Low 25-Hydroxyvitamin D and Risk of Type 2 Diabetes: A Prospective Cohort Study and Metaanalysis

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BACKGROUND: Vitamin D deficiency has been implicated in decreased insulin secretion and increased insulin resistance, hallmarks of type 2 diabetes mellitus. We tested the hypothesis that low plasma 25-hydroxyvitamin D [25(OH)D] is associated with increased risk of type 2 diabetes in the general population.

METHODS: We measured 25(OH)D in 9841 participants from the general population, of whom 810 developed type 2 diabetes during 29 years of follow-up. Analyses were adjusted for sex, age, smoking status, body mass index, income, physical activity, HDL cholesterol, and calendar month of blood draw.

RESULTS: Lower 25(OH)D concentrations, by clinical categories or seasonally adjusted quartiles, were associated with higher cumulative incidence of type 2 diabetes (trend, \( P = 2 \times 10^{-7} \) and \( P = 4 \times 10^{-10} \)). Multivariable adjusted hazard ratios of type 2 diabetes were 1.22 (95% CI 0.85–1.74) for 25(OH)D <5 vs ≥20 µg/L and 1.35 (1.09–1.66) for lowest vs highest quartile. Also, the multivariable adjusted hazard ratio of type 2 diabetes for a 50% lower concentration of 25(OH)D was 1.12 (1.03–1.21); the corresponding hazard ratio for those ≤58 years old was 1.26 (1.15–1.41). Finally, in a metaanalysis of 16 studies, the odds ratio for type 2 diabetes was 1.50 (1.33–1.70) for the bottom vs top quartile of 25(OH)D.

CONCLUSIONS: We observed an association of low plasma 25(OH)D with increased risk of type 2 diabetes. This finding was substantiated in a metaanalysis.

The pathogenesis of type 2 diabetes mellitus involves the development of a relative deficiency in insulin secretion and insulin resistance (1). Deficient vitamin D status has been associated with decreased insulin secretion and increased insulin resistance in animals and humans (2–9). Moreover, substitution with vitamin D in the deficient state has been associated with improvement in insulin secretion and glucose tolerance (5, 7, 9). These studies thus suggest a link between vitamin D deficiency and type 2 diabetes.

Observational and randomized studies on vitamin D concentrations or intake and risk of type 2 diabetes have been contradictory (10). In general, observational studies suggest that higher plasma 25-hydroxyvitamin D [25(OH)D] concentrations and higher vitamin D intake are associated with lower risk of type 2 diabetes. However, randomized studies do not show an effect of vitamin D supplementation on low risk of type 2 diabetes. Several factors have been proposed to explain these seemingly contradictory results, such as residual confounding in observational studies and insufficient doses in randomized studies. It is thus unclear at present whether low plasma 25(OH)D concentrations are associated with increased risk of type 2 diabetes.

We tested the hypothesis that low plasma 25(OH)D is associated with increased risk of type 2 diabetes in the general population. For this purpose, we studied 9841 white individuals from the Copenhagen City Heart Study followed for up to 29 years. We used seasonally unadjusted clinical categories of ≥20 µg/L [≥50 nmol/L] (sufficient), 10–19.9 µg/L [25–49.9 nmol/L] (insufficient), 5–9.9 µg/L [12.5–24.9 nmol/L] (deficient), and <5 µg/L [<12.5 nmol/L] (severely deficient), as well as concentrations adjusted for seasonal variation. Furthermore, the association of low plasma 25(OH)D concentrations with increased risk of type 2 diabetes was summarized in a metaanalysis including present and previous studies.

Materials and Methods

STUDY DESIGN
The Copenhagen City Heart Study is a prospective cohort study of the Danish general population initiated in

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provided written informed consent.

The present study included 9841 participants from the 1981–1983 examination (18 089 invited; 70% response rate) who were free of type 2 diabetes at baseline, had a nonfasting plasma glucose <198 mg/dL [<11 mmol/L] at baseline (fasting glucose concentrations were not available), and had available plasma samples for 25(OH)D measurement.

A Danish ethics committee approved the study (KF100.2039/91 and KF01–144/01). Participants provided written informed consent.

