1.88 (1.19–3.09) | 2.27 (1.41–3.76) | 0.34 (−0.25 to 1.00) | 21.42 | <0.0001 |
| β-Cell function | | | | | |
| IGI/IR | 9.55 (5.43–14.94) | 7.41 (4.49–13.70) | −1.30 (−4.62 to 1.53) | −20.80 | <0.0001 |
| ISSI-2 | 727.49 (568.74–907.48) | 613.51 (493.85–823.69) | −93.31 (−219.93 to 27.72) | −14.44 | <0.0001 |
| $AUC_{glucose}$ | 13.77 ± 2.29 | 14.82 ± 3.24 | 0.83 (−0.5 to 2.5) | 5.76 | <0.0001 |

Data are *n* (%) for categorical variables, means ± SD for continuous variables, or median (25 and 75% interquartiles) for non–normally distributed variables. Data are for all participants at follow-up with a baseline serum 25(OH)D measurement ($n = 489$). Tests of significance are the McNemar test for categorical variables, the paired Student *t* test for normally distributed variables, and the Wilcoxon signed-rank test for non–normally distributed variables. Percentage change was calculated as ([{follow-up – baseline}/baseline] × 100).

sufficient (≥50 nmol/L) 25(OH)D levels, respectively. Overall, participants gained weight over the 3 years and also reported significantly higher vitamin D supplement use at follow-up. In addition, the significant decrease in $IS_{OGTT}$ and significant increase in HOMA-IR over the 3 years indicate worsening IR. There also was a significant decrease in both IGI/IR and ISSI-2 and a significant increase in $AUC_{glucose}$, indicating that participants also had deteriorating β-cell function and glucose homeostasis over the follow-up period.

In the multivariate linear regression analyses (Table 2), baseline serum 25(OH)D was not significantly associated with follow-up $IS_{OGTT}$ in model 1 ($\beta = 0.002$, $P = 0.12$) and remained nonsignificant with additional covariate adjustment. Although an initial significant inverse association of baseline 25(OH)D with follow-up HOMA-IR was observed after adjustment for age, sex, ethnicity, season of the 25 (OH)D measurement, baseline HOMA-IR, and baseline and change in physical activity and supplement use, these findings were attenuated to nonsignificance after additional adjustment for baseline and change in BMI ($\beta = -0.001$, $P = 0.32$). In contrast, there was a significant positive association of baseline serum 25(OH)D with both measures of β-cell function at follow-up ($\beta = 0.005$, $P = 0.015$, and $\beta = 0.002$, $P = 0.023$, for IGI/IR and ISSI-2, respectively). There also was a significant inverse association of baseline 25 (OH)D with follow-up $AUC_{glucose}$ ($\beta = -0.001$, $P = 0.007$). Additional adjustment for serum PTH in these multivariate analyses did not significantly change the results (data not shown). A sensitivity analysis excluding subjects with diabetes at follow-up ($n = 30$) yielded similar findings for the above associations (data not shown). In addition, no significant interaction between 25(OH)D and sex, ethnicity, BMI, or season was found in any of the regression models ($P \geq 0.07$).

Baseline serum 25(OH)D according to glycemic progression status is described in Fig. 1. Those who remained normal glucose tolerant ($n = 352$) or regressed to NGT ($n = 6$) at follow-up had significantly higher serum 25(OH)D levels compared with those who were dysglycemic at follow-up ($n = 131$) (59.84 ± 23.07 nmol/L vs. 53.03 ± 23.16 nmol/L, respectively, $P = 0.0041$). Multivariate logistic regression analyses indicated a significant reduced risk of progression to dysglycemia per SD increase in baseline serum 25(OH)D after adjustment for age, sex, ethnicity, season, and baseline and change in both physical activity and vitamin D supplement use (Fig. 2). However, this association was attenuated with additional adjustment for baseline and change in BMI (odds ratio 0.78 [95% CI 0.59–1.02]). In addition, results were essentially the same in sensitivity analyses additionally adjusting for PTH or for family history of type 2 diabetes and baseline C-reactive protein.

