

# Decreased Serum Concentrations of 25-Hydroxycholecalciferol Are Associated With Increased Risk of Progression to Impaired Fasting Glucose and Diabetes

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**OBJECTIVE**—To study the association between vitamin D status and the risk of incident impaired fasting glucose (IFG) and diabetes in a population-based cohort of diabetes-free subjects.

**RESEARCH DESIGN AND METHODS**—In a historical prospective cohort study of subjects from the Clalit Health Services database, which includes information on nearly 4 million people, diabetes-free subjects aged 40–70 years with serum 25-hydroxycholecalciferol (25-OHD) measurements available were followed for 2 years to assess the development of IFG and diabetes in five 25-OHD subgroups:  $\geq 25$ , 25.1–37.5, 37.6–50, 50.1–75, and  $> 75$  nmol/L.

**RESULTS**—The baseline cohort included 117,960 adults: 83,526 normoglycemic subjects and 34,434 subjects with IFG. During follow-up, 8,629 subjects (10.3% of the normoglycemic group) developed IFG, and 2,162 subjects (1.8% of the total cohort) progressed to diabetes. A multivariable model adjusted for age, sex, population group, immigrant status, BMI, season of vitamin D measurement, LDL and HDL cholesterol, triglycerides, estimated glomerular filtration rate, history of hypertension or cardiovascular disease, Charlson comorbidity index, smoking, and socioeconomic status revealed an inverse association between 25-OHD and the risk of progression to IFG and diabetes. The odds of transitioning from normoglycemia to IFG, from normoglycemia to diabetes, and from IFG to diabetes in subjects with a 25-OHD level  $\leq 25$  nmol/L were greater than those of subjects with a 25-OHD level  $> 75$  nmol/L [odds ratio 1.13 (95% CI 1.03–1.24), 1.77 (1.11–2.83), and 1.43 (1.16–1.76), respectively].

**CONCLUSIONS**—Vitamin D deficiency appears to be an independent risk factor for the development of IFG and diabetes.

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The incidence of type 2 diabetes is increasing worldwide at an alarming rate (1). Although healthy diet, weight control, and physical activity remain the core of diabetes prevention and treatment (2,3), emerging modifiable risk factors are attracting more attention.

Recent studies suggested that hypovitaminosis D could be a risk factor for the metabolic syndrome and type 2 diabetes (4–10). In a prospective study, Mattila et al. (11) found an inverse association between serum 25-hydroxycholecalciferol (25-OHD) levels and the risk of incident

type 2 diabetes; however, this association became nonsignificant after adjustment for confounders. Kayaniyil et al. (12) did find, however, that low serum 25-OHD was a risk factor for the metabolic syndrome, a risk that remained significant after adjustment for confounders. A prospective population-based study of 552 people by Forouhi et al. (6) found increased 10-year risks for incident diabetes and insulin resistance in subjects with lower baseline 25-OHD levels; vitamin D was also an independent risk factor for diabetes. In contrast, others found no correlation between 25-OHD levels and the risk of the metabolic syndrome or diabetes (13–16). Notably, most studies were observational or cross-sectional, and a causal relationship between vitamin D status and development of diabetes therefore could not be determined. In addition, some studies were underpowered or lacked adequate covariate adjustment. Consequently, the impact of vitamin D on glucose tolerance and the risk of diabetes is not clear. Indeed, the 2011 Institute of Medicine Dietary Reference Intake for Calcium and Vitamin D and the Endocrine Society Task Force guideline concluded that the evidence for a causal relationship between vitamin D deficiency and cardiovascular disease or diabetes is inconclusive (17,18).

The objective of the current study was to assess the impact of vitamin D status, as measured by serum 25-OHD (19), on the risk of developing impaired fasting glucose (IFG) or diabetes. We hypothesized that subjects with low 25-OHD levels are at increased risk of progression to impaired glucose metabolism. We performed a large, population-based cohort study including adults free of diabetes with 25-OHD measurements and analyzed the progression to IFG or diabetes. The results were adjusted for a wide range of potential confounders to contribute convincing findings to the debate on whether low vitamin D is an independent risk factor for IFG and diabetes.

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**RESEARCH DESIGN AND**

**METHODS**—The study was a prospective historical study analyzing data from the Clalit Health Services (CHS) electronic database of nearly 4 million people in Israel.