**MEASUREMENTS OF 25(OH)D**

Plasma samples collected at baseline in 1981–1983 were stored at −20 °C until 2009–2010, when 25(OH)D was measured with the DiaSorin Liaison 25(OH)D Total assay (12). Assay precision was tested daily, and assay accuracy was tested monthly with an external quality control program. The interassay CV was 10% for low-concentration controls [approximately 54 nmol/L (135 g/L)] and 8% for high-concentration controls [approximately 54 μg/L (135 nmol/L)].

**POTENTIAL CONFOUNDERS**

Variables were ascertained in 1981–1983, 1991–1994, and 2001–2003 (11) and used as time-varying variables in multivariable adjusted models. Information on smoking habits was obtained from self-reported questionnaires completed together with an examiner on the day of attendance. Participants also reported their level of income (high, medium, or low) and duration and intensity of leisure-time physical activities (h/week) in self-reported questionnaires reviewed together with an examiner on the day of attendance. Body mass index (BMI) was calculated as measured weight (kilograms) divided by measured height (meters) squared.

**ENDPOINT**

Incident type 2 diabetes was self-reported diabetes and use of antidiabetic medicine at follow-up examination (1991–1994 or 2001–2003), nonfasting glucose >198 mg/dL [>11 mmol/L] at follow-up examination, or information on incident diagnoses of type 2 diabetes (WHO, *International Classification of Diseases, Revision 8*, code 250, and Revision 10, codes E11, E13, and E14) collected and verified by reviewing hospital admissions and diagnoses entered in the national Danish Patient Registry and by reviewing the national Danish Causes of Death Registry. Follow-up time for each subject began at the day of blood sampling in 1981–1983 and ended at diagnosis of type 2 diabetes (n = 810), death (n = 5908), emigration (n = 54), or August 2010, whichever occurred first. The median follow-up time was 20 years (range 0.03–29). Follow-up was 100% complete; that is, we did not lose track of even a single individual.

**STATISTICAL ANALYSES**

We divided baseline 25(OH)D into the following a priori seasonally unadjusted clinical categories of ≥20 μg/L [≥50 nmol/L] (sufficient), 10–19.9 μg/L [25–49.9 nmol/L] (insufficient), 5–9.9 μg/L [12.5–24.9] nmol/L (deficient), and <5 μg/L [<12.5 nmol/L] (severely deficient). In addition, because concentrations of 25(OH)D were expected to vary according to time of year due to the high-latitude geographical position of Denmark, we used seasonally adjusted 25(OH)D concentrations. Two strategies were applied to adjust for the seasonal variation in vitamin D. First, we used unadjusted 25(OH)D concentrations in regression analyses, while adjusting for calendar month of blood draw. Second, we obtained calendar month-specific cutpoints by assigning subjects to quartile categories within the same month of sample collection (see Supplemental Table S1, which accompanies the online version of this article at http://www.clinchem.org/content/vol59/issue2). For trend tests, individuals in each group were assigned the median value of their group, as either absolute values or percentiles. As a supplement to these analyses, we also compared participants with plasma 25(OH)D >30 μg/L [>75 nmol/L] with participants with plasma 25(OH)D of 20–30 μg/L [50–75 nmol/L], as it has been suggested that the non-calcemic benefits of vitamin D may be maximized when 25(OH)D is >30 μg/L [>75 nmol/L] (13). We chose to carry out analyses using both clinical categories with absolute values and month-specific quartiles. Although month-specific quartiles may be more suitable for biological hypothesis testing, the clinical categories give information that facilitates comparability between studies, and absolute values are also those used clinically, making absolute values transferable to the everyday activities of clinicians.

To evaluate whether storage time was associated with median concentrations of plasma 25(OH)D, we also measured plasma 25(OH)D in 400 participants without diabetes, cancer, heart disease, or other chronic diseases participating in the 1981–1983, 1991–1994, and 2001–2003 examinations of the Copenhagen City Heart Study.

We estimated cumulative incidences using the competing risk proportional subhazard models by the method
of Fine and Gray (14), in which competing risk of death was accounted for. The analyses were adjusted for age and year of birth to account for calendar effects. We used age as time scale. The cumulative incidence functions were plotted by seasonally unadjusted clinical categories and seasonally adjusted percentile categories.