## DISCUSSION

The current study found that baseline vitamin D status was an independent predictor of better β-cell function and $AUC_{glucose}$ after 3 years of follow-up in the PROMISE study cohort. This is the first study to examine the prospective association of serum 25(OH)D with β-cell function.

Previous studies assessing the association between 25(OH)D and β-cell function have used cross-sectional designs and have reported inconsistent findings (14,16–21,34). These inconsistencies may be attributed to the use of less-detailed, fasting-based surrogate measures of β-cell function (e.g., HOMA-β or C-peptide) in the majority of

TABLE 2
Multiple linear regression analysis of associations of baseline 25(OH)D (nmol/L) with measures of insulin sensitivity/resistance and β-cell function and $AUC_{glucose}$ at the 3-year follow-up

| Outcome per unit increase in baseline 25(OH)D | Model 1 | | | Model 2 | | | Model 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | β (95% CI) | *P* | $R^2$ | β (95% CI) | *P* | $R^2$ | β (95% CI) | *P* | $R^2$ |
| IS-OGTT* | 0.002 (−0.0004 to 0.004) | 0.12 | 0.49 | 0.002 (−0.0006 to 0.004) | 0.16 | 0.50 | 0.001 (−0.001 to 0.003) | 0.34 | 0.62 |
| HOMA-IR* | −0.003 (−0.005 to −0.0006) | 0.014 | 0.36 | −0.003 (−0.005 to −0.0003) | 0.028 | 0.37 | −0.001 (−0.003 to 0.001) | 0.32 | 0.53 |
| IGI/IR* | 0.007 (0.003–0.010) | 0.0003 | 0.18 | 0.007 (0.003–0.010) | 0.0006 | 0.18 | 0.005 (0.0009–0.008) | 0.015 | 0.29 |
| ISSI-2* | 0.002 (0.0004–0.003) | 0.014 | 0.36 | 0.002 (0.0005–0.004) | 0.009 | 0.36 | 0.002 (0.0003–0.003) | 0.023 | 0.44 |
| $AUC_{glucose}$* | −0.001 (−0.002 to −0.0005) | 0.0010 | 0.40 | −0.001 (−0.002 to −0.0004) | 0.0022 | 0.40 | −0.001 (−0.002 to −0.0003) | 0.007 | 0.45 |

Model 1: adjusted for age, sex, ethnicity, season of 25(OH)D measurement, and baseline outcome variable. Model 2: adjusted as in model 1 plus baseline physical activity, change in physical activity, baseline vitamin D supplement use, and change in vitamin D supplement use. Model 3: adjusted as in model 2 plus baseline BMI and change in BMI. *Log transformations.




**FIG. 1. Baseline serum 25(OH)D by glycemic progression status at follow-up. DM, type 2 diabetes; Pre-DM refers to IFG or IGT. None of the pairwise associations were statistically significant.**

previous studies (16–18,20,34). Only two studies have used gold-standard methods, including the hyperglycemic clamp (14) and the IVGTT (21), but no significant association between 25(OH)D and β-cell function was found after adjusting for potential confounders. In contrast, the findings in the current study support our cross-sectional results in the PROMISE study cohort (19), in which OGTT-based measures of β-cell function, specifically IGI/IR and ISSI-2, were significantly associated with 25(OH)D. Other cross-sectional studies also have reported a similar significant association of 25(OH)D with β-cell function (17,18). In addition to the observational literature, a limited number of intervention studies have examined the effect of vitamin D supplementation on measures of β-cell function (17,34–40); these studies have similarly yielded inconsistent results. However, most studies included small sample sizes, a short duration of intervention, variation in vitamin D doses, and surrogate measures of β-cell function, including C-peptide and HOMA-β, both of which on their own do not account for background IR. Three previous intervention studies (35,37,38) used gold-standard IVGTT-based measures of insulin secretion, with one study reporting that supplementation of 1,332 IU vitamin $D_3$ per day resulted in increased first-phase insulin secretion (35). However, given that this study was not a randomized controlled trial, that it included only 10 study participants, and that the IVGTTs were not performed according to standard procedures, their finding should be interpreted with caution. The remaining two studies using IVGTT (37,38) found no effect of supplementation with a synthetic analog of the active vitamin D metabolite, calcitriol [i.e., 1,25(OH)D], on insulin secretion in subjects with IGT. It is clear that current evidence is limited and inconsistent regarding the association of 25(OH)D with β-cell function.