CHS is one of the four not-for-profit health maintenance organizations that provide hospital- and community-based medical services, including diagnostic procedures, medical treatments, and hospitalizations, for the Israeli population. All Israeli residents are required by law to choose to be covered by one of the health maintenance organizations, of which CHS is the largest, providing medical services to more than 4 million people. On average, CHS members are slightly older than members of the other health maintenance organizations and somewhat lower in socioeconomic status (SES). Electronic medical records have been available for all CHS members since 1998. The electronic database includes detailed demographic and clinical data and sociodemographic information derived from Israel's Central Bureau of Statistics and the National Insurance Institute (Social Security). The data are compiled into a centralized data warehouse from electronic records from primary care and specialist clinics, hospitals, pharmacies, and laboratories. CHS also coordinates a chronic disease registry, and all affiliated primary care physicians provide clinical information on all subjects aged >20 years with any of 110 chronic diseases. Member records are comprehensive within the data warehouse because members receive most of their care and treatments within CHS. The data warehouse was made accessible for this study after approval by the Clalit ethics committee.

**Inclusion criteria**

The study population included all CHS members aged 40–70 years who were continuous members between July 2008 and June 2012, had at least one record of a 25-OHD measurement between July 2008 and June 2010 (baseline period), and had at least two blood glucose measurements, with the first being during the baseline period and the second from July 2010 until June 2012 (follow-up period). If more than one 25-OHD test was available, the first recorded result was selected as the baseline measurement.

**Exclusion criteria**

Subjects were excluded if during the baseline period they had a diagnosis of diabetes entered in their medical records, they purchased diabetes medications, or

they had blood glucose or HbA<sub>1c</sub> measurements compatible with diabetes (blood glucose level  $\geq 7$  mmol/L or HbA<sub>1c</sub>  $\geq 6.5\%$ ). A prolonged baseline period was used to accumulate sufficient data for definitive exclusion of subjects with diabetes. Other exclusion criteria were serum creatinine  $>133.0$   $\mu\text{mol/L}$ , serum calcium  $>2.7$  mmol/L, diagnosis of hyperparathyroidism, renal or liver failure, and steroid treatment for longer than 3 months. A total of 200,492 people had at least one 25-OHD test performed between July 2008 and June 2010. After exclusion of subjects who were not continuous members ( $n = 7,800$ ), those who had fewer than 2 glucose tests ( $n = 12,707$ ), members with diagnosis of diabetes ( $n = 49,269$ ), and those with the aforementioned comorbidities ( $n = 12,756$ ), 117,960 subjects finally fulfilled the study criteria. The cohort was then divided into two groups according to the glycemic status during the baseline period (July 2008–June 2010). The normal glucose (NG) group ( $n = 83,526$ ) had all glucose measurements  $<5.6$  mmol/L, and the IFG group ( $n = 34,434$ ) had one or more glucose measurements  $\geq 5.6$  mmol/L and  $<7.0$  mmol/L (20).

**Independent variables**

Demographic variables that were recorded closest to the index date (July 2010) were included in the multivariable analysis: age, sex, population group, immigrant status, and SES. Low SES was the designation for individuals who received National Insurance payment exemption because of low income. The following independent predictors were also included: BMI ( $<18.5$ ,  $18.5$ – $24.9$ ,  $25$ – $29.9$ , and  $\geq 30$  kg/m<sup>2</sup>), season of vitamin D measurement, triglyceride level ( $<1.69$ ,  $1.69$ – $2.25$ ,  $2.26$ – $3.38$ , and  $\geq 3.39$  mmol/L), HDL cholesterol (for men,  $\leq 1.03$ ,  $1.04$ – $1.55$ , and  $\geq 1.56$  mmol/L; for women,  $\leq 1.29$ ,  $1.30$ – $1.55$ , and  $\geq 1.56$  mmol/L), LDL cholesterol ( $\leq 3.36$  and  $>3.36$  mmol/L), hypertension, smoking status, estimated glomerular filtration rate (eGFR), cardiovascular disease (a diagnosis of ischemic heart disease, previous myocardial infarction, or coronary artery bypass grafting before index date), and Charlson comorbidity index (CCI) (21). There was no linear association between most potential variables included in our model and the outcome; we therefore divided these variables into clinically meaningful categories rather than expressing them as continuous variables.