We used Cox proportional hazards regression to estimate hazard ratios with 95% CI for incident type 2 diabetes. We used age as time scale with delayed entry (left truncation). Thus, age differences were automatically adjusted for, and analyses are referred to in text, tables, and figures as age adjusted. Multivariable adjusted Cox regression models included (a) risk factors for type 2 diabetes as age, sex, smoking status (never/ever), BMI, and duration and intensity of leisure time physical activities, (b) income as a measure of social status, and (c) calendar month of blood draw (the latter only for models with clinical categories) as a confounder for 25(OH)D concentrations. We tested for interactions using likelihood ratio tests with Cox regression models including and excluding multiplicative 2-factor interaction terms, the latter nested in the former model. In interaction analyses and stratified analyses, we used log2-transformed values of plasma 25(OH)D, whereby a 1-unit decrease corresponds to a 50% lower concentration of plasma 25(OH)D. The proportional hazards assumption was assessed in Cox regression models graphically by plotting \(-\ln(-\ln(\text{survival}))\) vs \(\ln(\text{analysis time})\); we detected no violations of the proportional hazards assumption. The data were 99.8% complete in relation to the included variables. The extracted data included first author; publication year; cohort size and source; reported follow-up time; design; method of vitamin D measurement; method of 25(OH)D categorization; estimates of the association between 25(OH)D concentrations and outcome; ascertainment of diagnosis; and adjustment for age, sex, overweight or obesity, smoking, and physical activity, as these variables are known risk factors for type 2 diabetes and vitamin D deficiency, and season of blood draw, which is associated with plasma vitamin D concentrations. We converted the risk estimates from individual studies to risk estimators for top vs bottom quartiles to obtain more robust synthesized risk estimates (28). For 3 studies, this conversion was not possible (15, 18, 21), and the corresponding authors were contacted to obtain the risk estimates. Some studies did not report mean or median concentrations of 25(OH)D, and in these studies mean concentrations were estimated from the reported distribution of 25(OH)D (17, 25, 26).

We performed the meta-analysis using fixed and random-effect models (29) and calculated random-effect weights using the DerSimonian and Laird model. Heterogeneity was assessed by the \(Q\) statistic and its extent was quantified by \(I^2\) (the fraction of between study variability due to heterogeneity) (30). Publication bias was evaluated by funnel plots, Begg rank correlation test, and Egger regression test.

Results

THE COPENHAGEN CITY HEART STUDY

Table 1 and online Supplemental Table S3 summarize baseline characteristics by plasma 25(OH)D concentrations. Low concentrations of 25(OH)D were associated with high age, smoking, high BMI, low income, low-duration leisure time physical activity, and blood sampling in winter. The association of 25(OH)D concentrations with BMI showed decreasing 25(OH)D in participants with increasing BMI (trend, \(P = 2 \times 10^{-41}\)), but underweight participants had lower concentrations of 25(OH)D than normal-weight participants (see online Supplemental Fig. S2). The median 25(OH)D concentration was 16 µg/L [41 nmol/L] among all participants and 14 µg/L [36 nmol/L] among
those who later developed type 2 diabetes. A total of 810 incident cases of type 2 diabetes occurred among 9841 participants during up to 29 years of follow-up. For 400 healthy participants, we had measurements of plasma 25(OH)D from 1981–1983, 1991–1994, and 2001–2003, which showed that median concentrations were relatively stable, i.e., storage time did not systematically associate with lower concentrations of 25(OH)D (see online Supplemental Fig. S3).