In contrast to the findings regarding β-cell function, the current study did not find a significant association of baseline 25(OH)D with follow-up measures of IR. Most cross-sectional studies have found significant inverse associations between 25(OH)D and IR (12–16,22,41,42),

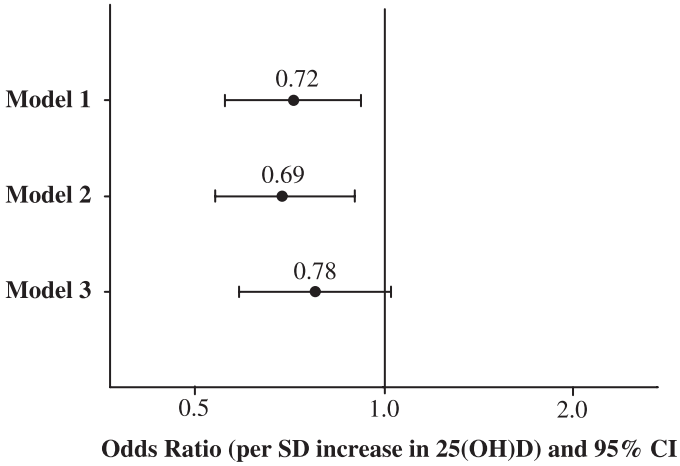


**FIG. 2. Multivariate logistic regression analysis of associations of baseline 25(OH)D with progression to dysglycemia at follow-up. Model 1: adjusted for age, sex, season, and ethnicity. Model 2: adjusted as in model 1 plus baseline physical activity, change in physical activity, baseline vitamin D supplement use, and change in vitamin D supplement use. Model 3: adjusted as in model 2 plus baseline BMI and change in BMI.**

including our recent study in the PROMISE study cohort (19). However, some studies have reported no association (20–22,43). In addition, only two prospective studies have been conducted to date (6,23). Forouhi et al. (23) reported a significant inverse association of baseline serum 25(OH)D with HOMA-IR after 10 years of follow-up in white subjects from the U.K. More recently, Gagnon et al. (6) found a significant positive association of baseline 25(OH)D with insulin sensitivity (HOMA-S) at 5 years in adults participating in the Australian Diabetes, Obesity, and Lifestyle Study. Likewise, we also report an initial significant inverse association of baseline 25(OH)D with follow-up HOMA-IR in the current study, which was attenuated to nonsignificance after adjustment for obesity. Given that our study population is more obese than the populations in these previous studies, it is possible that obesity was a stronger determinant of IR than 25(OH)D in this population.

The current study also found a significant inverse association of baseline serum 25(OH)D with $AUC_{glucose}$ at follow-up, indicating that those with higher baseline 25(OH)D had significantly better glucose homeostasis during the follow-up OGTT, even after adjusting for baseline $AUC_{glucose}$. Most previous studies have reported significant inverse associations of 25(OH)D with various continuous measures of glycemia, including fasting or 2-h glucose during the OGTT (14,16,22,43,44). Forouhi et al. (23) also reported a significant inverse association of baseline 25(OH)D with 2-h OGTT glucose, but not fasting glucose, after 10 years of follow-up, with multivariate adjustment.