**Main predictor**

Baseline level of 25-OHD (the first laboratory test recorded) was the primary independent variable in the model. Levels were divided into five 25-OHD subgroups:  $\leq 25$ ,  $25.1$ – $37.5$ ,  $37.6$ – $50$ ,  $50.1$ – $75$ , and  $>75$  nmol/L. We analyzed 25-OHD levels by these five strata to test for the impact of very low vitamin D levels, which are less common, on the risk of incident IFG or diabetes.

**Dependent variables**

Progression to IFG or diabetes during the follow-up period was the primary study outcome. Diabetes in the follow-up period was diagnosed by at least one of the following criteria: 1) fasting plasma glucose level  $\geq 7.0$  mmol/L at least twice ( $n = 725$ ), 2) glucose level  $\geq 11.1$  mmol/L 120 min after an oral glucose tolerance test ( $n = 156$ ), 3) one blood glucose measurement  $\geq 7.0$  mmol/L and HbA<sub>1c</sub>  $\geq 6.5\%$  ( $n = 314$ ), 4) diagnosis of diabetes in the medical records and one glucose measurement  $\geq 7.0$  mmol/L, ( $n = 432$ ), and 5) diagnosis of diabetes in medical records and HbA<sub>1c</sub>  $\geq 6.5\%$  ( $n = 535$ ). IFG was diagnosed if at least one blood glucose measurement was  $>5.6$  mmol/L without fulfilling the diagnostic criteria for diabetes.

**Laboratory methods**

Biochemical analyses were performed at CHS laboratories with routine standardized methodologies on fresh samples of blood obtained after an overnight fast. Glucose was measured in plasma, and all other biochemical analyses were performed on serum. The laboratories are authorized to perform tests according to the international quality standard ISO 9001. Periodic assessment of quality control is performed on a regular basis. The accuracy of the measurements in the individual laboratory is confirmed by in-house daily quality control monitoring (OLIMPUS and BIO-RAD) and by monthly external quality control program (NEQAS). The 25-OHD level was tested by the LIAISON 25-OH Vitamin D TOTAL Assay (DiaSorine), a competitive two-step chemiluminescence assay that measures both the D<sub>2</sub> and D<sub>3</sub> 25-OHD metabolites. The measuring range is 10–375 nmol/L; the analytical sensitivity is  $<2.5$  nmol/L, and the functional sensitivity is  $<10.0$  nmol/L. The intra-assay coefficients of variation for low (18 nmol/L) and high (320 nmol/L) 25-OHD concentrations were 5.5% and 4.8%, respectively. The interassay coefficients

of variation were 12.7% and 7.9% for low and high 25-OHD concentrations, respectively. Performance characteristics of the vitamin D assay were evaluated by the CHS laboratory and were comparable to the manufacturer's specifications.

### Statistical analyses

The cohort at baseline was divided into five 25-OHD subgroups. Descriptive statistics were then assessed for both the NG and IFG groups across the vitamin D subgroups. Evidence of a trend across vitamin D groups was assessed with univariate linear regression for each covariate. Multivariable logistic regression assessed the association between IFG (relative to NG) and vitamin D levels while controlling for the covariates. All covariates were checked against one another for collinearity.

The proportions of members at baseline who underwent one of the three glycemic transitions (NG to IFG, NG to diabetes, and IFG to diabetes) were tested across the five baseline 25-OHD groups. Differences across groups were assessed by  $\chi^2$ , Student *t*, and rank sum tests for categorical, normally distributed, and nonnormally distributed parameters, respectively. Three logistic regression models were conducted to assess the association between 25-OHD groups and the odds of progression across the three glycemic categories while controlling for age, sex, population group, immigrant status, BMI, season of vitamin D measurement, LDL and HDL cholesterol, triglycerides, estimated GFR, history of hypertension or cardiovascular disease, CCI, smoking, and SES. A subgroup analysis was performed among subjects with low 25-OHD levels at baseline ( $\leq 37.5$  nmol/L) who had at least two 25-OHD measurements during the study period to evaluate the impact of changes in vitamin D levels on the risk of development of diabetes. For this analysis, we included subjects of the two lowest vitamin D categories ( $\leq 25$  and  $25.1-37.5$  nmol/L) and pooled the baseline IFG and NG groups to increase the number of participants. The first 25-OHD measurement was used as the baseline indicator, and the subsequent measurements were averaged to create the follow-up indicator. The odds of developing diabetes were assessed. Statistical analysis was performed with SPSS17 software (IBM Corporation, Armonk, NY). Data are expressed as mean  $\pm$  SD. Significance was set at  $P = 0.05$  level.