The cumulative incidence of type 2 diabetes increased with decreasing concentrations of baseline plasma 25(OH)D expressed in clinical categories (trend, \( P = 3 \times 10^{-5} \)) and expressed in seasonally adjusted quartiles (\( P = 2 \times 10^{-5} \)) (Fig. 1). Multivariable adjusted hazard ratios for type 2 diabetes increased with decreasing concentrations of 25(OH)D by clinical categories and expressed in seasonally adjusted quartiles (\( P < 0.001 \)) (Fig. 1). Multivariable adjusted hazard ratios for type 2 diabetes increased with decreasing concentrations of 25(OH)D by clinical categories and expressed in seasonally adjusted quartiles, and were 1.22 (95\% CI 0.85–1.74) for 25(OH)D <5 \( \mu g/L \) [\(<12.5 \text{ nmol/L} \)] vs \( \geq 20 \ \mu g/L \) [50 \text{ nmol/L}], and 1.35 (1.09–1.66) for lowest vs highest quartile (Fig. 2). Additional analyses including the clinical category of 25(OH)D >30 \( \mu g/L \) [\( >75 \text{ nmol/L} \)], consisting of 985 participants, showed multivariable adjusted hazard ratios for type 2 diabetes of 0.91 (0.67–1.25) for 25(OH)D >30 \( \mu g/L \) [\( >75 \text{ nmol/L} \)] vs 30 \( \geq 25(\text{OH})D \geq 20 \ \mu g/L \) [75 \( \geq 25(\text{OH})D \geq 50 \text{ nmol/L} \]). The use of 25(OH)D >30 \( \mu g/L \) [\( >75 \text{ nmol/L} \)] as the reference value showed results similar to those of the above analyses (see online Supplemental Fig. S4).

The multivariable adjusted hazard ratio for type 2 diabetes for a 50\% lower concentration of 25(OH)D was 1.12 (1.03–1.21) (Fig. 3). A 50\% lower concentration of 25(OH)D was associated with a hazard ratio \( \leq 1.0 \) in most strata; however, not all individual risk estimates were significant. Nevertheless, as tests of interaction were nonsignificant for all stratifications, except age, after correction for 7 parallel tests using the Bonferroni correction, this implies that low 25(OH)D concentrations associate with increased risk of type 2 diabetes irrespective of category levels of other vari-

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**Table 1. Baseline characteristics according to clinical cutpoints for plasma 25(OH)D concentrations.**

<table>
<thead>
<tr>
<th>Plasma 25(OH)D, ng/mL</th>
<th>&lt;5</th>
<th>5–9.9</th>
<th>10–19.9</th>
<th>( \geq 20 )</th>
<th>Trend, ( P^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>458</td>
<td>1805</td>
<td>3932</td>
<td>3646</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>209 (46)</td>
<td>797 (44)</td>
<td>1680 (43)</td>
<td>1561 (43)</td>
<td>0.29</td>
</tr>
<tr>
<td>Age, years</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>59</td>
<td>58</td>
<td>58</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Interquartile range</td>
<td>50–65</td>
<td>49–65</td>
<td>48–65</td>
<td>47–64</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>62 (14)</td>
<td>308 (17)</td>
<td>857 (22)</td>
<td>850 (23)</td>
<td></td>
</tr>
<tr>
<td>Ever</td>
<td>396 (86)</td>
<td>1497 (83)</td>
<td>3075 (78)</td>
<td>2796 (77)</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>24.9</td>
<td>25.5</td>
<td>25.1</td>
<td>24.2</td>
<td></td>
</tr>
<tr>
<td>Income</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>206 (45)</td>
<td>663 (37)</td>
<td>1218 (31)</td>
<td>977 (27)</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>190 (42)</td>
<td>806 (46)</td>
<td>1828 (47)</td>
<td>1700 (47)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>57 (13)</td>
<td>306 (17)</td>
<td>834 (22)</td>
<td>936 (26)</td>
<td></td>
</tr>
<tr>
<td>Duration of leisure time physical activity, h/week</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \leq 2 )</td>
<td>147 (32)</td>
<td>417 (23)</td>
<td>646 (16)</td>
<td>406 (11)</td>
<td></td>
</tr>
<tr>
<td>2–4 (light activity)</td>
<td>198 (43)</td>
<td>887 (49)</td>
<td>1953 (50)</td>
<td>1788 (49)</td>
<td></td>
</tr>
<tr>
<td>( \geq 4 ) or 2–4 (heavy activity)</td>
<td>113 (25)</td>
<td>501 (28)</td>
<td>1328 (34)</td>
<td>1450 (40)</td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May–October (summer)</td>
<td>114 (25)</td>
<td>667 (37)</td>
<td>2016 (51)</td>
<td>2329 (64)</td>
<td></td>
</tr>
<tr>
<td>November–April (winter)</td>
<td>344 (75)</td>
<td>1138 (63)</td>
<td>1916 (49)</td>
<td>1317 (36)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Data are n (%) unless noted otherwise. 
\(^b\) Cuzick nonparametric trend test.
ables. Concerning age, the multivariable adjusted hazard ratio for type 2 diabetes for a 50% lower concentration of 25(OH)D was 1.50 (1.33–1.70) and 1.00 (0.88–1.15) for those ≤58 years and >58 years old, respectively (interaction, \( P = 10^{-8} \)).