In addition to the continuous outcome measures assessed, this study also examined the association between baseline serum 25(OH)D and the risk of progression to dysglycemia at follow-up. The initial multivariate logistic regression analyses indicated a significant reduced risk of progression to dysglycemia with greater baseline 25(OH)D, but this association was attenuated to nonsignificance with additional adjustment for BMI in model 3. Although there is limited evidence, most previous studies have found an inverse association between vitamin D and diabetes risk (7–9,45–47), but negative findings also have been reported (10,11,48). As was documented in the current study, some investigators also have reported attenuation of an initial significant association after BMI adjustment (10,11), but most previous studies have reported a significant association between 25(OH)D and diabetes even after accounting for body composition (6–9,45,47). Vitamin D is a fat-soluble vitamin, and the consistently observed inverse association between 25(OH)D and adiposity is thought to be largely a result of the sequestering of 25(OH)D in adipose tissue, where it is no longer bioavailable (49). PROMISE study participants are primarily overweight or obese, with 72.8% having a BMI $\geq$27 kg/m$^2$, and thus the sequestering effect of adipose tissue on vitamin D bioavailability is one potential explanation for the nonsignificant association with dysglycemia, after BMI adjustment in this cohort. However, given that the current study did find a significant prospective association of baseline 25(OH)D with β-cell function and continuously measured glycemia, increased power provided through a longer follow-up duration may be needed to detect a significant association of 25(OH)D with risk of progression to dysglycemia.

The current study has a number of potential limitations. First, only baseline 25(OH)D was collected, and an additional serum 25(OH)D measurement at follow-up to examine the effect of longitudinal changes in 25(OH)D on the outcome measures would have strengthened the study. Second, no information on diet was collected, but we did have information on participants' vitamin D supplement use, which is an important contributor to 25(OH)D levels. In addition, gold-standard measures of IR and β-cell function were not used because these procedures are costly and invasive and therefore not feasible for large epidemiological studies. However, the current study used extensively validated proxy measures to determine IR and β-cell dysfunction based on glucose and insulin values from multiple time points in the OGTT. In addition, we did not have glucose data for 60 and 90 min during the OGTT, which would have allowed for increased accuracy in the calculation of $AUC_{glucose}$. It also is important to note possible bias in our results given that those who returned for the follow-up clinic visit were more likely to be older, female, and white than those who did not return. However, we did adjust for these variables in our multivariate analyses. Last, because this was an observational study, residual confounding is possible because unmeasured confounders may impact the association of serum 25(OH)D with the outcomes. Strengths of this study include its prospective design, which allows for the temporality of the associations to be observed. In addition, the current study examined a multiethnic cohort, whereas most previous studies have focused solely on white populations. Examining non-white populations is valuable, considering these individuals are at high risk for type 2 diabetes and are known to have low 25(OH)D concentrations. The current study also included the direct measurement of serum 25(OH)D, versus reliance on diet and sun-exposure data. In addition, multivariate analyses were adjusted for numerous potential confounders, including vitamin D supplement use, which has been excluded in most previous studies (14,16,20,23).

In conclusion, the current study found that higher baseline 25(OH)D independently predicted better β-cell function and lower $AUC_{glucose}$ after 3 years of follow-up, even after

adjustment for baseline β-cell function and $AUC_{glucose}$, respectively. Higher 25(OH)D levels also were associated with a reduced risk of progressing to dysglycemia, although this association was not statistically significant after adjustment for obesity (adjusted odds ratio 0.78 [95% CI 0.59–1.02]). Longer follow-up of this cohort may reveal a significant inverse association of 25(OH)D with risk of type 2 diabetes. These results support a potential role for vitamin D in the etiology of type 2 diabetes.

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S.K. researched data; wrote, reviewed, and edited the manuscript; and contributed to discussion. R.R. reviewed and edited the manuscript and contributed to discussion. S.B.H. researched data, reviewed and edited the manuscript, and contributed to discussion. R.V., J.A.K., H.C.G., and B.A.P. reviewed and edited the manuscript and contributed to discussion. B.Z. and A.J.H. researched data, reviewed and edited the manuscript, and contributed to discussion.

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