## RESULTS

### Baseline analysis

Our study included a cohort of 117,960 members free of diabetes on as of July 2010, with 71% in the NG group and 29% in the IFG group. Baseline characteristics of the cohort are shown in Table 1. The mean age of the subjects included in the cohort was 56.6 years, 76.6% were females, and the mean BMI was 27 kg/m<sup>2</sup>. IFG subjects were more likely to be men than NG subjects and were also older. A multivariable analysis showed that IFG was also associated with high BMI [odds ratio (OR) 2.36 (95% CI 2.28–2.45)], hypertension [1.41 (1.37–1.45)], low HDL cholesterol [1.37 (1.32–1.43)], and high triglyceride levels [1.49 (1.35–1.64)] (Table 2).

One-third of the subjects had 25-OHD level  $\leq 37.5$  nmol/L, and only 13% had levels  $>75$  nmol/L. Subjects with low

25-OHD levels were younger and had lower SES and CCI. In addition, these subjects were more likely to have IFG, higher BMI, and decreased HDL cholesterol (not shown). The 25-OHD levels were inversely associated with IFG. The odds of IFG among subjects with 25-OHD levels  $\leq 25$  nmol/L were 1.21 (95% CI 1.14–1.27) relative to those with 25-OHD levels  $>75$  nmol/L ( $P < 0.001$ ) (Table 2).

### Follow-up analysis

During the 2 years of follow-up, 8,629 subjects (10.3%) developed IFG and 2,162 subjects developed diabetes; among these, 334 (0.4%) were from the NG group and 1828 (5.3%) were from the IFG group. The intervals between initial 25-OHD measurement and diagnosis of IFG or diabetes were  $16.5 \pm 7.7$  and  $25.1 \pm 8.5$  months, respectively. Progression to IFG or diabetes occurred

**Table 1—Baseline characteristics of 117,960 CHS members by glycemic status**

Variables	NG	IFG	Total
N	83,526	34,434	117,960
Age (years)	55.8 $\pm$ 8	58.5 $\pm$ 7.3	56.6 $\pm$ 7.9
BMI (kg/m <sup>2</sup> )	26.4 $\pm$ 4.9	28.5 $\pm$ 5.4	27.0 $\pm$ 5.1
Female (%)	78.2	72.6	76.6
25-OHD <sup>a</sup> (nmol/L)	50.3 $\pm$ 23.0	49.3 $\pm$ 22.6	50.0 $\pm$ 22.9
Population group (%)			
General <sup>b</sup>	85.6	88.8	86.5
Ultra-Orthodox Jews	3.9	2.9	3.6
Arabs	10.5	8.3	9.9
Low SES <sup>c</sup> (%)	14.6	19.0	15.9
Immigrant <sup>d</sup> (%)	39.8	49.9	42.8
Smoking status (%)			
Past or current	25.7	27.0	26.1
Never	74.3	73.0	73.9
Hypertension (%)	24.9	41.3	29.7
Cardiovascular disease (%)	5.0	9.0	6.1
Triglyceride <sup>a</sup> (mmol/L)	1.36 $\pm$ 0.71	1.55 $\pm$ 0.77	1.42 $\pm$ 0.73
LDL cholesterol <sup>a</sup> (mmol/L)	3.02 $\pm$ 0.75	2.97 $\pm$ 0.78	3.01 $\pm$ 0.76
HDL cholesterol <sup>a</sup> (mmol/L)	1.42 $\pm$ 0.36	1.34 $\pm$ 0.34	1.4 $\pm$ 0.35
CCI <sup>e</sup>	0.68 $\pm$ 1.04	0.89 $\pm$ 1.22	0.74 $\pm$ 1.10
eGFR (mL/min)	92.4 $\pm$ 13.1	89.1 $\pm$ 13.7	91.5 $\pm$ 13.6
Season <sup>f</sup> (%)			
Autumn	20.5	19.4	20.1
Winter	25.7	26.5	26.0
Spring	28.4	29.4	28.7
Summer	25.4	24.7	25.2

Data are means  $\pm$  SD for continuous variables and % for categorical variables. <sup>a</sup>To convert 25-OHD concentration from nmol/L to ng/mL, divide by 2.5. To convert HDL and LDL cholesterol concentrations to mg/dL, multiply by 38.67. To convert triglycerides to mg/dL, multiply by 88.57. <sup>b</sup>General population group represents the whole population not including ultra-Orthodox Jews and Arabs. <sup>c</sup>Low SES refers to individuals who receive governmentally determined exemptions from medical copayments because of poverty. <sup>d</sup>Immigrant status was defined according to the country of birth (Israel or elsewhere). <sup>e</sup>CCI reflects burden of disease according to a summation of scores for individual chronic diseases. <sup>f</sup>Season refers to the percentage of tests performed in each season.