METAANALYSIS

A total of 14 studies representing 16 cohorts were included in the metaanalysis, with a total of 72,204 participants and 4,877 type 2 diabetes events. The characteristics of the studies are summarized in Table 2 and
Fig. 4. The odds ratios of type 2 diabetes comparing low vs high concentrations of 25(OH)D were 1.50 (95% CI 1.33–1.66, fixed effect) and 1.50 (1.33–1.67, random effect) (Fig. 4). Further analyses restricted to studies of the general population or studies with complete adjustment did not change the estimates appreciably. Analyses stratified according to study design likewise did not alter the associations substantially. There was no evidence of between-study heterogeneity ($I^2 = 1.4\%$, $P = 0.44$) or publication bias (Begg rank correlation test, $P = 1.00$, and Egger regression test, $P = 0.58$) (see online Supplemental Fig. S5). The Anderson et al. study (15) differed from the other studies with regard to population, follow-up (mean 1.3 years), adjustment, and ascertainment of diabetes; thus the metaanalysis was repeated without this study resulting in a odds ratio for type 2 diabetes of 1.39 (1.21–1.58).

**Discussion**

In the largest general population study to date, we observed an increasing risk of type 2 diabetes with decreasing plasma 25(OH)D concentrations. These findings were confirmed in a metaanalysis of prospective cohort and nested case-control studies published until July 2012.

Biologically, our results make sense, since vitamin D status has been implicated in 2 essential processes linked to type 2 diabetes, i.e., insulin secretion and insulin resistance. (1) Evidence supporting a role for vitamin D in insulin secretion: the vitamin D receptor and the 1-α-hydroxylase enzyme, the enzyme that converts 25(OH)D into the active hormone 1,25-dihydroxyvitamin D, are present in β-cells (31, 32); in vitro and in vivo studies show that vitamin D receptor
knockout or vitamin D deficiency impairs glucose-induced insulin secretion (5, 6, 8, 9, 33); and the insulin secretory response improves after vitamin D supplementation in both animals and humans (5, 6, 8, 9, 34). (2) Evidence supporting a role for vitamin D in insulin sensitivity: the vitamin D receptor is present in skeletal muscle cells (35); vitamin D stimulates insulin receptor expression and insulin-induced glucose transport in vitro (36, 37); vitamin D directly regulates pathways implicated in the regulation of fatty acid metabolism in skeletal muscle and adipose tissue (38); and low concentrations of vitamin D are associated with impaired insulin sensitivity, whereas substitution with vitamin D in the deficient state improves insulin sensitivity (2–4, 9, 39). However, several randomized studies have also shown contrasting results with no improvement in insulin secretion or sensitivity after vitamin D supplementation (10).

Our metaanalysis shows that low concentrations of 25(OH)D are robustly associated with increased risk of type 2 diabetes irrespective of population, level of adjustment, or study design. The estimate from the present metaanalysis is comparable to previous metaanalyses with fewer studies and not including the present study (10, 16). Interestingly, there were no signs of statistical heterogeneity or publication bias in our metaanalysis. Further studies should be randomized intervention studies or genetic epidemiological studies designed to establish causality rather than association as in the present study.

A potential limitation is that our cohort consists of whites of Danish descent living in Denmark (latitude 55–58 degrees north) with less sun exposure than closer to the equator; consequently, our findings would be most applicable to individuals with a similar skin color and a similar level of sun exposure. The delay in measurement from 1981–1983 to 2009–2010 could raise concern of potential decay of plasma 25(OH)D, but this seems unlikely to have distorted our analyses for several reasons: we noticed the expected seasonal