Table 2—Multivariable logistic regression for IFG group compared with NG group at baseline

Characteristic	B	OR	95% CI	P value
25-OHD <sup>a</sup> (nmol/L; reference >75)				<0.0001
0–25	0.19	1.21***	1.14–1.27	
25.1–37.5	0.13	1.14***	1.08–1.12	
37.5–50	0.08	1.08*	1.03–1.14	
50.1–75	0.04	1.04	0.99–1.09	
Age group (years; reference 40–45)				<0.0001
45–50	0.19	1.21***	1.13–1.29	
51–55	0.45	1.58***	1.48–1.67	
56–60	0.60	1.82***	1.71–1.93	
61–65	0.68	1.98***	1.86–2.11	
66–70	0.68	1.98***	1.85–2.12	
Male sex (reference female)	0.20	1.22***	1.18–1.27	
Immigrant <sup>b</sup> (reference nonimmigrant)	0.15	1.16***	1.13–1.19	
Population group (reference general <sup>c</sup> )				<0.0001
Ultra-Orthodox Jews	–0.43	0.65***	0.61–0.71	
Arabs	–0.36	0.70***	0.66–0.74	
Normal SES level (reference low SES <sup>d</sup> )	0.08	1.09***	1.05–1.13	
CCI <sup>e</sup>	0.08	1.08***	1.07–1.09	
BMI (kg/m <sup>2</sup> ; reference 18.5–24.9)				<0.0001
<18.5	–0.25	0.78**	0.67–0.91	
25–29.9	0.45	1.56***	1.51–1.61	
>30	0.86	2.36***	2.28–2.45	
Triglyceride <sup>a</sup> (mmol/L; reference <1.69)				<0.0001
1.69–2.25	0.17	1.18***	1.14–1.23	
2.26–3.38	0.25	1.28***	1.23–1.35	
>3.39	0.40	1.49***	1.35–1.64	
HDL cholesterol (reference low)				<0.0001
Medium	–0.16	0.86***	0.87–0.93	
High	–0.32	0.73***	0.70–0.76	
LDL cholesterol <sup>a</sup> (mmol/L; reference ≤3.36)	–0.03	0.96*	0.94–0.99	
eGFR	–0.00	0.97**	0.94–0.99	
Smoking status (reference never)				
Past or current	–0.01	0.98	0.95–1.01	
Hypertension	0.34	1.41***	1.37–1.45	
CVD	0.12	1.13***	1.07–1.19	
Season (reference summer)				
Autumn	–0.05	0.95*	0.91–0.99	
Winter	0.01	1.01	0.97–1.05	
Spring	0.03	1.03	0.99–1.07	

For each covariate, the coefficient of logistic regression (B), coefficient *P* value, OR, and 95% CI are presented. The multivariate logistic regression model was adjusted for age (45–50, 51–55, 56–60, 61–65, 66–70 years), sex, immigrant status, population group (ultra-Orthodox Jews, Arabs, general); SES (normal, low); CCI; BMI (<18.5, 18.5–24.9, 25–29.9, ≥30 kg/m<sup>2</sup>); triglyceride level (<1.69, 1.69–2.25, 2.26–3.38, ≥3.39 mmol/L); HDL (men ≤1.03, 1.04–1.55, ≥1.56 mmol/L; women ≤1.29, 1.30–1.55, ≥1.56 mmol/L); LDL (≤130, >130 mmol/L); eGFR (continuous); smoking status (past or current smoker, nonsmoker); hypertension; history of cardiovascular disease; and season of 25-OHD sampling. \**P* < 0.05. \*\**P* < 0.001. \*\*\**P* < 0.0001. <sup>a</sup>To convert 25-OHD concentration from nmol/L to ng/mL, divide by 2.5. To convert LDL cholesterol concentration to mg/dL, multiply by 38.67. To convert triglycerides to mg/dL, multiply by 88.57. <sup>b</sup>Immigrant status was defined according to the country of birth (Israel or elsewhere). <sup>c</sup>General population group represents the whole population not including ultra-Orthodox Jews and Arabs. <sup>d</sup>Low SES refers to individuals who receive governmentally determined exemptions from medical copayments because of poverty. <sup>e</sup>CCI reflects burden of disease according to a summation of scores for individual chronic disorders.

23.4 ± 6.7 and 32.4 ± 8.1 months, respectively, after initial blood glucose measurement. The percentage of subjects progressing to diabetes or to IFG continuously decreased with increasing 25-OHD

levels (Fig. 1). Univariate logistic regression to assess the association between 25-OHD and transition from NG to IFG yielded an OR of 1.16 (95% CI 1.06–1.27) when comparing the ≥25 nmol/L group

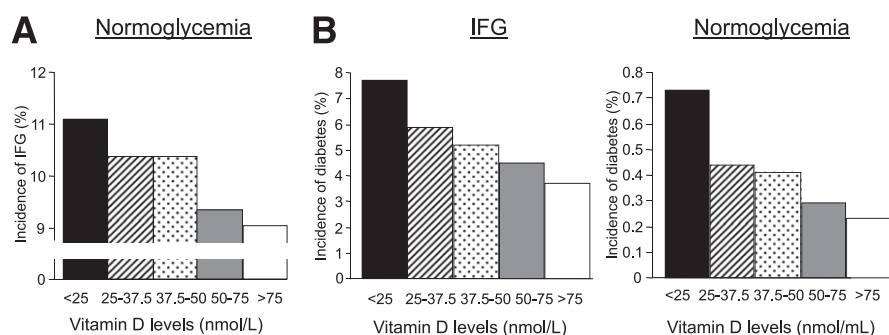
with the >75 nmol/L 25-OHD group, with an OR of 2.17 (1.79–2.64) among those transitioning from IFG to diabetes and an OR of 2.88 (1.87–4.41) among those transitioning from NG to diabetes.

After adjustment for all covariates, including BMI, the association between low 25-OHD levels and the risk of progression to IFG or diabetes was attenuated but remained significant for 25-OHD levels ≥25 nmol/L (NG to IFG or diabetes) and for 25-OHD levels ≥37.5 nmol/L (IFG to diabetes) (Table 3). The progression of NG to IFG had an OR of 1.13 (95% CI 1.03–1.24), NG to diabetes had an OR of 1.77 (1.11–2.83), and IFG to diabetes had an OR of 1.43 (1.16–1.76) when comparing subjects with 25-OHD ≥25 nmol/L with those with 25-OHD >75 nmol/L.

The majority of subjects included in our cohort were females, which may have strongly influenced the findings in the whole cohort. Stratification of the data according to sex showed that low vitamin D was also associated with a higher risk of progression from IFG to diabetes in males [OR 1.82 (95% CI 1.21–2.73); *P* < 0.01] when comparing subjects with 25-OHD ≥25 nmol/L versus those with 25-OHD <25 nmol/L.

The subgroup analysis examining whether a change in 25-OHD levels affected the progression to diabetes included 19,664 subjects with a baseline 25-OHD ≤37.5 nmol/L and average subsequent follow-up measurements ≤37.5 nmol/L (persistent low vitamin D) or ≥50 nmol/L (corrected vitamin D). In 53.6%, 25-OHD levels increased to ≥50 nmol/L during follow-up. In the corrected vitamin D group, average vitamin D level increased by 41.3 ± 18.5 nmol/L (*P* < 0.001). This correction was associated with a 40% reduction in the odds of incident diabetes relative to subjects whose low 25-OHD levels persisted (*P* < 0.001). After covariate adjustment, the reduction in the odds was 26% and remained statistically significant (*P* < 0.005).

**CONCLUSIONS**—Previous studies suggested that low vitamin D levels are associated with diabetes and the metabolic syndrome (4–12); however, the question of whether low vitamin D is an independent risk factor for diabetes remains open. To our knowledge, this is the largest longitudinal study published to date on the link between vitamin D and glycemic status. A large database of real-life data on close to 4 million



**Figure 1**—Incidences of transitions between glycemic states by 25-OHD level. Graphs show unadjusted incidences of transitions from NG to IFG (A) and from NG or IFG to diabetes (B) according to 25-OHD level at baseline divided into five subgroups:  $\geq 25$ , 25.1–37.5, 37.6–50, 50.1–75, and  $>75$  nmol/L. The incidences of IFG and diabetes are expressed as percentages of the NG and IFG groups, as indicated.

individuals allowed assessment with reduced bias of the association between vitamin D concentrations and the risk of diabetes, with rigorous adjustment for multiple confounders. The main findings of this large-scale cohort of Israeli adults are as follows: 1) 25-OHD levels are inversely associated with an increased risk of the presence and future development of IFG, 2) low 25-OHD levels are associated with an increased risk of future development of diabetes, and 3) increasing 25-OHD levels in subjects with vitamin D deficiency may reduce the risk of diabetes.