Fig. 3. Hazard ratios for type 2 diabetes by a 50% lower concentration of plasma 25(OH)D overall and in strata. Analyses were adjusted for sex, age, smoking status (never/ever), BMI, income, and duration and intensity of leisure time physical activities (except the one stratified for). Age and BMI were categorized by use of the approximate median. Based on 9841 individuals from the Danish general population, the Copenhagen City Heart Study, followed for up to 29 years after blood sampling for measurement of 25(OH)D. NS = not significant (P > 1.0) after multiplication of P value by 7 according to the Bonferroni correction.
Table 2. Observational prospective studies of the association of plasma 25(OH)D with risk of type 2 diabetes.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Women, %</th>
<th>Mean age, years</th>
<th>Mean BMI, kg/m(^2)</th>
<th>White, %</th>
<th>Adjustment, (0–6)\textsuperscript{b}</th>
<th>Design</th>
<th>Population setting</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fourouhi et al. (16)</td>
<td>2008</td>
<td>58</td>
<td>64</td>
<td>ND\textsuperscript{c}</td>
<td>99</td>
<td>6</td>
<td>Cohort</td>
<td>General practice population</td>
<td>OGTT</td>
</tr>
<tr>
<td>Pilz et al. (23)</td>
<td>2012</td>
<td>61</td>
<td>68</td>
<td>27</td>
<td>ND</td>
<td>5</td>
<td>Cohort</td>
<td>(Middle-aged) general</td>
<td>OGTT, fasting glucose, glycosylated hemoglobin</td>
</tr>
<tr>
<td>Knekt et al. (22)</td>
<td>2008</td>
<td>54</td>
<td>ND</td>
<td>ND</td>
<td>100</td>
<td>6</td>
<td>Nested case-control</td>
<td>General</td>
<td>Medication treated, registry-based</td>
</tr>
<tr>
<td>González-Molero et al. (18)</td>
<td>2012</td>
<td>57</td>
<td>50</td>
<td>ND</td>
<td>ND</td>
<td>6</td>
<td>Cohort</td>
<td>General</td>
<td>OGTT, glycosylated hemoglobin</td>
</tr>
<tr>
<td>Grimnes et al. (smokers only) (19)</td>
<td>2010</td>
<td>60</td>
<td>57</td>
<td>24.7</td>
<td>100</td>
<td>6</td>
<td>Cohort</td>
<td>General</td>
<td>Questionnaire, OGTT, glycosylated hemoglobin, glucose, registry-based</td>
</tr>
<tr>
<td>Hurskainen et al. (20)</td>
<td>2012</td>
<td>54</td>
<td>63</td>
<td>27.8</td>
<td>ND</td>
<td>6</td>
<td>Cohort</td>
<td>(Middle-aged) general</td>
<td>OGTT, fasting glucose, medication treated</td>
</tr>
<tr>
<td>Thorand et al. (26)</td>
<td>2011</td>
<td>47</td>
<td>52</td>
<td>27.1</td>
<td>ND</td>
<td>6</td>
<td>Case-cohort</td>
<td>General</td>
<td>Validated questionnaire</td>
</tr>
<tr>
<td>Pittas et al. (24)</td>
<td>2010</td>
<td>100</td>
<td>56</td>
<td>27.8</td>
<td>98</td>
<td>6</td>
<td>Nested case-control</td>
<td>US female nurses</td>
<td>Validated questionnaire</td>
</tr>
<tr>
<td>Fourouhi et al. (16)</td>
<td>2012</td>
<td>58</td>
<td>58</td>
<td>26.0</td>
<td>99</td>
<td>6</td>
<td>Case-cohort</td>
<td>General practice population</td>
<td>Self-report with linkage to general, hospital, and death registries</td>
</tr>
<tr>
<td>Deleskog et al. (27)</td>
<td>2012</td>
<td>40</td>
<td>48</td>
<td>26.3</td>
<td>ND</td>
<td>5</td>
<td>Nested case-control</td>
<td>Population enriched with familial diabetes</td>
<td>OGTT, fasting glucose</td>
</tr>
<tr>
<td>Husemoen et al. (21)</td>
<td>2012</td>
<td>52</td>
<td>46</td>
<td>26</td>
<td>100</td>
<td>6</td>
<td>Cohort</td>
<td>General</td>
<td>OGTT, fasting glucose, glycosylated hemoglobin, diagnosis</td>
</tr>
<tr>
<td>Gagnon et al. (17)</td>
<td>2011</td>
<td>55</td>
<td>51</td>
<td>26.6</td>
<td>92</td>
<td>5</td>
<td>Cohort</td>
<td>General</td>
<td>OGTT, fasting glucose, medication treated</td>
</tr>
<tr>
<td>Grimnes et al. (nonsmokers) (19)</td>
<td>2010</td>
<td>62</td>
<td>60</td>
<td>26.3</td>
<td>100</td>
<td>6</td>
<td>Cohort</td>
<td>General</td>
<td>Questionnaire, OGTT, glycosylated hemoglobin, glucose, registry-based</td>
</tr>
<tr>
<td>Robinson et al. (25)</td>
<td>2011</td>
<td>100</td>
<td>66</td>
<td>28.1</td>
<td>90</td>
<td>5</td>
<td>Nested case-control</td>
<td>Postmenopausal women</td>
<td>Medication treated, self-report</td>
</tr>
<tr>
<td>Anderson et al. (15)</td>
<td>2010</td>
<td>75</td>
<td>55</td>
<td>ND</td>
<td>ND</td>
<td>2</td>
<td>Cohort</td>
<td>Health care population</td>
<td>Physician diagnoses</td>
</tr>
<tr>
<td>This study\textsuperscript{d}</td>
<td>2012</td>
<td>56</td>
<td>56</td>
<td>25.3</td>
<td>100</td>
<td>6</td>
<td>Cohort</td>
<td>General</td>
<td>Self report, medication treated, nonfasting glucose, registry-based</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Studies are ranked as in Fig. 4 based on the fixed-effect weight in the metaanalysis.