Corroborating previous studies, we found that low 25-OHD levels at baseline were associated with a lower SES and with a higher prevalence of different components of the metabolic syndrome, including IFG, higher BMI, elevated triglycerides,

and low HDL (7,12,22–27). During follow-up, low serum 25-OHD concentrations were associated with increased risk of progression to IFG and diabetes, an association that remained significant even after adjustment for various socioeconomic, anthropometric and metabolic confounders. Low vitamin D thus seems to be an independent risk factor for prediabetes and diabetes.

This is the first study to demonstrate that vitamin D concentrations are significantly associated with incident IFG. In quantitative terms, the impact of vitamin D deficiency on the risk of IFG was relatively small but significant. In the Framingham Offspring Study cohort, a higher predicted 25-OHD score was associated with a lesser increase in fasting plasma glucose concentration during 7

years among subjects who were diabetes free at baseline, further suggesting that vitamin D might be an important determinant for change in fasting glucose in NG subjects (28).

In our cohort, increased incidence of diabetes among subjects with low vitamin D is remarkable considering the relatively short follow-up period. The risk of progression to diabetes was higher among subjects with 25-OHD levels  $\leq 25$  nmol/L (NG to diabetes) and 37.5 nmol/L (IFG to diabetes) but not among those with higher 25-OHD levels. This is most likely explained by the short follow-up, which may not reveal the deleterious effects of mild vitamin D insufficiency, thus leading to underestimation of the impact of vitamin D on the risk of diabetes; however, the presence of a vitamin D level threshold above which there is no impact on the development of diabetes cannot be excluded.

In the Diabetes Prevention Program, the association between vitamin D levels and the risk of diabetes was linear in subjects at risk for diabetes (23). We maintain that future studies are needed to clarify whether our finding that vitamin D levels influence the risk of IFG or diabetes only below certain thresholds in fact holds true in other contexts.

Vitamin D insufficiency has been implicated in  $\beta$ -cell dysfunction and insulin resistance (8), which may increase blood glucose at all stages of diabetes. The precise mechanisms involved are currently not known. Further studies are

**Table 3**—Adjusted odds of progression between glycemic states by 25-OHD level

	25-OHD <sup>a</sup> group (nmol/L)				
	$\leq 25$	25.1–37.5	37.6–50	50.1–75	$>75$
NG subjects	11,887	14,457	17,212	28,786	11,184
IFG subjects	4,951	6,311	7,408	11,623	4,141
Glycemic status conversion					
NG to IFG					
Subjects	1,323 (11.13)	1,540 (10.65)	1,830 (10.63)	2,851 (9.90)	1,085 (9.70)
OR	1.13 [1.03–1.24]*	1.03 [0.94–1.12]	1.02 [0.94–1.11]	0.98 [1.06–1.37]	1
NG to diabetes					
Subjects	87 (0.73)	63 (0.44)	71 (0.41)	84 (0.29)	29 (0.26)
OR	1.77 [1.11–2.83]*	1.18 [0.74–1.88]	1.17 [0.75–1.84]	0.97 [0.63–1.49]	1
IFG to diabetes					
Subjects	383 (7.74)	375 (5.94)	390 (5.26)	526 (4.50)	154 (3.72)
OR	1.43 [1.16–1.76]**	1.23 [1.00–1.50]*	1.16 [0.95–1.41]	1.08 [0.89–1.30]	1