\textsuperscript{b} Age, sex, season of blood draw, BMI, smoking, and physical activity.

\textsuperscript{c} ND, no data; OGTT, oral glucose tolerance test.

\textsuperscript{d} Copenhagen City Heart Study.
variation of 25(OH)D concentrations; median concentrations of plasma 25(OH)D across plasma samples from 3 different examinations on the same healthy participants with storage times of 10, 20, and 30 years were similar; previous studies have shown high stability during storage (40); the median concentration observed in our study of 16.926 μg/L [41 nmol/L] was similar to that in comparable populations (22, 26); and a low sample quality for the 25(OH)D measurement would tend to weaken rather than inflate an association. Similarly, the diagnoses were obtained from self-report, hospital discharge, and death registries, thus postponing diagnoses made by the general practitioner alone and leading to potential underreporting by participants. However, this potential underreporting would only tend to weaken rather than inflate an association.

Our study has several strengths: our population was homogeneous, we had up to 29 years of follow-up with no loss to follow-up, we could account for other major risk factors associated with risk of type 2 diabetes, and we had the highest statistical power to date to examine the associations of low plasma 25(OH)D concentrations with risk of type 2 diabetes. Furthermore, in Northern Europe, UV-B radiation from the sun is adequate for sufficient endogenous vitamin D production in the skin only during the summer months, and food has never been fortified with vitamin D in Denmark. Thus, this cohort from the Danish general population allows determination of the natural history of the association of vitamin D deficiency with risk of type 2 diabetes.

Clinical applications of the present study should be considered cautiously, as this is an observational study. Randomized interventional trials are needed before supplementation with vitamin D can be recommended for prevention of diabetes.

In conclusion, we observed an association between low plasma 25(OH)D and increased risk of type 2 diabetes in the general population. This finding was substantiated in a metaanalysis.

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**Fig. 4.** Metaanalysis of prospective studies on plasma 25(OH)D and risk of type 2 diabetes.

The reference category is the highest category of 25(OH)D in each study, and risk estimates are versus the lowest category of 25(OH)D in each study. On the forest plot, black box areas are proportional to the fixed-effect weight of the individual studies. The white diamonds represent the summary estimate, and CIs correspond to the width of the diamonds. Complete adjustment included adjustment for age, sex, season of blood draw, BMI or other obesity measures, smoking, and physical activity. The Knekt study includes both the Finnish Mobile Clinic Health Examination Survey and the Mini-Finland Health Survey. *The Copenhagen City Heart Study, the present study. ND = no data. **
or revising the article for intellectual content; and (c) final approval of the published article.

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References