Data represent n (%) or OR [95% CI]. The multivariate logistic regression model was adjusted for age (45–50, 51–55, 56–60, 61–65, 66–70 years), sex, immigrant status, population group (general, ultra-Orthodox Jews, Arabs); SES level (regular, low); CCI; BMI ( $<18.5$ , 18.5–24.9, 25–29.9,  $\geq 30$  kg/m<sup>2</sup>); triglyceride level ( $<1.69$ , 1.69–2.25, 2.26–3.38,  $\geq 3.39$  mmol/L); HDL (men  $\leq 1.03$ , 1.04–1.55,  $\geq 1.56$  mmol/L; women  $\leq 1.29$ , 1.3–1.55,  $\geq 1.56$  mmol/L); LDL ( $\leq 3.36$ ,  $>3.36$  mmol/L); eGFR (continuous); smoking status (past or current, never); season of initial 25-OHD sampling; hypertension; and history of cardiovascular disease. \* $P < 0.05$ . \*\* $P < 0.001$ . <sup>a</sup>To convert the 25-OHD levels from nmol/L to ng/mL, divide by 2.5.

required to shed light on the role of vitamin D in the pathophysiology of diabetes.

Despite the mounting evidence supporting the relationship between vitamin D and the risk of diabetes, the results of clinical trials and post hoc analyses on the effect of vitamin D supplementation on glycemic outcomes have been inconclusive (4,29–33). Notably, in our cohort the risk of diabetes was reduced in subjects with initial low serum 25-OHD who had a documented increase in 25-OHD level relative to those with persistent low levels. In this retrospective population-based study, no information was available on how vitamin D deficiency was corrected in this group, precluding definitive conclusions as to the impact of vitamin D supplementation on the risk of diabetes. Collectively, our findings suggest that vitamin D is an important determinant of glycemia along the whole spectrum of glucose metabolism and that increasing vitamin D level may reduce the risk of diabetes. Large-scale, randomized, controlled trials targeting different 25-OHD levels are required to assess the impact of vitamin D supplementation on diabetes prevention.

This study has several limitations that warrant consideration. First, the analysis is retrospective, and therefore a causal relationship between vitamin D deficiency and diabetes cannot be definitively determined. Nevertheless, the longitudinal collection of data and the adjustment for multiple confounders reduce the impact of potential biases and strongly suggest that vitamin D status affects the risk of diabetes. Second, the analysis could be overly influenced by the findings among females, who comprised the majority of our cohort. We believe that the dominance of female sex in our sample is explained by physicians' awareness of poorer vitamin D status among females. Stratification of the data according to sex showed that low vitamin D was also associated with a higher risk of progression to diabetes in males, and we therefore believe that the impact of vitamin D status on the risk of diabetes is not limited to females. Third, no information is available on the indication for measuring vitamin D in a given subject. During the last few years, primary care physicians, specialists, and the general public became aware of the high prevalence of vitamin D deficiency and its potential implications for public health. In addition, 25-OHD testing became more accessible; we therefore assume that the majority of tests were

performed as screening and not for any specific medical indication. We cannot rule out, however, the possibility that subjects selected for vitamin D and repeated blood glucose testing were those already at increased risk of diabetes. Future studies will clarify whether vitamin D deficiency increases the risk of diabetes in the general population or only in subjects more likely to develop diabetes. Fourth, CHS members are slightly older and have somewhat lower SES relative to the general population in Israel. Still, our analyses have controlled for these variables and should reduce potential confounding effects, thus allowing conclusions that are largely generalizable. Fifth, we did not have information on calcium and vitamin D intake and on physical exercise; however, the large number of subjects in this cohort minimizes the likelihood that the results were biased by differences in these parameters. Seasonality is an important predictor of vitamin D status. In our cohort, the number of tests increased steadily with time; however, there was no seasonal variation in the frequency of blood sampling, and the impact of vitamin D status on the risk of IFG or diabetes was not affected by the season in which blood samples were obtained.

In conclusion, vitamin D concentrations are inversely associated with future incidence of IFG and diabetes. Hypovitaminosis D, especially at low levels ( $\leq 37.5$  nmol/L) is an independent risk factor for diabetes, and increasing 25-OHD may partially minimize the risk of diabetes. Confirmation of these results in large-scale randomized, controlled trials will have important implications for diabetes prevention and public health, because vitamin D supplementation is easy to implement, inexpensive, and safe. Further studies are required to assess the link between 25-OHD and other metabolic anomalies that are common in diabetes (e.g., hyperlipidemia) and to determine the optimal vitamin D target in the general population and among those at risk for diabetes.

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A.T. designed the study and wrote the manuscript. B.S.F. was involved in the design of the study, research of the data, and discussion. I.F. and M.B.H. researched data. G.L. was involved in the interpretation and discussions of the data and wrote part of the manuscript. R.D.B. was involved in the design of the study and discussion of the data. B.S.F. is the guarantor

of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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